The addition of different starters on characteristics

*Sargassum* sp. liquid fertilizer

Eko Nurcahya Dewi1*, Laras Rianingsih1, Apri Dwi Anggo1

1Departemen Fisheries Processing Technology, Faculty of Fisheries and Marine Science, Diponegoro University, JL. Prof. H. Soedarto.SH. Tembalang, Semarang, 50275, Indonesia
Corresponding author: murdewisatsmoko@yahoo.com

Abstract. *Sargassum* sp. is a macroalgae contains of nutrients and stimulating plant growth regulators that can be used as organic fertilizer. The purpose of the study was to determine the effect addition of three different starters to the seaweed liquid fertilizer on producing C-organic, nitrogen, phosphorus, potassium, pH on *Sargassum* sp seaweed for organic fertilizer. The addition of different starters were to enhanced fermentation process. Seaweed were chopped and dried for around 2 days, lamtoro leaves as source of nitrogen compound was added for 25% of total amount of seaweed. Water was added to the mixture and then fermented for 14 days with addition of 3 different type of starters namely bread, *tape* and *tempe* starter. The data were then analysed by ANOVA and Tukey test in order to know which treatments were different. The result showed that the addition 3 different starters gave differences on pH and yield, total solid content, macronutrient and micronutrient of organic fertilizer. The addition of bread starter to seaweed gave the highest N content (2.7%), pH (5.43), yield (50.44%) and total solid content (94.36), while seaweed fertilizer added with *tempe* starter gave the highest micromineral content Fe (38.77 ppm), Zn (4.2 ppm) and Mn (4.74 ppm).

1. Introduction

*Sargassum* sp is a macroalgae contains of nutrients and stimulating plant growth regulators (PGRs), cytokinin and gibberellins substances that can be used as organic fertilizer [1]. *Sargassum* sp seaweed were abundant and collected along Indonesia island.

The use of organic fertilizer recently increased because the negative effect from chemical fertilizer to the agricultural ecosystem. Finding chemical fertilizer is easier in the market but the price is expensive [2]. A harmful impact on human health also detected in the use of chemical fertilizer [3]. Furthermore, some chemical contributed to a runoff problem that leads to many nutrients leached out, this is potentially a major environmental issue.

Organic fertilizer can improve soil properties (texture, structure, porosity etc) since organic substances are adhesive loose grain, it also is a source of plant nutrients and energy source to most soil organisms, the use of organic fertilizer can reduce excessive application of anorganic [4,5]. Seaweed extracts have also been widely marketed as additives in plant fertilizers [6,7]. It contains important minerals and growth-stimulating hormones [8,9,10]. Seaweed extract different from chemical fertilizers, since this extract can be degraded naturally, non-toxic, does not contaminate and is safe for humans and animals [11]. Seaweed had been used directly as a soil conditioner and fertilizer in various place in the world

Several studies have been conducted to make seaweed fertilizer but the presence of macro and micro nutrients from liquid fertilizers has not been fulfilled the requirement, because the levels are still very low [12-13]. Macro elements in liquid fertilizer are N, P and K. Research by Sigit et al., [14] on the addition of EM4 and Biosca as bioactivators on seaweed fermentation, were not satisfactory, since some of macro and micro elements in seaweed liquid fertilizer did not meet the requirements by Permentan Indonesia (Indonesian Agriculture Minister Regulation).

Mold and yeast were known as high enzyme producers and it were expected to breakdown seaweed cellwall so that the minerals inside on it seaweed can be released to liquid fertilizer. The minerals can
be expected will increase the levels of macro and micronutrient elements of organic seaweed liquid fertilizer. The components of C-organic, nitrogen, phosphorus, potassium will be produced during seaweed fermentation [15].

The purpose of this study was to determine the effect addition of different starters namely bread, tape and tempe to the quality (macro and micro elements production) of organic liquid fertilizer Sargassum sp. The microorganism starters usually contents of mold and yeast bioactivator (Sacharomyces cereviceae) that accelerated fermentation liquid fertilizer process.

2. Research Methods
2.1. Raw material
Sargassum sp. were collected from Gunung Kidul coast, South Coastal of Yogyakarta, Indonesia. The three different starters (bread, tape and tempe) were purchased from local market in Semarang, Indonesia.

