Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

ISSN: 0972-6268	Vol. 15	No. 4	pp. 1153-1160	2016

Original Research Paper

Combination of Vermicompost Fertilizer, Carbon, Nitrogen and Phosphorus on Cell Characteristics, Growth and Quality of Agar Seaweed *Gracilaria verrucosa*

Andi Rahmad Rahim*†, Endang Yuli Herawati, Happy Nursyam*** and Anik Martinah Hariati******

*Doctoral Program, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang-65145, East Java, Indonesia

**Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang-65145, East Java, Indonesia

***Department of Fishery Product Technology, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang-65145, East Java, Indonesia.

****Laboratory of Fish Nutrition, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang-65145, East Java, Indonesia

†Corresponding author: Andi Rahmad Rahim

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 11-11-2015
Accepted: 10-12-2015

Key Words:
Vermicompost
Fertilizer
Gracilaria verrucosa
Quality of agar

ABSTRACT

Seaweed is one of the marine and fishery commodities which encounter many problems in spurring growth and obtain high quality of agar, especially in Takalar District South Sulawesi Province, Indonesia. The combined use of vermicompost organic fertilizer, carbon, nitrogen and phosphorus are a source of much-needed nutrients by the seaweed to expand the network of cells that affect the growth and quality of agar. The purpose of this study was to see the influence of the combination of vermicompost fertilizer, carbon, nitrogen and phosphorus on the characteristics of the cells, the growth and the quality of agar seaweed *G. verrucosa*. This study was conducted for 42 days. The treatment ratio of carbon% : nitrogen% : phosphorus% are 93:6:1, 92:7:1, 91:8:1, 90:9:1, 89:9:2 and 88:10:2. Treatment ratio of fertilizer was best obtained at treatment 91:8:1, the cell number 39-104 mm^{-2}, cell size from 25.189 to 400.213 ¼m, cell shape oval and cell wall thickness, weight biomass 0.65 to 2.14 g, the daily specific growth from 2.29 to 7.99%, thallus length from 2.5 to 3.8 cm, quality of agar from 9.98 to 26.52%, quality of agar viscosity of 43-90 cps, quality of agar gel strength of 49.0 to 80.4 g/cm^2. The range of water quality during the study was 25-26°C for the temperature, water salinity was 25 ppt, pH 7.50 to 7.87, carbon 734.51 to 4754.12 ppm, nitrogen from 14.91 to 98.91 ppm, and phosphorus of water from 19.84 to 38.39 ppm.

INTRODUCTION

Seaweed is a potential commodity of marine and fisheries in Takalar District South Sulawesi Province with an area of aquaculture ponds *Gracilaria verrucosa* 4,100 ha, and the production of 1,300 tons per 40-60 days of maintenance (Arhan 2008). During this particular type of seaweed culture of *G. verrucosa* many problems are faced in developing, growth and high quality of agar. The low quality of agar was due to the quality of the soil of ponds that has been decreased because of the use of synthetic fertilizers that contain many chemicals (Rahmat 2012). Aquaculture ponds productivity can be improved with enhancing the farm management techniques, e.g. using the environmental friendly fertilizer. The quality of seaweed is determined by the total gel or agar content which is the photosynthesis product of the seaweed.

Agar utilization for food is one important part of the industry producing colloid of red algae. Many of agar products in the market with a variety of shapes, brands, and prices, cause consumers to find it difficult to make the selection. A solution is to select agar commercial products with the quality characteristics, which is expected to provide an overview to determine high quality of agar products (Anggadireja et al. 2006).

One of the factors that determine the quality of agar seaweed is fertilizing with the addition of nutrients to the cultivation media. Vermicompost is an organic fertilizer that is environmental friendly and contains good nutrients for plant growth (Hernandez et al. 2014). In the vermicompost fertilizer, nutrients that are important such as C, N, and P are needed by plants, contained in foods modified by the activity of microorganisms into a form that is absorbed easier by plants (Morales et al. 2014). Vermicompost, carbon, nitrogen and phosphorus have a function to arrange the plasma cells in the form of agar seaweed *G. verrucosa* (Rahmat 2012). *G. verrucosa* particular intensification farming in the province of South Sulawesi is being encouraged in order to fulfil industry needs of agar. An effort to improve the quality of agar seaweed *G. verrucosa* is by using a combination of vermicompost organic fertilizer, carbon, nitrogen, phosphorus and supported with good land cultivation pond. Based on the description thus we need to investigate the necessary combination of vermicompost fertilizer, carbon, nitrogen and phosphorus on the growth and quality of agar seaweed *G. verrucosa*.

