The effect of addition of fish bone meal on the concentration of nitrogen (N), phosphorus (P), and potassium (K) in seaweed liquid organic fertilizer of Gracilaria sp.

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Abstract. Gracilaria sp. contains macro and micronutrients needed by plants, such as phosphorus, potassium, calcium, magnesium, manganese, zinc, and boron. Seaweed-based liquid organic fertilizer currently on the market has low phosphorus and potassium content. One ingredient that can increase phosphorus and potassium content is fishbone. This study aimed to determine the effect of fishbone meal addition to Gracilaria sp. liquid organic fertilizer on the concentration of nitrogen, phosphorus, and potassium. The method used in this study was the experimental method with four treatments and five replications on each treatment. These treatments were P0 (0% fish bone meal), P1 (25% fish bone meal), P2 (50% fish bone meal), and P3 (75% fish bone meal). The main parameters in this study were the concentration level of nitrogen, phosphorus, and potassium. The addition of fishbone meal increased nitrogen, phosphorus, and potassium concentration. The highest nitrogen, phosphorus, and potassium contents were found in the treatment with the addition of 75% fish bone meal, producing 0.69% nitrogen, 0.42% phosphorus, and 0.43% potassium content.

1. Introduction
Seaweed has many benefits and values for agriculture. It has been applied for agricultural crop cultivation on some countries in the world. Types of seaweed preparation such as liquid seaweed fertilizer (LSF), seaweed liquid fertilizer (SLF), liquid fertilizer (LF), and chopped powered algal manure have been widely utilized in several countries with beneficial impact on plants [1]. One kind of seaweed that can be utilized as liquid organic seaweed fertilizer is Gracilaria sp. The advantage of using seaweed as a raw material for making fertilizer is an easy to obtain raw material, while it can also reduce seaweed waste on the beach and it can also utilize the potassium, calcium, and magnesium content in seaweed to produce hygienic and contaminant-free quality compost such as heavy metals and phytotoxic compounds [2].

The current problem with liquid organic fertilizer made from seaweed is low concentrations of phosphorus and potassium. Phosphorus and potassium are the main elements in fertilizer that can help plant growth. The function of phosphorus and potassium in fertilizer is to strengthen roots and stems, and accelerate the formation of roots and leaves [3]. Seaweed contains low phosphorus and potassium, it is necessary to add an ingredient that can increase phosphorus and potassium to produce a good
seaweed liquid organic fertilizer product. One of the ingredients needed to increase the concentration of phosphorus and potassium in seaweed liquid organic fertilizer is fish bone flour. According to [4], fish bones has 59.7% calcium content and 35.8% phosphorus content. Fish bone flour added to seaweed liquid organic fertilizer can increase the concentration of phosphorus and potassium and produce the best seaweed liquid organic fertilizer on the market that can accelerate plant growth.

2. Materials and methods

2.1 Study design

This study used the experimental method with completely randomized design method [5]. The concentration treatment of fish bone meal addition on *Gracilaria* sp. liquid organic fertilizer comprised:

- P₀: 500 g *Gracilaria* sp. + 10 ml EM4 + 1 L aquades + 0% fishbone meal
- P₁: 500 g *Gracilaria* sp. + 10 ml EM4 + 1 L aquades + 25% fishbone meal
- P₂: 500 g *Gracilaria* sp. + 10 ml EM4 + 1 L aquades + 50% fishbone meal
- P₃: 500 g *Gracilaria* sp. + 10 ml EM4 + 1 L aquades + 75% fishbone meal

2.2 Liquid fertilizer production

*Gracilaria* sp. seaweed was obtained from Kalialo Village, Kupang, Jabon, Sidoarjo Regency, while fish bone waste was obtained from PT. Alam Jaya, Tenggilis Mejoyo, Surabaya. *Gracilaria* sp. seaweed with 500 g weight and fish bone waste were washed using running water, then cut to approximately five centimeters length. Seaweed was inserted into the plastic container and added with EM4. Cleaned fish bone waste was grinded to become meal. Fish bone meal was added to the plastic container containing seaweed based on the treatments given and fermented for 14 days in anaerobic conditions.

2.3 Nitrogen assay

Sample was hydrolyzed with sulfuric acid until forming ammonium sulfate compound. Nitrate obtained from the sample was reacted with salicylic acid to form nitrosalicylate, then reduced with sodium thiosulfate until forming ammonium compounds. Ammonium was distilled and titrated with sulfuric acid solution until the green color turned pink [6].