2.2. Preparations of seaweed (12) with modifications
Sargassum sp., was washed thoroughly with seawater to remove impurities and then packed in plastic bags and transported to the Department of Fisheries Processing Diponegoro University. The seaweed then washed in the tap water in order to remove salt and the dirt and let it dried.

2.3. Preparation of seaweed liquid fertilizer (12) with modifications
Dried seaweed were soaked in tap water for 2 hours and chopped for around 1-2 cm. Wet seaweed weighed 150 gram and added with 500 ml aquadest. The mixture of seaweed and water were heated for 90 °C and hold for 15 min. After finished, the mixture was added with 50 ml mollase and cooled down. Fresh lamtoro leafs as source of nitrogen compound was added for 25% of total seaweed raw material. Three different starters namely bread, tape and tempe starter (10%) were added to the mixture and then fermented for 14 days.

2.4. Collection of liquid
After fermented for the designated time (14 days), all the liquid were collected and filtered using tea strainer and it were used for samples analysis.

2.5. Determination of pH
The pH of seaweed fertilizer was determined directly using pH meter.

2.6. Determination of C content (16)
The organic matter was tested using Walkey and Black methods [16]. Liquid fertilizer was weighed 1 g and put in a 100 measuring flask. 10 ml K2Cr2O7 1 N and H2SO4 were added and it left for 30 minutes. Sampel was added with 100 ml distilled water and let it for 12 hours and finally the sampel was measured at λ 561 nm.

2.7. Determination of N content (16)
Total nitrogen in each sampel was determined using Kjeldahl method and it was expressed as %.

2.8. Determination of yield
Yield can be determined with the material weighed before being processed (a) and the weight of the finished material (b).

\[
\text{Yield} = \frac{b}{a} \times 100\% 
\]  

(1)

2.9. Determination of phosphor content (16)
A 5 g liquid fertilizer is included in the Kjeldahl flask digestion, then added HNO3 5 ml, shaken and left overnight. Flask was heated until temperature increased from 100°C to 200°C. Destruction ended when fluid in pumpkin flask remaining around 5 ml. Solution cooled down and diluted with aquadest
and volume be adjusted to be 50 ml, shaken to homogeneous, leave it overnight and obtained clear extract (extract A).

1 ml extract A was pipetted into a 10 ml volume flask added 1 drops indicator PP and NaOH 40% to arise pink color. After that added 2 ml of reagent vanadate into the flask measuring and dilute use aquadest, let stand for 20 minutes and measured by spectrophotometer at a wavelength of 400 nm.

2.10. Analysis kalium content [16].
The determination of potassium levels was carried out using the Flame Photometry method.

2.11. Analysis micromineral content (16)
Cu, Pb, Zn analyzis uses a method AAS graphite method with a detection limit of up to 0.01 ppb.

2.12. Planting the crop
The crop selected for the research was on Chinesse cabbage (Brassica rapa.). The plastic polybag were used for planting the crops, it were filled with 3 kg garden soil and rearranged at regular intervals to ensure uniform environmental impact on the plant growth. The total composition of each treatment was 100%. For example for addition 50% ragi tempe the urea have to be added in the soil was 50%. The liquid seaweed fertilizer were poured on the soil surrounded the crops. The crops were harvested after 21 days plantation time.

2.13. Statistical analysis
The data in tables were expressed as mean ± SD. Analysis of variance was performed by ANOVA procedure. Tukey test was used to determine the differences between the samples treatments. P values <0.05 were regarded as significant different.