MATERIALS AND METHODS

Time and place: The study was conducted at the Laboratory of Tissue Culture, Faculty of Agriculture Studies Program, Agricultural Cultivation University of Brawijaya from January to March 2015.

Preparation of seeds: We used fresh seeds. Healthy parent plants were selected from cultivation instead of the stock. The seed characteristics were having many branches with ends coloured rather dark brownish, young thallus, rigid and fresh, free from other plant or foreign objects, no spots and flaky, minimum aged 2 weeks, and weight of 50-100 g.

Tissue culture: Containers used in the form of Petri dishes, flasks, bottles, tubes, pipettes, tweezers and the airstone. It was washed and rinsed thoroughly with distilled water, then sterilized by using an autoclave at a temperature of 121°C for 15 minutes with a pressure of 20 psi. After autoclaved, it was put in the culture room with a temperature of 25°C.

Sea water was subsequently sterilized with a salinity of 25 ppt, by filtering it, using a sieve/filter and put into Erlenmeyer flasks of 1 litre. The sea water was filtered again using a vacuum pump through the sterile filter paper of 0.45 µm. The filtered seawater was then autoclave at a temperature of 121°C for 15 minutes at a pressure of 20 psi.

Furthermore, sterilization of seaweed explants (cell) was processed through four stages of sterilization, namely (1) thallus of seaweed were taken with a length of 10 cm and then washed with detergent at a dose of 5 mL/100 mL of distilled water (5%). (2) We used fungicide at a dose of 3 g/L and then shaken for 5 minutes. (3) We used a 10% Clorox (10 mL/100 mL of distilled water) and then shaken for 5 minutes. (4) Thallus sterilized at stage 3, was then washed thoroughly with sterile water. Then it was rinsed with distilled water and shaken each for 1 minute as not to disturb the rest of Clorox that was added to seaweed cells.

Conway media, a liquid tissue culture media, was used for cell culturing. The sea water was used to maintain the results of sterilization explant source after being taken from the field and placed on media acclimatization. Conway media composition (volume 250 mL sea water) is as follows: macro-density, 10 × 25 mL, 2.5 mL micro concentrations 100 ×, 100 × concentration of Fe solution of 2.5 mL, vitamin MS concentrations 100× 2.5 mL, growth regulator 100× concentration of 5 mL, and 25 ppt sea water is added NaOH until the pH of seawater reaches 7.5.

We cut cells (explants) of seaweed with a size of approximately 2 cm at the end of the seaweed thallus. Then it was planted in each vial of sterile sea water salinity of 25 ppt and media Conway, placed at room temperature 25-27°C, conducted in Laminar Flow chamber (Fig. 1).

The explants were placed on the mixer shelf of seaweed tissue culture (Fig. 2). Glass bottles that had been planted with explants (cell) seaweed, were put on the rack in a sterile mixer, given the lighting in the form of fluorescent lamps of 40 watts. The lights were put on the top shelf stirrer and given a distance of 30 cm from the culture bottle, lighting for 24 hours continuously.

When explants showed thallus growth, it means the tissue culture process conducted has been running properly. Culture bottles containing media and explants were sprayed with 70% alcohol every day to keep it sterile. Media and explants that were contaminated with bacteria and fungi then removed from the room and replaced with new ones (Hayashi et al. 2008).

Growth: Calculation of length and weight used a ruler and analytical balance. The growth of biomass and daily specific growth rate of *G. verrucosa* seaweed was determined by following formula (Dawes et al. 1994):

Biomass Growth (g) = final weight – initial weight

$$\text{Specific Growth Rate (\%)} = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{Time of observation}} \times 100$$

Characteristics of cells: observation on cell seaweed was done manually using occular microscope (Fig. 3).

