The Influence of PES (Provassoli’s Enriched Seawater) media and modification of vitamin B\textsubscript{12} on technical culture for the growth of Sargassum sp.

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Abstract. One medium for algae culture is Provassoli’s Enriched Seawater (PES), which is the best medium for most marine algae. In this study, enrichment media used with vitamin B\textsubscript{12} elements were used to support the growth of Sargassum sp. This study was conducted to obtain a good concentration of vitamin B\textsubscript{12} in Provassoli’s Enriched Seawater (PES) media and its effect on the growth of Sargassum sp. This study uses an experimental method with Completely Randomized Design (CRD), There were 4 treatments for administering doses of vitamin B\textsubscript{12} (2 g; 2.5 g; 3 g and controls) and 5 replications. If the treatment given gives a significantly different effect, it will be continued with Duncan's Multiple Distance Test (DMRT) to determine the differences between treatments. The results showed Provassoli’s Enriched Seawater (PES) media with a modification of vitamin B\textsubscript{12} on technical culture media has a very significant effect (P <0.05) on the growth of Sargassum sp. Treatment with the addition of 3 grams of vitamin B\textsubscript{12} is the best growth treatment. At the fourth week the daily growth rate is 0.33%, relative growth rate 0.027 gram and average value of chlorophyll a content of 2.295 mg/L.

1. Introduction
Indonesia is a maritime country with waters covering two thirds of the sea. The length of Indonesia's coastline reaches ± 81,000 km and occupies the second country with the longest coastline in the world after Canada. With this vast sea area, Indonesia is expected to be able to produce fisheries products with high economic value with potential food sources. One product that can be produced from Indonesian marine waters with high potential is seaweed. Indonesia is one of the seaweed exporting countries namely Euchema sp., Gracillaria sp., Gelidium sp. and Sargassum sp. [1].

Seaweed is one of the fisheries products that is easily available, affordable, easy to cultivate, tastes delicious, and is easily processed. Seaweed can also be processed into various products such as karageenan, gelatin, cosmetics and other industrial odor materials [2]. With the many uses and benefits of seaweed, more and more demand for seaweed from outside and within the country. Based on data from the Central Statistics Agency (BPS) the value of seaweed exports as of July 2017 increased by 20.88% or worth 69.9 million USD. This condition reflects that Indonesia has competitive competitiveness in the international market.
Sargassum sp. is a brown alga with the largest genera of Sargassaceae [3]. The abundance and distribution of Sargassum sp. is the largest in all Indonesian marine areas compared to other species and lives on coral with a depth of 10-12 m. Sargassum sp. is a role in the formation of coral reefs and as a place to care for small biota. From several studies, the nutritional content of Sargassum sp. is very good as is the protein content and some essential minerals, it's just that the analysis of its composition is incomplete. Research conducted by Handayani et al. [4] Sargassum sp. protein content is 5.19%; ash content (mineral) 36.93%; vitamin A 489.55 g/100g and vitamin C at 49.01 mg/100g; fat content of 1.63%; alginate levels 37.91% and vitamin B₁₂ 1 g/100g. Sargassum sp. algae also contain choline which is good for fetal and infant health for brain growth [5].

One way to produce seaweed with high nutritional value is to maintain it in a medium that contains nutrients or good nutrition, seed selection, and seawater. Seaweed seeds are derived from healthy plants, do not contain disease, many branches, elastic, and free from other plants that stick [2]. Medium is a material that contains several nutrients used for algal growth [6]. To get sea water free of pollutants, seawater extraction in the oligotrophic part is highly recommended because of the low metal and sedimentary material [6].

One medium for algal culture is Provassoli’s Enriched Seawater (PES) which is the best medium for most marine algae. PES medium can provide good growth and can increase the content of metabolite compounds [7].

Enrichment medium is a medium enriched with several nutrients to support the growth of organisms used for culture. Medium enrichment is a base that is highly nutritious. In this study used enrichment media added with the element vitamin B₁₂ which can support the growth of Sargassum sp. Vitamin B₁₂ is a type of vitamin that is water soluble. Vitamin B₁₂ plays a role in the regeneration of methionine [8]. Without vitamin B₁₂, methionine regeneration cannot occur so that the amount of methionine present in cells is limited. This study was conducted to obtain good concentration of vitamin B₁₂ in Provassoli’s Enriched Seawater (PES) media and its effect on the growth of Sargassum sp.