3. Results and discussion
3.1. Effect of starter addition on pH, total solid and yield in seaweed fertilizer
The standart of Permentan [17] on liquid fertilizer pH is 4-9. The value in pH seaweed fertilizer with different starter were presented in Table 1. It can be seen from the data that all the seaweed fertilizer pH were meet the standart of Permentan. Seaweed fertilizer pH with bread starter addition greater than other treatments (p<0.05). The microorganism in bread starter is S. cereviceae where tape starter is consist of mixed culture from mold, yeast (S.cereviceae) and bacteria. The bacteria namely is Enterococcus faecium and Bacillus licheniformis [18]. Rhizopus oligosporus is the main microorganism in tempe fermentation, but there are also found yeast and lactic acid bacteria [19]. During fermentation process macromolecule will be converted to simple molecule and most of them will produce organic acid and reduce the pH [18]. S. cereviceae will convert sugar glucose in to etanol, CO2 and organic acid (pyruvic acid and lactic acid) [20]. Data on pH, total solid and yield in seaweed fertilizer were presented on Table 1.

| Table 1. pH, Total solid content and Yield in Sargassum sp Seaweed fertilizer |
|-----------------|-----------------|------------------|
| pH | Total Solid Content (%) | Yield (%) |
| Control | 4.13±0.32<sup>a</sup> | 93.70±0.44<sup>ab</sup> | 49.12±4.97<sup>b</sup> |
| Bread starter | 5.43±0.06<sup>b</sup> | 94.36±0.61<sup>b</sup> | 50.44±7.61<sup>b</sup> |
| Tape starter | 4.23±0.15<sup>a</sup> | 93.24±1.05<sup>ab</sup> | 42.62±7.22<sup>ab</sup> |
| Tempe starter | 4.17±0.32<sup>a</sup> | 92.57±1.08<sup>a</sup> | 30.87±5.21<sup>a</sup> |
| Permentan 2011 | 4.9 | | |
Values represent mean ±SD from triplicates determination.
Different superscript between column characterize significant differences (p<0.05).

3.2. Effect of starter addition on seaweed fertilizer on macronutrient

The changes in macronutrient of seaweed fertilizer with different starters addition were shown in Table 2. Nitrogen (N) content seaweed fertilizer with bread starter was higher than other treatment (p<0.05). Nitrogen in seaweed fertilizer comes from the decomposition of raw material during fermentation process. During fermentation process the enzyme will hydrolyze the macromolecule into simple molecule. The bread starter gave higher N content and it is showed that bread starter hydrolyze N-macromolecule greater than other treatments. While the addition tape starter and tempe starter have no effect in enhancing macronutrient decomposition from raw material and remain equally effective as that of control.

Nitrogen source of this fertilizer is came from Sargassum sp. and Lamtoro (L. leucocephalata). Lamtoro leaves contain 3.84% nitrogen, 0.2% P and 2.06% K [22]. and Sargassum sp contain 5.19% protein (b/b), mineral 36.93% (w/w), Fe 132.65 mg/100g, and P 474.03 mg/23].

Phosphorus (P) of seaweed fertilizer all treatments were generally higher than control (p<0.05). The bread starter addition gave the highest phosphor content while addition tape starter and tempe starter showed lower phosphor content. Phosphorus content is in line with the nitrogen content in liquid fertilizer. The value of compost prepared from spent mushroom substrate, nitrogen content is likely related to the microorganisms that grew during fermentation process [24]. Kalium (K) content of seaweed fertilizer all treatments gave the same result (p<0.05).

Table 2. Macron mineral content in Sargassum sp seaweed fertilizer

|        | N (%)       | P (%)       | K (%)       | C (%)       |
|--------|-------------|-------------|-------------|-------------|
| Control| 1.4±0.03 a  | 0.019±0.001 a | 0.3607±0.05 a | 2.21±0.28 a |
| Bread Starter | 2.7±0.04 b | 0.045±0.002 c | 0.5020 ±0.24 a | 2.65±0.49 a |
| Tape Starter | 1.6±0.02 a  | 0.032±0.004 b | 0.373±0.03 a | 2.31±0.23 a |
| Tempe Starter | 1.8±0.02 a | 0.030±0.000 b | 0.4310±0.06 a | 2.57±0.36 a |
| Permentan | 3-6 a       | 3-6 a       | 3-6 a       |

Values represent mean ±SD from triplicates determination.
Different superscript between column characterized significant difference (p<0.05).

3.3. Effect of starter addition on seaweed fertilizer on micronutrient

During the fermentation process, addition of different starter gave different result in micronutrient content (Table 3). Ferrum (Fe) in seaweed fertilizer with bread starter addition showed the lowest among treatments (p<0.05), it was probably Fe was used by the S. cervicenae during the fermentation.
process. *S. cereviceae* needs an iron compound in the metabolism and in the presence of oxygen it will have high affinity iron uptake by this yeast [25]. Mangan (Mn) and Zink (Zn) content in *tempe* starter and *tape* starter treatment were higher than control and bread starter (p<0.05). Cu content was no significantly different among the sampel (p<0.05).

