Characteristics of liquid organic fertilizer from different composition types of seaweed between *Gracilaria* sp. and *Sargassum* sp.

A R Tsaniya, E N Dewi, A D Anggo
Departemen Fisheries Processing Technology, Faculty of Fisheries and Marine Science, Diponegoro University, JL. Prof. H. Soedarto.SH. Tembalang, Semarang, 50275, Indonesia

Corresponding author: artsaniya@gmail.com

Abstract. *Gracilaria* sp. and *Sargassum* sp. contain growth hormones such as auxins, gibberellins, and cytokinins. A combination of them is expected to complement each other as raw material for organic fertilizers to improve soil structure and help plant growth. The purpose of this study is to determine the characteristics of organic fertilizer from different seaweed composition. Reduction the size of sample and soaking with 1% phosphoric acid solution to soften the seaweed. Homogenization and heating with the addition of molasses 3% as microbial nutrition and KOH 1% to maximize mineral content. The fermentation process is carried out for 14 days as microbial exponential phase of *Azospirillum* sp as nitrogen fixation also phytohormones producer and *Trichoderma* sp. as an organic material decomposer. The results of ANOVA analysis and Tukey test showed that the treatment of raw material composition using different types of seaweed gives significantly different in C-Organic, nitrogen, potassium, phosphorus, pH, color, and aroma. The composition of *Gracilaria* sp. and *Sargassum* sp. give the highest value for C-Organic (1.15%) and Nitrogen (0.67), as well as phosphorus (0.45%), potassium (0.48%), and pH (4.48). Color and aroma are maturity indicators of organic fertilizers. This composition of liquid fertilizer has a pungent aroma and a dark brown color.

1. Introduction
Seaweed contains minerals, organic compounds, and growth promoting hormones (phytohormones). The types of seaweed that are usually used as raw material for organic fertilizers are *Gracilaria* sp. and *Sargassum* sp. Both of them macroalgae are currently known as a biostimulant plant which can increase plant growth and nutrients in crop yields. It contains phytohormones, including auxins, cytokinins, and gibberellin. Brown seaweed, one of them is *Sargassum*, is rich in alginate and fucoidan, which can stimulate plant root growth, while *Gracilaria* contains 10-47% protein which can increase organic compounds to stimulate plant growth [1].

Organic fertilizer is environmentally friendly and can prevent land degradation. Organic fertilizer is divided into two types, that are solid and liquid. The advantages of liquid organic fertilizer can be applied by pouring it on the roots and plants at once. Hence it can keep the soil moist. Liquid organic fertilizer makes it easier for plants to absorb nutrition, so increasing plant growth [2]. Fertilization using liquid organic fertilizer is distributed effectively because it’s 100% soluble.

Potential of *Gracilaria* sp. and *Sargassum* sp. as raw material for liquid organic fertilizer because of the high nutrient content and plant growth hormone. Plant growth hormone is found in *Gracilaria* sp.
including 144 ppm auxin, 1,552 ppm gibberellin and cytokinins consisting of kinetin 65 ppm and zeatin 81. Plant growth hormone content is also found in *Sargassum*, namely auxin 148 ppm, gibberellin 160 ppm and cytokinins consisting of 71 ppm kinetin and 86 ppm zeatin. The value of growth hormone is higher than commercial organic fertilizers with auxin values of 130 ppm, gibberellin 122 ppm and cytokines consisting of 61 ppm kinetin and 67 ppm zeatin [3].

The combination of seaweed *Gracilaria* sp. and *Sargassum* sp. through a fermentation process which is accelerated by bioactivators from *Trichoderma* sp. and *Azospirillium* sp. *Trichoderma* sp. is a group of fungi that acts as a decomposer of organic matter as well as controlling pests and plant diseases [4]. *Azospirillium* sp. is one of the aerobic bacteria that can increase nitrogen level and associate with plants [5]. *Azospirillium* sp. can decompose nitrogen in higher plants such as seaweed.

Aim of this study is to determine the characteristics of organic fertilizer from different composition of seaweed raw materials, namely the comparison of *Gracilaria* sp. and *Sargassum* sp. Liquid organic fertilizer formulations with different types of seaweed treatment have an effect on level of N, P, K, C-Organic, pH, and sensory tests (color and aroma) of liquid organic fertilizers produced in order to obtain liquid organic fertilizer with the best characteristics.