Quality of agar yield: The yield referred in this research is

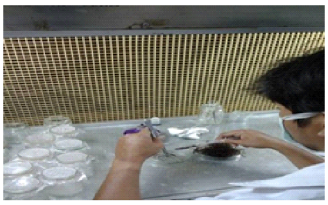


Fig. 1: Planting seaweed in Laminar Flow.

Fig. 2: Rack mixer of seaweed tissue culture.

the weight that is contained in dried raw material of seaweed divided by the weight of dried seaweed and expressed as a percent. Before testing, the seaweed was washed and cleaned from sand, dirt and debris matter and then dried. Seaweed was taken in 2 parts at the base and the tip, then weighed as much as 5 g, washed and soaked for 12 hours. After that, it was sterilized in an autoclave at a temperature of 121°C for 30 minutes. Further it was heated in water bath until the filtrate remained 150 mL, then crushed with a blender. Performed filtering into a plastic container and then precipitated with iso-propanol ($CH_3CH(OH)CH_3$) of 100 mL and then dried under the sun light (SNI 1998).

Quality of agar viscosity: Viscosity (thickness) of a solution is condition that can be described as a solution which is difficult to handle. The intent of this measurement is to determine the value of a solution viscosity expressed in centipoise (cps). Solution with a concentration of 1.5% that is heated in a water bath, stirring regularly until it reaches a temperature of approximately 75°C. Then it was attached to the measuring instrument viscosimeter Brookfield. The position of the spindle in the hot solution is set up right, viscosimeter is turned on and the temperature of the solution is measured. When the solution temperature reached 75°C, and the value of viscosity is known by reading viscosimeter on a scale of 1 to 100. The reading is done after one minute full rotation. Readings duplication used in accordance with the spindle at 60 rpm. This serves to express the absolute viscosity in centipoises (cps) (AOAC 1990).

Quality of agar gel strength: Gel strength is the maximum load required to break the polymer matrix in burdened areas. Polysaccharide gel is a structure that is formed from the polymer solution, and then form a solid or gel stiffness and elastic properties. Gelatin solution with a concentration of 1.5% is heated for 10 minutes while stirring with a temperature of 75°C. The total weight both before and after heating is kept constant. Hot gelatin solution is inserted into the mold with a diameter of 3 cm and 4 cm high, then left for one night to form a gel. Gel strength measurement was done by using Curd meter. Gel inside the mold is placed on the gauges. The measuring device in the form of Rods suppressor 5.0 mm diameter with a surface area (S) 19,635 mm^2 and the circumference (L) 15,708 mm. Spring load of 100 g and a pressure rod penetration rate of 0.36 cm/sec. If the position of a pressure rod has been right in the middle surface of the gel, Curd meter then activated until the middle of the rod-suppressing penetrate the surface of the gel (Kumar & Fotedar 2009).

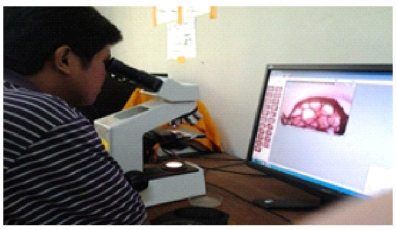


Fig. 3: Observation on the cell characteristics of *G. verrucosa* seaweed.

Design of the study: this study used a treatment ratio carbon% : nitrogen% : phosphorus% on media fertilizer vermicompost as 93:6:1, 92:7:1, 91:8:1, 90:9:1, 89:9:2 and 88:10:2. Vermicompost fertilizer used has a carbon content of 20%, 2% nitrogen and 1% phosphorus, with a dose of 450 ppm as best vermicompost fertilizer was obtained from the results of the preliminary study in 2014.

Data analysis: The experimental design used in this research is completely randomized design (CRD) with 6 different treatments ratio carbon%: nitrogen%: phosphorus% vermicompost fertilizer, with three replications, thus total of each treatments was 18 experimental units. Each parameter, e.g. number of cells, growth and quality of the seaweed at any ratio of vermicompost fertilizer was evaluated using analysis of variance (ANOVA) to see whether the data have significant effect or not, followed by Tukey's test if the effect is significant ($p<0.05$).