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\text{Total Nitrogen (\%) = } \frac{(V_1 - V_2) \times N \times 14.008 \times P \times 100 \times 100}{W(100 - KA)}
\]

Note:
- \(V_1\): H₂SO₄ solution for sample titration
- \(V_2\): H₂SO₄ volume for blank titration
- \(N\): H₂SO₄ normality solution
- 14.008: Nitrogen atom weight
- \(P\): Dilution
- \(W\): Sample weight
- \(KA\): Moisture content

2.4 Phosphorus assay

Phosphorus concentration was measured using spectrophotometry method reference. Phosphorus assay was performed by adding 45 mL aquadest to measuring flask containing sample solution and kept for 5 minutes. Molybdovanadat reagent was added to the sample solution, then optimized with spectrophotometer on 400 nm wavelength. The absorbance of the sample solution was read on the spectrophotometer based on the standard curve [6].
Note:
C : Total phosphorus on the standard curve reading
P : Dilution factor
W : Sample weight, mg
KA : Moisture content, %

2.5 Potassium Assay
Potassium concentration was measured based on flame photometry method. The sample solution was inserted into 100 mL measuring flask. Suppressor solution was added as much as 1 – 10 mL and homogenized. Potassium concentration was measured by flame photometer or SSA at 766.5 nm wavelength [6].

Note:
C : Potassium concentration, mg.l⁻¹
P : Dilution factor
1.2046 : Conversion factor of K₂O against K
W : sample weight, mg

2.6 pH and total plate count (tpc) assay
Acidity degree (pH) on Gracilaria sp. liquid organic fertilizer was performed using pH meter. According to the Indonesian Ministry of Agricultural Affairs Policy No. 70/PERMENTAN/SR. 140/10/2011, the optimum pH in liquid organic fertilizer is 4-9.

Total plate count assay (TPC) was performed to determine the microbe number contained in the fertilizer by calculating the bacterial colony grown in the media. This method used the serial dilution. The purpose of serial dilution was to reduce the microbe number suspended in liquids [7]. TPC was performed using spread plate method. Spread plate method was performed by taking 1 mL sample using micropipette. Sample was transferred to PCA (Plate Count Agar) medium and aligned with Drigalski. Media were inserted into the incubator at 37°C for 24 hours.

2.7 Data analysis
Data obtained from the study result were analyzed using ANOVA (Analysis of Variance) to determine whether there were any significant differences among treatments based on the design performed, namely completely randomized design.

3. Result and discussion
3.1 Result
The addition of fish bone meal on Gracilaria sp. liquid organic fertilizer showed significant different result against increased concentrations of nitrogen, phosphorus, and potassium. Gracilaria sp. liquid organic fertilizer with 75% fish bone meal addition produced the highest concentration of nitrogen (0.69%), phosphorus (0.42%), and potassium (0.43%). Treatment without fish bone meal addition produced the lowest concentration of nitrogen (0.048%) and potassium (0.24%), while 50% fish bone meal produced the lowest phosphorus concentration (0.33%).

| Treatment            | Nitrogen (%)       | Phosphorus (%) | Potassium (%)     |
|----------------------|--------------------|----------------|------------------|
| 0% fish bone meal    | 0.048 ± 0.01d      | -              | 0.24 ± 0.02d     |
25% fish bone meal 0.27 ± 0.05<sup>c</sup> - 0.34 ± 0.01<sup>c</sup>
50% fish bone meal 0.52 ± 0.03<sup>b</sup> 0.33 ± 0.04 0.37 ± 0.01<sup>b</sup>
75% fish bone meal 0.69 ± 0.07<sup>a</sup> 0.42 ± 0.02 0.43 ± 0.03<sup>a</sup>

Note: Data were presented as average value of five replications ± standard deviation. Different notation showed significant differences.

pH assay on *Gracilaria* sp. liquid organic fertilizer with fish bone meal addition showed significant different result (Table 2). *Gracilaria* sp. liquid organic fertilizer with 0% and 25% fish bone meal had the same pH value (7.08). *Gracilaria* sp. liquid organic fertilizer with 50% fish bone meal addition had pH 7.12, while the highest pH value was obtained from the addition of 75% fish bone meal with 7.24.

**Table 2. pH value of *Gracilaria* sp. liquid organice fertilizer**

| Treatment            | pH     |
|----------------------|--------|
| 0% fish bone meal    | 7.08 ± 0.08<sup>b</sup> |
| 25% fish bone meal   | 7.24 ± 0.11<sup>a</sup> |
| 50% fish bone meal   | 7.12 ± 0.08<sup>ab</sup> |
| 75% fish bone meal   | 7.08 ± 0.08<sup>b</sup> |

Note: Data were presented as average value of five replications ± standard deviation. Different notations showed significant differences.

Total bacterial number assay in *Gracilaria* sp. liquid organic fertilizer with fish bone meal addition (Table 3) indicated different results among treatments during the fermentation process from day 0 to 15.

**Table 3. Total Plate Count (TPC) assay result on *Gracilaria* sp. liquid organic fertilizer**

| Treatment            | TPC (Colony.ml<sup>-1</sup>) |
|----------------------|-------------------------------|
|                      | Day 0                         | Day 15                        |
| 0% fish bone meal    | 2.70 x 10<sup>4</sup>         | 8.01 x 10<sup>5</sup>         |
| 25% fish bone meal   | 1.50 x 10<sup>4</sup>         | 9.10 x 10<sup>5</sup>         |
| 50% fish bone meal   | 2.20 x 10<sup>4</sup>         | 1.22 x 10<sup>6</sup>         |
| 75% fish bone meal   | 1.80 x 10<sup>2</sup>         | 6.46 x 10<sup>2</sup>         |

3.2 Discussion

The addition of fishbone meal in the production process of *Gracilaria* sp. liquid organic fertilizer affected significantly (p<0.05) towards increased concentration of nitrogen, phosphorus, and potassium. The addition of 75% fish bone meal was the best treatment applied as it produced the highest concentrations of nitrogen, phosphorus, and potassium, namely 0.69%, 0.42%, and 0.43% respectively. However, the addition of 75% fish bone meal was unable to fulfill the regulatory standards based on the Indonesian Ministry of Agricultural Affairs Policy No.70/Permentan/SR. 140/10/2011.