2. Research methods

2.1. Raw material

*Sargassum* sp. were collected from Gunung Kidul coast, South Coastal of Yogyakarta, Indonesia. *Gracilaria* sp. were harvested from Tambak Harja, Semarang, Indonesia.

The seaweed treatment is washed until clean from the dirt. After that, reducing the size of seaweed was done by cutting the seaweed into small pieces 1-2 cm. Immersion of the seaweed was carried out in a 1% solution of phosphoric acid with 1:10 for 18-24 hours. The treatment of liquid organic fertilizer are comprised:

A : 100% *Gracilaria* sp. + *Trichoderma* sp. 1 CFU/ml + *Azospirillium* sp. 1 CFU/ml + 3% Molase
B : 100% *Sargassum* sp. + *Trichoderma* sp. 1 CFU/ml + *Azospirillium* sp. 1 CFU/ml + 3% Molase
C : 50% *Gracilaria* sp. + 50% *Sargassum* sp. + *Trichoderma* sp. 1 CFU/ml + *Azospirillium* sp. 1 CFU/ml + 3% Molase

2.2. Preparation of microba

The starter of bioactivator was *Trichoderma* sp. that obtained from Central of plant and disease laboratory, Ungaran, Semarang, Indonesia, and *Azospirillium* sp. was obtained from agricultural microbial laboratory, agriculture faculty, Gadjah Mada University, Yogyakarta, Indonesia. Culture of *Azospirillium* sp. and *Trichoderma* sp. are activated or refreshed with liquid media according to its microbial specifications, namely *Azospirillium* sp. in Merck Nutrient Broth (made in Germany) and *Trichoderma* sp. in the media of Merck Potato Dextrose Broth (made in Germany). A colony was activated in 9 ml of liquid media for 24 hours for *Azospirillium* sp. and *Trichoderma* sp. for 3x24 hours [6, 7].

2.3. Liquid fertilizer production

The seaweed was re-weighed after soaking with phosphoric acid according to the treatment in the study. Next process was addition of the 1% phosphoric acid solution to the sample with a ratio of 1:10, which then subjected to refinement and homogenization. The addition of 3% molasses was carried out when the fertilizer formulation with each treatment was completely dissolved, and heating was carried out again to a temperature of 100°C.

2.4. Microbial induction in liquid fertilizer

Each treatment was added with a bacterial inoculant with 10 ml of bacterial colonies mixed with the liquid organic fertilizer. The adaptation process was carried out for 2x24 hours for *Azospirillium* sp. and 3x24 hours for *Trichoderma* sp [8, 9].
2.5. Fermentation process
The fermentation process occurred during 14 days to obtain liquid organic fertilizer from different seaweeds. Fermentation was carried out using an aerator to provide oxygen during the aerobic fermentation process.

2.6. Determination of C-organic
Measurement of C-Organic concentration was carried out based on the Walkey and Black method [11]. This measurement was carried out by sample digestion using K₂Cr₂O₇ 1N and H₂SO⁴ dilution, homogenization and left for 12 hours. The next step was measurement with UV/VIS spectrophotometer (Mattler Toledo made in Europe) at a wavelength of 561 nm.

2.7. Determination of nitrogen
Nitrogen concentration measurement was carried out based on the Kjeldahl method, titrimetry and Devarda method [10].

2.8. Determination of phosphorus
Measurement of phosphorus concentration used UV/VIS Spectrophotometric (Mattler Toledo made in Europe) with a wavelength of 650 nm [10].

2.9. Determination of potassium
Measurement of potassium concentration was carried out by Flamephotometry (A.Kruss made in Germany) [10].

2.10. Determination of pH
pH testing was carried out using a pH meter (Mattler Toledo made in Europe) [10].

2.11. Sensory test
The sensory test was a test of the quality of a material with the help of the human senses. Sensory testing method for organic fertilizers that consist of aroma and color parameters ranging 1-9 based on preferences to capture liking data [11].

2.12. Data analysis
Data analysis for parametric statistical testing used ANOVA followed by the Tukey test, while non-parametric was performed using the Kruskal-Wallis method, followed by the Man-Whitney test.