RESULTS AND DISCUSSION

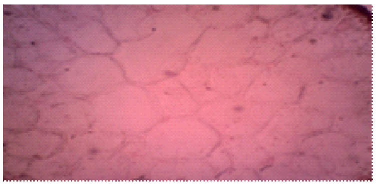
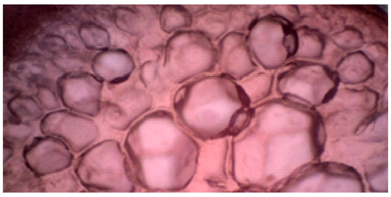
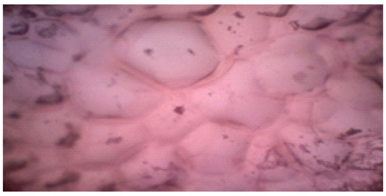
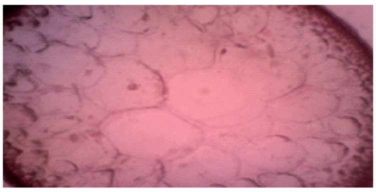
Cell Characteristics of *G. Verrucosa* Seaweed

The size and shape of the cell: from the provision of vermicompost fertilizer ratio on seaweed *G. verrucosa* it was found that administration of vermicompost fertilizer ratios give effect to changes in shape and size of *G. verrucosa* seaweed cells (Table 1). With an average of each treatment cell lines that tend to be oval and rounded, has a thick cell wall and smaller than its original size. The range of cell sizes of *G. verrucosa* seaweed during the study was ranging from 28.114 to 421.315 μm for the axis length and 25.189 to 400.183 μm for the axis short.

In this study, it was found that the best ratio of C:N:P for vermicompost fertilizer to change the shape and size of cells of *G. verrucosa* seaweed is 91:8:1. Seaweed *G. verrucosa* utilize the content of carbon, nitrogen and phosphorus to produce more changes in the shape and size of the cell.

Shewfelt et al. (2005) stated that nutrients such as carbon, nitrogen and phosphorus are needed by plants in the cell synthesis and growth. These three elements must be present in the proper ratio to achieve the best multiplication of cells. The carbon use of plants is the main source in the process of photosynthesis to produce carbohydrates and was instrumental in the process of plant respiration. Other-

Table 1: Cell characteristics of *G. verrucosa* seaweed.

Treatment Ratio C%:N%:P% Vermicompost Fertilizer (ppm)	Figure Cells	Description
93:6:1		1. Cells form: tend to be elongated, cell wall thicker and smaller than its original size. 2. Cell size: - Axis length: 35.114 – 400.183 µm. -Axis short: 20.143 – 363.147 µm. 3. Cells weight: - Initial explant weight: 0.58 g (20 clumps). - Final explant weight: 2.27 g (20 clumps). - Maintenance time: 42 days.
92:7:1		1. Cells form: tend to be rounded and smaller than its original size. 2. Cell size: - Axis length: 32.183 – 421.315 µm. - Axis short: 29.183 – 400.183 µm. 3. Cell weight: - Initial explant weight: 0.58 g (20 clumps). - Final explant weight: 2.40 g (20 clumps). - Maintenance time: 42 days.
91:8:1		1. Cells form: tend to be oval, cell wall thicker and smaller than its original size. 2. Cell size: - Axis length: 28.114 – 400.213 µm. - Axis short: 25.189 – 383.114 µm. 3. Cell weight: - Initial explant weight: 0.58 g (20 clumps). - Final explant weight: 2.72 g (20 clumps). - Maintenance time: 42 days.
90:9:1		1. Cells form: polygonal cells tend to be rounded and smaller than its original size. 2. Cell size: - Axis length: 31.485 – 383.114 µm. - Axis short: 28.183 – 341.233 µm. 3. Cell weight: - Initial explant weight: 0.58 g (20 clumps). - Final explant weight: 2.01 g (20 clumps). - Maintenance time: 42 days.
89:9:2		1. Cells form: polygonal cells tend to be rounded and smaller than its original size. 2. Cell size: - Axis length: 33.247 – 415.211 µm. - Axis short: 28.214 – 391.412 µm. 3. Cell weight: - Initial explan weight: 0.58 g (20 clumps). - Final explant weight: 1.94 g (20 clumps). - Maintenance time: 42 days.
88:10:2		1. Cells form: tend to be oval and smaller than its original size. 2. Cell size: - Axis length: 48.314 – 413.213 µm - Axis shor : 38.214 – 390.224 µm 3. Cell weight: - Initial explant weight: 0.58 g (20 clumps) - Final explant weight: 2.07 g (20 clumps) - Maintenance time: 42 days

wise, nitrogen and phosphorus compounds are important constituents of plant cells in the formation of proteins, DNA and RNA.