*Gracilaria* sp. liquid organic fertilizer without the addition of fishbone meal produced the lowest nitrogen concentration with 0.048%, while the highest nitrogen concentration was obtained from the addition of 75% fish bone meal with 0.69%. This means that increased addition of fishbone meal affects nitrogen concentration improvent on *Gracilaria* sp. liquid organic fertilizer. The mechanism of nitrogen formation in fertilizer formed as protein in organic material was converted into amino acids with aminization reaction, then converting amino acids into ammonia (NH₃) and ammonium (NH₄⁺) through ammonification process and nitric oxide reaction to convert into nitrate [8]. This condition was reinforced by [9], who stated that increased nitrogen content is caused by nitric bacteria activity that convert ammonia into nitrates at the end of the composting process.
The highest concentration of phosphorus was discovered on the addition of 75% fish bone meal in Gracilaria sp. liquid organic fertilizer, producing 0.42%, then followed with 50% fish bone meal addition (0.33%). Treatment with 0% and 25% fish bone meal showed no influence on the concentration of phosphorus produced. This happened as the absence of phosphorus sources from other materials added to the production of liquid organic fertilizer due to low phosphorus content on Gracilaria sp. Nevertheless, [10] mentioned that phosphorus derived from algae group has an advantage as more easily absorbed by plants than from inorganic phosphate. Based on [11], the use of liquid organic fertilizer made from seaweed can increase the phosphorus content in hybrid watermelon.

The highest concentration of potassium was discovered on the addition of 75% fish bone meal in Gracilaria sp. liquid organic fertilizer, reaching 0.43% concentration. Although the highest potassium produced in Gracilaria sp. liquid organic fertilizer was only 0.43%, this result was still higher than the previous studies conducted by [1] on liquid fertilizer produced from Eucheuma cottonii, Sargassum sp., and Gracilaria sp. seaweed with the composting process, producing potassium only 3.4x10^{-5}% and [9] on the influence of EM4 bioactivator and fish meal addition to Gracilaria sp. seaweed liquid organic fertilizer, producing potassium concentration only 0.4%. According to [12], fish bones contain 6.7 mg.kg^{-1} potassium, therefore the addition of fish bone meal to produce Gracilaria sp. liquid organic fertilizer can increase the potassium concentration in the fertilizer. Potassium has an important role in regulating the photosynthesis and protein synthesis process [13].

Gracilaria sp. liquid organic fertilizer with 25% fish bone meal addition produced the highest pH value with 7.24. Increased pH value in liquid organic fertilizer was caused as fish waste product added contained high salt content [3]. Treatment with 50% and 75% fish bone meal addition produced reduced pH value, i.e 7.12 and 7.08 respectively. Acidity is associated with the microorganism habitat. This study results indicates that the total number of bacteria in Gracilaria sp. liquid organic fertilizer added with fish bone meal is directly proportional to pH value. Increased microorganism number in Gracilaria sp. liquid organic fertilizer on the early fermentation process until 15th day indicated the presence of induced microorganism number among all treatments. The most significant microorganism number was obtained from 50% fish bone meal addition with 2.20 x 10^{5} at the beginning of the fermentation process, then reaching to 1.22 x 10^{6} when Gracilaria sp. liquid organic fertilizer was harvested.

Decreased pH value in fertilizer with the addition of 50% and 75% fish bone meal was thought to be derived from the bacterial content in EM4, namely lactic acid bacteria, photosynthetic bacteria, and yeast [14]. This was in line with [15], who stated that pH value reduced along induced lactic acid formation as the result of lactic acid bacteria presence to improve remaining metabolism, thereby causing pH value tends to be acidic. pH value change is influenced by the activity of microorganisms contained in EM4 and early stages of fermentation process on liquid organic fertilizer added with sugar as an energy source for microorganism growth [16]. Liquid organic fertilizer that has susceptible pH value 6-7 will facilitate the plant in absorbing nutrients [17].

Gracilaria sp. liquid organic fertilizer added with fish bone meal produced improved concentration of nitrogen (0.69%), phosphorus (0.42%), and potassium (0.43%). Gracilaria sp. liquid organic fertilizer is potentially adequate to provide nutrients in plants and able to replace the chemical fertilizer damaging the soil ecosystem.

4. Conclusion
The addition of fishbone meal on the production of Gracilaria sp. liquid organic fertilizer increases nitrogen, phosphorus, and potassium concentration. The highest concentration of nitrogen, phosphorus, and potassium were obtained from 75% fish bone meal addition with 0.69%, 0.42%, and 0.43% respectively.

5. References
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