3. Results and discussion

3.1. C-organic content
Carbon is an important element for microorganisms as a source of energy to form new cells and growth during the decomposition process [12]. Liquid organic fertilizers with different types of seaweed as raw material, namely *Sargassum* sp. and *Gracilaria* sp. have levels of C-Organic shown in Figure 1.
C-Organic level of liquid organic fertilizer with the highest level were from a mixture of Gracilaria sp. and Sargassum sp. 1.15%. The results showed that the liquid organic fertilizer from Sargassum sp. contains 0.06% C-Organic higher than the liquid organic fertilizer from Gracilaria sp., 0.67%. That is due to the differential of organic matter content. Sargassum contains higher organic matter content than Gracilaria [13]. However, the difference in the amount of organic matter from both Gracilaria and Sargassum seaweed are not significantly different, but both can increase the organic content for soil fertility.

Liquid organic fertilizer from Gracilaria sp. and Sargassum sp. higher than the C-Organic content of liquid organic fertilizer from vegetables, namely 0.17% [13]. The low C-Organic value in liquid organic fertilizer from Gracilaria sp. and Sargassum sp. is due to the role of Trichoderma and Azospirillum as decomposers of organic compounds including carbohydrates, cellulose and hemicellulose from seaweed both Sargassum sp. and Gracilaria sp. into CO₂ and water which can be lost to the air and cause carbon levels to decrease [14]. The decomposition process through the activity of these microorganisms utilizes the element carbon as an energy source. Therefore it has an effect on reducing the resulting C-Organic content.

3.2. Nitrogen content
Nitrogen (N) plays a role in the plant metabolic system, especially in essential protein constituents [12]. The results of nitrogen content in liquid organic fertilizers by treating different types of seaweed as raw material are presented in Figure 2.
The nitrogen content of liquid organic fertilizers are the highest in liquid organic fertilizers with mixing between Sargassum and Gracilaria. Based on these results, it can be seen that the nitrogen content of liquid organic fertilizer from Sargassum sp. lower than Gracilaria sp. The research directly proportional to the protein content of the dry samples from Gracilaria sp. which is 11.78% higher than the dry sample of Sargassum sp. that is 9.75%. The protein content of brown seaweed is lower than that of red seaweed [13]. Therefore, Gracilaria can increase the levels of protein and nitrogen in the soil for plant growth.

Nitrogen content in liquid organic fertilizer from Gracilaria sp. and mixture of two types of seaweed, namely 0.56% and 0.67%. The nitrogen content of the two treatments meets the requirements for liquid organic fertilizer from the Decree of the Minister of Agriculture of the Republic of Indonesia concerning Minimum Technical Requirements for Organic Fertilizers, Biological Fertilizers and Soil Improvement which states that the minimum content of N-Total in Liquid Organic Fertilizer is 0.5% [15].

Nitrogen level is related to the presence of plant growth hormone (phytohormone). The advantage of organic fertilizer from seaweed is the phytohormone content in the raw material itself. The high role of phytohormones will help plants to get optimal nutrition. Phytohormone does not affect the specific nutrition of plant growth, but coordinates growth and development with nutrients in plant organs and acts together to trigger increased nutrition for plant growth [16].

3.3. Phosphorus content

Liquid organic fertilizers from different types of seaweed have different levels of phosphorus in each treatment given. The results of testing the levels of phosphorus in liquid organic fertilizers with different raw material treatments are presented in Figure 3.

![Figure 3. Phosphorus content of liquid organic fertilizer](image)

The phosphorus content in the liquid organic fertilizer from Gracilaria sp. was higher than the phosphorus content in the liquid organic fertilizer from Sargassum sp. Based on these results, it shows that the phosphorus content formed in liquid organic fertilizers from Gracilaria sp. was higher than liquid organic fertilizers from Sargassum sp. Analysis of phosphorus content in liquid organic fertilizers from Gracilaria sp. 0.045% higher than the liquid organic fertilizer from Sargassum sp. 0.0078% [3]. The levels of phosphorus in organic fertilizers with the lowest treatment of different types of seaweed as raw material, namely the combination treatment of Sargassum sp. and Gracilaria sp. with an average phosphorus content of 0.45%. The increasing of phosphorus content in this study can be caused by the multiplication of microorganisms as a decomposer of organic matter. Therefore the higher the organic matter in the material, the higher the phosphorus content [17].

The phosphorus content in liquid organic fertilizer in this study is higher than Sargassum liquid organic fertilizers with different types of starters with a phosphate value between 0.019% - 0.03% [18]. This is due to the role of microba as organic matter decomposer, namely Azospirilium sp. Apart from
acting as nitrogen fixing bacteria, these bacteria also play a role in solvent phosphorus so that it can increase the phosphorus content in fertilizers [19].