The number of cells: Fig. 4 shows the number of cells of *G. verrucosa* seaweed on the ratio of different vermicompost fertilizer. The best cell numbers were obtained in the treat-

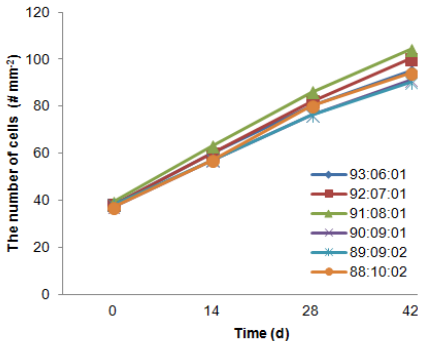


Fig. 4: The number of cells (# mm^{-2}) *G. verrucosa* seaweed during the study.

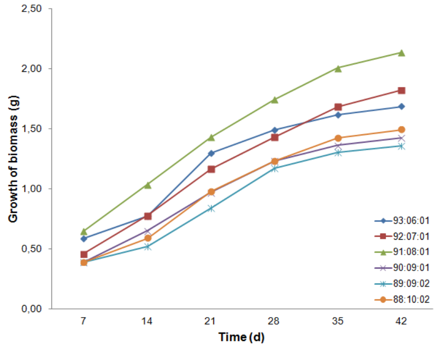


Fig. 5: Average growth of biomass (g) of *G. verrucosa* seaweed.

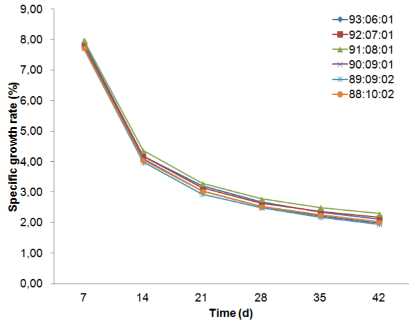


Fig. 6: Average daily specific growth rate (%) of *G. verrucosa* seaweed.

ment ratio of C:N:P, 91:8:1 with a total cell count during the study ranged 39-104 #mm^{-2}.

The results of ANOVA showed that the ratio of C:N:P fertilizer vermicompost gives significant effect to increase the number of cells in *G. verrucosa* seaweed ($p < 0.05$). From the test results of further Tukey analysis, treatment of vermicompost fertilizer ratio C:N:P, 91:8:1, gives a real difference compared to the treatment ratio 90:9:1 and 89:9:2 to increase the number of cells in *G. verrucosa* seaweed ($p < 0.05$). The best ratio of C:N:P fertilizer vermicompost is 91:8:1, because it gave the best overall number of cells compared with other vermicompost fertilizer ratio. The content of carbon, nitrogen and phosphorus in the best ratio accelerate cell multiplication process to improve the growth and quality of crops (Sari et al. 2012).

The growth of seaweed *G. verrucosa*: The observation of vermicompost fertilizer ratio on the growth of *G. verrucosa* seaweed during the study showed that the ratio of fertilizer give effect to the increased growth of seaweed. In this case, it is the growth of biomass, specific growth rate, and length of seaweed. The best ratio of C:N:P fertilizer vermicompost is 91:8:1, with a range of growth of *G. verrucosa* as follows: biomass growth from 0.65 to 2.14 g, specific growth rate from 2.29 to 7.99% and thallus length from 2.5 to 3.8 cm.

Biomass growth, specific growth rate and thallus length: The analysis on a variety of biomass growth (Fig. 5) and daily specific growth rate (Fig. 6) showed that vermicompost fertilizer ratios give real effect to the increased weight of the biomass and daily specific growth rate of *G. verrucosa* seaweed ($p < 0.05$). Tukey's test results showed that the ratio C:N:P, 91:8:1 gave a real difference to the treatment ratio 90:9:1, 89:9:2 and 88:10:2 ($p < 0.05$). The ratio of vermicompost fertilizer treatment did not give significant effect on increasing the length of seaweed thallus *G. verrucosa* (Fig. 8) ($p > 0.05$). The high growth in the form of biomass, daily growth and length thallus on the ratio of C:N:P, 91:8:1, can not be separated from the role of macro elements carbon, nitrogen and phosphorus which are key elements which are indispensable for the growth of seaweed.