2. Material and methods
2.1. Procedures of research
The research activity was carried out on a laboratory scale using 20 bottles as a medium container with a size of 600 mL as a growth container for Sargassum sp. Each bottle consists of 5 thallus and size 2 cm on technical culture media. Research work procedures include making nutrient enrichment stock in 800 mL deonized water (dH₂O). Ingredients consist of NaNO₃, Na₃β-glycerophosphate, Fe-EDTA solution, trace metal solution, thiamine (vitamin B₁), cyanocobalamin (vitamin B₁₂). The first was the preparation of Fe-EDTA solution as a nutrient enrichment stock mixture which was mixing EDTA and Fe components into 950 mL dH₂O. After being added dH₂O to a volume of 1L then homogenize. Making trace metal solution as part of the enrichment stock mixture, EDTA and other mixtures enter 900 mL then add dH₂O to 1 liter then homogenize. All ingredients are put together to become a nutrient enrichment stock and then take 10 mL of stock and put it in 990 mL into filtered seawater [9].

The materials used for making modified technical culture media are urea, TSP, ZA, MgSO₄, EDTA and vitamin B₁₂. The first step is to weigh the material with an analytical balance and then wrap it with aluminum foil or sterile plastic. Then, add autoclave seawater and technical media materials, urea, TSP, ZA, MgSO₄, EDTA and vitamin B₁₂ in a sterile bottle. Culture media were differentiated in addition of vitamin B₁₂ at a dose of 2 g/L, 2.5 g/L, and 3 g/L. Thallus is uniformed with a size of 2 cm. Thallus is grown on culture media with optimum temperature and salinity of 30-35 ppt. Light intensity uses 1500 lux lights for 12 hours of light and 12 hours of darkness. Giving aeration is used to homogenize water masses and transport nutrients. The water content in thallus has interaction with the turgor pressure in the plant tissue and affects the photosynthesis [10]. Subculture have performed using with new media that nutrients every 3 days. The value of each of these quality parameters has been adjusted to the optimal water range for the growth of Sargassum sp.
2.2. Research parameters
The main parameter observed the weight of the thallus Sargassum sp. Furthermore, the data is processed in the form of Specific Growth Rate and Growth Rate values. Another data measured the amount of chlorophyll-α. Calculation of Specific Growth Rate, Growth Rate and chlorophyll-α Sargassum sp. conducted at the beginning and end of the study. Thallus measurement is done once every 3 days for 15 days.

2.3. Statistical analysis
Observation data in the form of weight and chlorophyll-α calculation data were analyzed using One way Analysis of Variance (ANOVA). Data analysis using the latest version of SPSS computer software. If the results obtained have the effect of giving different dose of culture media to the results, then a further test using Duncan's multiple range test (DMRT) is carried out to determine the difference between one treatment and the other [11].

3. Result and discussion
3.1. Growth of Sargassum sp.
Sargassum sp. growth after treatments in different technical culture media was represented on Table 1 and Table 2.

| Treatment | SGR (%/day) |
|-----------|-------------|
| P0        | 0.07±0.392  |
| P1        | 0.10±0.231  |
| P2        | 0.13±0.180  |
| P3        | 0.33±0.147  |

Note: Superscript abc showed comparisons between treatments that were significantly different (p <0.05), in Duncan's multiple test.

| Treatment | GR (grams) |
|-----------|------------|
| P0        | 0.004±0.0025 |
| P1        | 0.008±0.0018 |
| P2        | 0.010±0.0014 |
| P3        | 0.027±0.0013 |

Note: Superscript abc showed comparisons between treatments that were significantly different (p <0.05), in Duncan's multiple test.

ANOVA analysis result showed every treatment of technical culture media gave high significant difference Sargassum sp. growth (p<0.05). DMRT statistical analysis result showed that SMW media was highly significant difference from seawater without additiona and technical culture media P1, P2 and P3 (p<0.05).

3.2. Containing of chlorophyll-α
Sargassum sp. growth after treatments in different technical culture media was represented on Table 3.

| Treatment | Chlorophyll-α (mg/l) |
|-----------|----------------------|
| P0        | 1.630±0.0196         |
| P1        | 2.058±0.0254         |
| P2        | 2.234±0.0278         |
| P3        | 2.295±0.0284         |

Note: Superscript abc showed comparisons between treatments that were significantly different (p <0.05), in Duncan's multiple test.
Based on the table above shows that the highest observation value of chlorophyll-a was obtained from Sargassum sp. with a dose of Vitamin B$_{12}$ added which is equal to 3 g with an average value of chlorophyll a content of 2.295 mg/L. The results of spectrophotometric measurements of chlorophyll levels in Lamber-Beer Law. As for several methods for calculating total chlorophyll, chlorophyll-a and chlorophyll-b levels that have been formulated.