3.4. Potassium content
The results of the potassium test in liquid organic fertilizer with different types of seaweed treatment as raw material are presented in Figure 4.

![Figure 4. The potassium content of liquid organic fertilizer](image)

Potassium levels in organic fertilizers from mixing of *Gracilaria* sp. and *Sargassum* sp. almost 1.5 times higher than the liquid organic fertilizer from *Gracilaria* sp. Liquid organic fertilizer from *Sargassum* sp. has the highest potassium content of 0.68%. This is related to the mineral content in the seaweed. *Sargassum* sp. (brown seaweed) has an ash content is higher than that of red seaweed, *Gracilaria* sp. Brown seaweed contains high ash (45.04%), followed by red seaweed (28.79%) and green seaweed (14.10%) [20].

Potassium plays a role in the rate of photosynthesis for the formation of protein and carbohydrates, which also maintains plant turgor, while gibberellin plays a role in cell division and enlargement, which means increasing plant turgor. Based on their roles, it can be seen that there is a synergistic relationship between potassium content phytohormone especially gibberellin [21]. The higher the potassium content, the higher the gibberellin content. This is evidenced by the gibberellin content in organic fertilizer from *Sargassum* of 160 ppm and *Gracilaria* of 1,552 ppm [3].

3.5. Degree of acidity (pH)
Liquid organic fertilizer is made from different types of seaweed, namely *Gracilaria* sp. and *Sargassum* sp. those fermented for 14 days have the acidity levels (pH) shown in Figure 5.
Figure 5. Degree of acidity (pH) in Liquid Organic Fertilizer

Degree of Acidity (pH) affects the quality of the soil which means it also affects plant growth [22]. Liquid organic fertilizer with different treatment that have the lowest pH, namely liquid organic fertilizer from a mixture of composition between Gracilaria sp. and Sargassum sp. with pH level in 4.48. Liquid organic fertilizer from Sargassum sp. amount 7.03 higher than liquid organic fertilizer with a composition of Gracilaria sp, which is 6.49. The degree of acidity (pH) in liquid organic fertilizers is influenced by the decomposition process of C-Organic by microbes [23]. The higher the amount of C-Organic, the lower the pH of the organic fertilizer.

Liquid organic fertilizers from three different raw material compositions produce different pH values with intervals between 4.48-7.03. The pH value is in accordance with the requirements for liquid organic fertilizer from the Decree of the Minister of Agriculture of the Republic of Indonesia concerning Minimum Technical Requirements for Organic Fertilizers, Biological Fertilizers and Soil Improvement which states that the pH value of Liquid Organic Fertilizers between 4-9 [15].

3.6. Sensory test

The average results of sensory testing on liquid organic fertilizers with differences in the composition of seaweed as raw materials are presented in Table 1.

| Treatment                  | Parameter   | Trust Interval |
|----------------------------|-------------|----------------|
|                            | Appereance  | Aroma          |
| Gracilaria                 | 6.97±1.07a  | 5.20±1.30b     | 6.02 <µ < 6.14 |
| Sargassum                  | 4.43±1.33c  | 4.27±1.17a     | 4.42 <µ < 4.48 |
| Gracilaria and Sargassum   | 7.43±1.01b  | 6.40±0.89c     | 6.85 <µ < 6.99 |

Note: 4 “dislike slightly”, 5 “Netral”, 6 “like slightly”, 7 “Like moderately”

The aroma of organic fertilizer is a parameter that affects the maturity of organic fertilizer from the fermentation process. Unacceptable aroma produced in the liquid organic fertilizer of the composition of 100% Gracilaria sp. and 100% Sargassum sp. because the aroma produced tends to sting. This can be attributed to the lower content of organic matter so that microbial activity during the decomposition process of organic matter into organic acids is not optimal. The aroma is an important factor in the
maturity level of fertilizers in the decomposition process. The decomposition process can remove the organic compound that causes strong odors, which is ammonia [24].

![Figure 6](image_url)

**Figure 6.** Liquid organic fertilizer from difference type of Seaweed

Note: (A) Gracilaria sp., (B) Sargassum sp., (C) Mix Gracilaria sp. and Sargassum sp.