Parnata (2004) stated that during the growth process, the plants need the elements of carbon, nitrogen and phosphorus. If one element is not available, it can inhibit the growth, development and productivity of cells. Sutedjo (2002) also stated that the elements of carbon, nitrogen and phosphorus have function to accelerate the establishment and extension of thallus kelp, raising the osmotic pressure, improve cell permeability to water, improve protein synthesis, and promote the development of the cell walls of seaweed; all of which support growth and development of *G. verrucosa* seaweed.

Quality of agar seaweed *G. verrucosa*: Average quality of agar yield, viscosity and gel strength of seaweed *G. verrucosa* on the ratio of C:N:P fertilizer vermicompost was different. Quality of agar yield and viscosity was best in the treatment ratio of C:N:P, 91:8:1 with yield values ranging from 9.98 to 26.52% and the viscosity from 43-90 cps. The highest quality of agar gel strength was in the treatment ratio of C:N:P, 90:9:1 with values ranging from 68.2 to 101.8 g/cm^2.

Yield, viscosity and gel strength: The results of ANOVA on ratio C:N:P of vermicompost fertilizer give significant effect on the quality improvement of agar viscosity (Fig. 9) in *G. verrucosa* seaweed ($p < 0.05$) and no significant effect on the quality of agar yield (Fig. 8) and quality of agar gel strength (Fig. 10) ($p > 0.05$). Tukey test results showed that, the treatment ratio of C:N:P, 91:8:1 gave a significant difference in the treatment ratio of C:N:P, 90:9:1 to the improvement of the quality of agar viscosity ($p < 0.05$). The high quality of agar yield on the treatment ratio of C:N:P fertilizer vermicompost 91:8:1 due to the elements carbon,

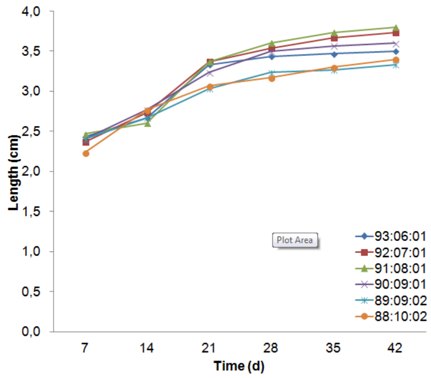


Fig. 7: Average length (cm) of *G. verrucosa* seaweed.

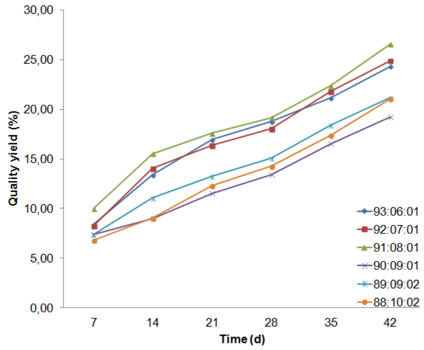


Fig. 8: Average quality of agar yield (%) *G. verrucosa* seaweed.

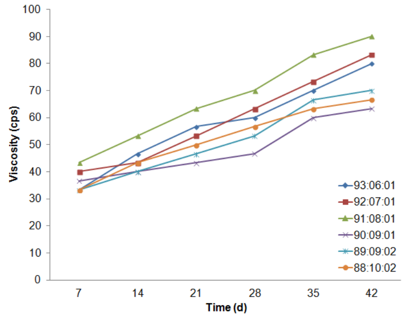


Fig. 9: Average quality of Agar viscosity (cps) *G. verrucosa* seaweed.

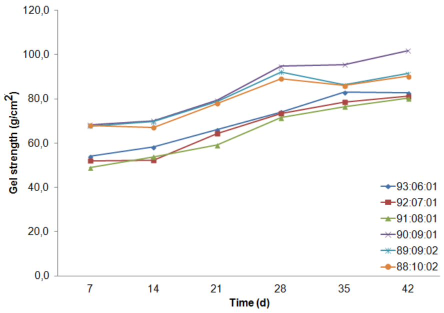


Fig. 10: Average quality of Agar gel strength (g/cm^2) *G.verrucosa* seaweed.