3.3. Water quality parameters

Controlling of water quality on Sargassum sp. carried out for fifteen days with parameters consisting of DO, temperature, pH and salinity.

| Treatment | Parameter | DO (mg/L) | Temperature (°C) | pH | Salinity (ppt) |
|-----------|-----------|-----------|-----------------|----|----------------|
| P0        |           | 7.32      | 21.5            | 8.09 | 30             |
| P1        |           | 7.29      | 21              | 8.05 | 30             |
| P2        |           | 7.28      | 20.9            | 8.07 | 30             |
| P3        |           | 7.29      | 21              | 8.10 | 30             |

Note: P0=control; P1=Technical Culture Modified vitamin B$_{12}$ 2 g/l; P2= Technical Culture Modified vitamin B$_{12}$ 2.5 g/l; P3= Technical Culture Modified vitamin B$_{12}$ 3 g/l.

According to Samsuari [12] water quality can affect the growth of seaweed which covers temperature, salinity, pH, and dissolved oxygen. The degree of acidity (pH) is the concentration of hydrogen ions present in the waters. The degree of acidity (pH) also affects the growth process of Sargassum sp. during the study ranged from 8-8.1. According to Anggadiredja [13], the optimum pH of seaweed cultivation ranges from 6-9. Alkaline waters 7-9 are productive waters whose role is to encourage the process of growth of organic matter in water into minerals that can be assimilated by phytoplankton.

According to Anggadiredja [13], good salinity for seaweed growth ranges from 30-33.5 ppm. So it can be stated that the salinity range during the study is below the optimum salinity range for seaweed growth. Salinity can limit the growth of seaweed if the growing media of seaweed is mixed with fresh water.

Environmental temperature has an important role in the photosynthesis cycle where the higher the intensity of light and temperature, the higher the results of photosynthesis. In addition to photosynthesis, temperature has an important role for seaweed life. Water temperature can affect the physiological functions of seaweed such as metabolism, respiration, growth and reproduction. Temperature range during this study was relatively optimal for seaweed growth. This is in accordance with the statement Anggadiredja [13] that is a good temperature for seaweed cultivation around 27-30°C.

Dissolved oxygen is a basic need for the life of living things in water. The concentration of dissolved oxygen at the study site was 7.27-7.32 mg/L, this is in accordance with the statement of the [14] that dissolved oxygen to support seaweed cultivation is 3-8 mg/L.

Provassoli’s Enriched Seawater (PES) is the best medium for most marine algae. PES medium can provide good growth and can increase the content of metabolite compounds. In the opinion of James [7] the composition of PES medium for 100 ml dH$_2$O include: NaNO$_3$ 350 mg, C$_3$H$_7$Na$_2$O$_6$PxH$_2$O 50 mg, Fe-EDTA 2.5 mg, vitamin B$_{12}$ 10 μg, 0.5 mg thiamine, Biotin 5 μg, Triss-Buffer 500 mg.

Vitamin B$_{12}$ plays an important role in the regeneration of methionine for algal growth. Without vitamin B$_{12}$, methionine regeneration cannot occur so that the amount of methionine present in cells is limited. The results of this study are in accordance with Ohwada and Taga [15] which states that by adding a dose of vitamin B$_{12}$ as much as 3 grams from the optimal dose of 1 gram, methionine which
is an essential amino acid that will combine with other amino acids to form proteins that are important for the growth process.

Treatment with different doses of vitamin B\textsubscript{12} results in different daily growth rates. In the P3 treatment with the addition of 3 grams of vitamin B\textsubscript{12} dose obtained a daily growth rate of 0.33%, the results of the daily growth rate in the P3 treatment were higher than P2 treatment which was 0.13%, P1 treatment was 0.10% and treatment P0 (control) was 0.07%.

Rapid growth in P3 treatment (0.33%) was thought to be due to nutrients absorbed by \textit{Sargassum} sp. Seaweed more than P2, P1 and P0 treatments. In this treatment it is suspected that increasing the dose of vitamin B\textsubscript{12} also affects the process of faster growth. This is because vitamin B\textsubscript{12} has a nutritional element that is high in protein for the growth process.

Photosynthesis will increase in line with an increase in light intensity at a certain optimum value. The intensity of light is also directly related to the primary productivity of a waters. The higher the intensity of light, the higher the primary productivity at a certain limit [16].

Growth rates in treatments P2 (2.5 g), P1 (2 g) and P0 (control) are smaller because of the location of the treatment that affects the photosynthesis process so that the sunlight obtained is uneven so that photosynthesis results are less than optimal and too tight aeration settings result the rate of air bubbles flowing through the aeration stone is too fast.

4. Conclusion
\textit{Sargassum} sp. on difference media affect specific growth rate and growth rate. Enrichment nutrients on media such as nitrate, phosphat, EDTA and vitamin B\textsubscript{12} is needed for growth \textit{Sargassum} sp. The complete nutrients, PES media, gave the greatest effects on the growth. However, the technical culture media modified is slow on the growth, it can substitute PES media as a cheaper and easy obtain for substrate media.

5. References
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