The results of the appearance assessment of liquid organic fertilizers by semi-trained panelists showed that the panellist that 'like moderately' the mixture composition of *Gracilaria* sp. and *Sargassum* sp., the panelist rate “like slightly” the composition of *Gracilaria* sp., while the composition of *Sargassum* sp. Is judged 'rather disliked' by the panelists. Composition of a mixture of *Gracilaria* sp. and *Sargassum* sp. is preferred compared to other compositions because the color of the fertilizer is dark brown, like the color of the soil. This is in accordance with the provision of the color of fertilizers on the Indonesian National Standard of organic fertilizer, namely blackish brown color [25]. The color of the fertilizer is a physical indicator of the maturity level of the fertilizer. One of the characteristics of good compost is that it has a brown color [11].

4. Conclusion

The composition of seaweed as raw material for making the best liquid organic fertilizer is the mixture of *Gracilaria* sp. 50% and *Sargassum* sp. 50% because it has different characteristics either chemically and physically of liquid organic fertilizer. The nutrient content of liquid organic fertilizer from mixing *Gracilaria* and *Sargassum* is higher than other compositions, then physically, it has a standard color and doesn't smell strong.

Acknowledgments

The authors are grateful Microbial laboratory of Fisheries and Marine Faculty, Diponegoro University, Semarang and Central Java Agricultural Technology Research Center, Semarang, for supporting the research.

References

[1] Raj T S, Nisthanthi P, Graff K H and Suji H A 2018 Int. J. Trop. Agric. 36 563-580

[2] Madusari S 2019 J. Appl. Sci. Adv. Technol. 1 81-90

[3] Sedayu B, Erawan I M S and Assadad L 2014 JPB of Fish. 9 61-68.

[4] Thaha A R, Umrah U, Asrul A, Rahim A, Farji F and Nurzakia N 2020 J. Agric. Food 5 825-834

[5] Zeffa D M, Perini L J, Silva M B, Sousa N V D, Scapimj C A, Oljivjerjira A L M J D, Junior A T D A and Goncalves L S A 2019 Plos one 32 1-19

[6] Palealu J B, Butarbutar R B and Tallei T E 2017 Bioslogos Journal 7 36-40

[7] Harlis R, Budiarti S, Kapli and Sanjaya M E 2019 Biospecies 12 40-48

[8] Rizki U and Agustini R 2018 J. Chem. 748-52
[10] AOAC 2012 *Association of Official Analytical Chemists* 19th Edition (US: Washington, DC)
[11] Mustika A M, Suryani P and Aulawi T 2019 *Agrotechnology* 9 13-20
[12] Leghari S J, Wahocho N A and Leghari G M 2016 *J. Adv. Environ. Biol* 10 209-218
[13] Izzati M, Haryanti S and Setiari N 2019 *J. Phys.* 1217 1-7
[14] Muliarta I N, Agung I G A M S, Adnyana I M and Diara I W 2019 *Int. J. Life Sci.* 3 56-70
[15] Agriculture Departement 2019 261/KPTS/SR.310/M/4/2019 Jakarta.
[16] Kiba T, Kudo T, Kojima M and Sakakibara H 2011 *J. Exp. Bot.* 62 1399-1409
[17] Ratrinia P W, Uju and Suptijah P 2016 *J. Indonesian Fish. Proc. Prod* 19 309-320
[18] Dewi E N, Rianingsih L and Anggo A D 2019 *Int. Confe. Trop. Coast. Reg. Eco Dev.* 248 1-9
[19] Fendrihan S, Sorina D and Constantinescu F 2018 *J. Adv. Agric.* 71096-1110
[20] Manteu S H, Nurjanah and Nurhayati T 2018 *J. Indonesian Fish. Proc. Prod* 21 396-405
[21] Hasanuzzaman M M H, Bhuyan M B, Nahar K, Hossain M S, Jubaer A M, Hossen A A, Masud C, Moumita and Fujita M 2018 *Agronomy* 8 1-29
[22] Zakiyah Z N, Rahmawati C and Fatimah I 2014 *Indonesian J. Chem. Res.* 3 38-48
[23] Sastro Y, Bakrie B and Sudolar N R 2013 *J. Indonesian Trop. Anim. Agric* 38 257-263
[24] Nuraini Y and Asgianingrum R E 2017 *Indonesian Horticulture J.* 8 183-191
[25] Kusmiyarti T 2013 *Agrotech.* 383-92