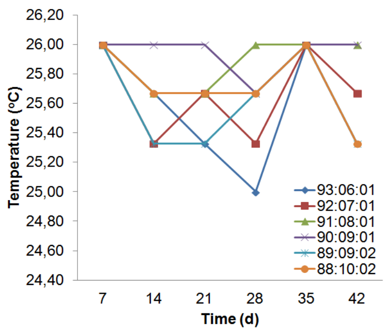


Fig. 11: The average temperature (°C) of water.

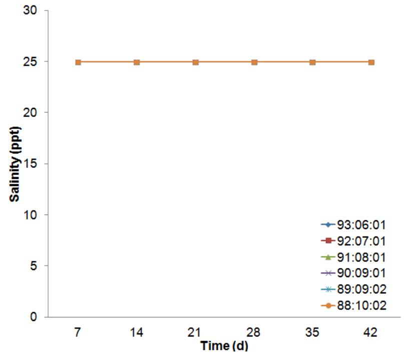


Fig. 12: The average salinity (ppt).

nitrogen and phosphorus are needed in the right quantities by seaweed in generating strong cell wall structure, multiplication of cells and the formation of young cells, which affects the growth and quality of seaweed (Aslan 2005).

The best quality of the viscosity is in treatment ratio of C:N:P fertilizer vermicompost 91:8:1. The best ratio of C:N:P has very active role in the process of photosynthesis to produce the best cell counts as well as essential elements in producing qualified and best plant growth (Sari et al. 2012). The high quality of agar viscosity will be followed by high quality of agar yield of seaweed, this is because the polymer structure that is surrounded by negative charges along the polymer chain in order, resulting in a chain of molecules surrounded by water mobilized or catch the water so that the polymer structure that was once a strong change becomes viscous properties or viscosity increases (Guiseley et al. 2000).

The best of quality of agar gel strength in the treatment ratio of C:N:P fertilizer vermicompost 90:9:1, the results indicate that the quality of agar gel strength seaweed *G. verrucosa* is inversely proportional to the quality of agar yield and viscosity means the higher quality of agar gel strength of seaweed *G. verrucosa* will lower the quality of agar yield and viscosity seaweed *G. verrucosa*. This was confirmed by Guiseley et al. (2000) that, polymer structure that is composed of carbohydrates and sulphates, the higher the nutrient absorbed by the seaweed, it will cause the power of attraction between the groups sulphate-laden different, resulting in the outbreak of the polymer structure of agar and form a structure which is thick or increased viscosity and lower gel strength.

Water Quality for Seaweed *G. verrucosa*

Temperature, salinity and pH: The temperature range in treatment ratio of C:N:P fertilizer vermicompost is 25-26°C (Fig. 11). Temperature plays a role in metabolic processes that determine the speed of seaweed to fulfil the needs of nutrients such as carbon, nitrogen and phosphorus for cell growth and division. Necessity for various types of seaweed temperature ranges 20-30°C (Boyd 1990). Salinity in this study was 25 ppt (Fig. 12). Extreme changes in salinity will result in inhibition of growth for seaweed. *G. verrucosa* has a good adaptability to salinity changes between 17-40 ppt

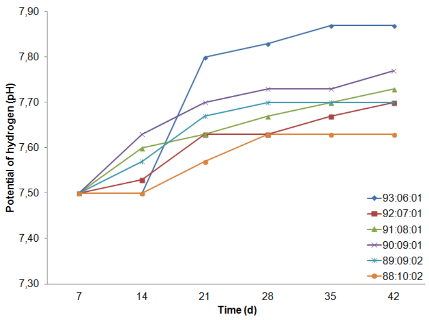


Fig. 13: The average pH of water.

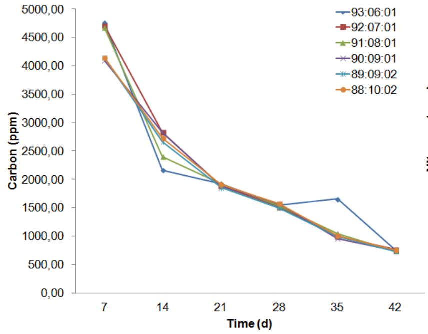


Fig. 14: The average carbon (ppm).