**Table 3. Micronutrient content in Sargassum sp seaweed fertilizer**

|                | Fe (ppm)   | Mn (ppm)   | Zn (ppm)   | Cu (ppm)   |
|----------------|------------|------------|------------|------------|
| Kontrol        | 35.87±5.35b| 3.12±0.68a | 2.96±0.75a | 0.58±0.11a |
| Bread starter  | 22.17±2.01a| 2.07±0.31a | 3.03±0.08a | 0.63±0.05a |
| *Tape* starter | 34.14±5.72b| 3.40±1.11ab| 3.78±0.76ab| 0.93±0.27a |
| *Tempe* starter| 38.77±8.54b| 4.74±0.92b | 4.20±0.41b | 0.57±0.33a |
| *Permentan* 2011| 5.50      | 250-5000   | 250-5000   | 250-5000   |
| *Sargassum* sp.| 632        | 27.2       | 4.4        | 32         |

Values represent mean ±SD from triplicates determination.
Different superscript between column characterize significant differences (p<0.05).

### 3.4. Effect of starter addition on seaweed fertilizer on heavy metal

There were no significant different on Cd and Pb values on measurement for all the samples. Cadmium (Cd) samples were low in all treatments and Plumbum (Pb) in all samples were not detected. All samples were within the recommendation of the standar of Permentan Indonesia (Indonesian Agriculture Minister Regulation) since the Permentan showed that the maximum Cd is 0.50 ppm while Cd in samples were vary only from 0.11 to 0.20 ppm. Cadmium (Cd) is one of the most toxic heavy metals in human body, it should appeared in low value in liquid fertilizer. In high value it adversely affects kidneys and bones mechanism [26].

The changes in heavy metal of seaweed fertilizer with different starters addition were shown in Table 4.

**Table 4. Effect of starter addition on Sargassum sp seaweed fertilizer on heavy metal content**

|                | Cd (ppm)   | Pb (ppm)   |
|----------------|------------|------------|
| Control        | 0.11±0.08a | 0.00±0.00a |
| Bread Starter  | 0.12±0.02a | 0.00±0.00a |
| *Tape* Starter | 0.20±0.04a | 0.00±0.00a |
| *Tempe* Starter| 0.18±0.03a | 0.00±0.00a |
| *Permentan* 2011 | Max 0.50 | Max 12.5   |

Values represent mean ±SD from triplicates determination.
Different superscript between column characterize significant differences (p<0.05).

### 3.5. Effect fertilizer on Chinese cabbage (*Brassica rapa*) growth

The data on average root length, leaves counts, leaves width and leaves length were showed in Table 5. The application of three different liquid seaweed fertilizers have no effect on leaf wide among the treatments after 21 day cultivation (p<0.05), but it have significant effect on leaves length, leaves count and root length (p<0.05). Addition of 100% *tempe* starter to liquid fertilizer gave the longest in root length (p<0.05) after 21 day cultivation. A combination formulation of urea 50% and liquid fertilizer with *tempe* starter 50% gave the highest leaves count after 21 day cultivation (19.67±1.53 cm).

**Table 5. Effect fertilizer on Chinese cabbage (*Brassica rapa*) growth**
The root is an important part of plant growth to support the plant.

The proportion 100% addition three different starters were gave statistically different effect on root length. The root is an important part of plant growth to support the plant. The addition combination 50% tempe starter and 50% urea have the highest on leaves count after 21 days plantation (19.67±1.53 pieces). Those finding were agree with those reported by other researcher (27, 28) since the growth of the root length may affected by N content. However addition liquid fertilizer with different starters were shown less effective to leaves wides. The crop growth can be related to micro and macroelement components (29).

4. Conclusion
Bread starter addition in Sargassum sp. liquid fertilizer gave higher N, pH, yield and total solid while tempe starter addition gave higher micronutrient on Fe, Mn and Zn.

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