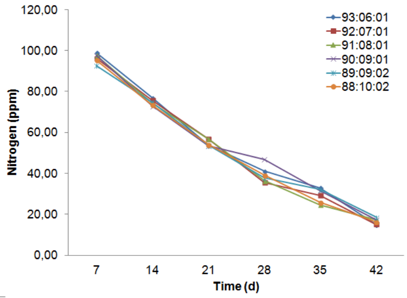


Fig. 15: The average nitrogen (ppm).

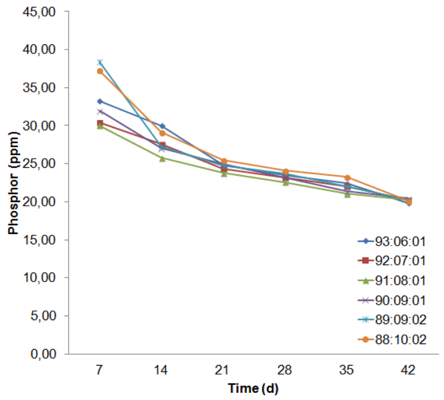


Fig. 16: The average of phosphorus (ppm).

(Komarwidjaja 2003). pH in this study ranged from 7.50 to 7.87 (Fig. 13). An increase in the pH value is influenced by several factors such as the biological activity, e.g. photosynthesis, respiration, temperature and nutrients in water. pH optimum in water for the growth of seaweed is around 7-8 (Meiyana et al. 2001).

Carbon, nitrogen and phosphorus: Based on observations, we found decreased concentration of carbon, nitrogen and phosphorus in the treatment ratio of C:N:P in vermicompost fertilizer throughout the study, with a range of water carbon 734.51 to 4754.12 ppm, air nitrogen 14.91 to 98.91 ppm and water phosphorus 19.84 to 38.39 ppm. This is caused by to the addition ratio of C:N:P in the vermicompost fertilizer which lead to large concentrations of carbon, nitrogen and phosphorus in the waters. According to Komarawidjaja (2003), an increase in the carbon, nitrogen and phosphorus in the water due to the addition ratio of C:N:P, with carbon as the main element for the growth very much dissolved in the water and unlimited, whereas nitrogen and phosphorus are elements that are limited in the waters. The source comes from the plants decomposition, domestic sewage, agricultural and industrial wastes. The addition of carbon, nitrogen and phosphorus are needed by plants to fulfil the nutritional needs of the cell division.

CONCLUSION

The conclusion from this study is:

1. The results of distributing ratio C%:N%:P% vermicompost fertilizer in each treatment give effect to changes in the shape and size of cells of *G. verrucosa* seaweed. The shape of cells in each treatment fertilizer ratio rounded or oval, thick cell walls and the cell size tends smaller than the original size. With the size of the cells to the axis length ranging from 28.114 to 421.315 µm and axis short is ranging from 25.189 to 400.183 µm.
2. Best ratio of C%:N%:P% in vermicompost fertilizer to the growth of *G. verrucosa* seaweed was a ratio of 91:8:1, with the biomass growth range from 0.65 to 2.14 g, the daily specific growth rate from 2.29 to 7.99% and thallus length from 2.5 to 3.8 cm.
3. The best ratio of C%:N%:P% in vermicompost fertilizer in the term of agar yield quality and viscosity of *G. verrucosa* seaweed was the treatment ratio of 91:8:1, with the value of quality of agar yield ranging from 9.98 to 26.52%, the value of quality of agar viscosity ranges from 43-90 cps. The best ratio of C%:N%:P% for qualified agar gel strength was the ratio of 90:9:1 with values ranging from 68.2 to 101.8 g/cm^2.
4. Water quality in each treatment ratio of C%:N%:P% in vermicompost fertilizer are: temperature ranges from 25 - 26°C, salinity of 25 ppt, pH ranged from 7.50 to 7.87, carbon water ranged from 734.51 to 4754.12 ppm, nitrogen water ranged from 14.91 to 98.91 ppm and phosphorus water ranged from 19.84 to 38.39 ppm.

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