Identification and characterization of solid waste from 
Gracilaria sp. extraction

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Abstract. The identification and characterization of chemical composition of solid agar extracted solid waste were carried out. The method used was to take samples randomly in several seaweed processing industries to analyze their chemical composition. The parameters tested included the micro-nutrient and macro-nutrient content, growth-stimulating hormone level, and ash contents. The results showed that solid waste from agar extraction consist of micronutrients such as: Cu (4.80 ppm), Fe (0.24 ppm), Zn (8.42 ppm), Mn (57.58 ppm), B (32.32 ppm) as well as the macronutrients such as: N (0.20%), P (0.12%), K (0.17%), C-organic (10.96%), Na (0.66%), Ca (0.61%), Mg (0.09%), CEC (13.5 me/100g), and N/C ratio of 54:7. Growth hormones auxins (191 ppm), gibberellin / GA3 (509.5 ppm), cytokinin-kinetin (244.5 ppm) and cytokinin-zeatin (70.5 ppm). The ash content was 60.19%, a slightly acidic pH of 6.3, diluted in cold water (11.67%) and hot water (23.17%) as well as in 1% sodium hydroxide solution (35.32%). Based on the results of identification and characterization, the solid waste of agar extraction was useful as a raw material for plant fertilizer.

Keyword: agar extraction, Gracilaria sp., growth hormones, micro and macromineral, solid waste

1. Introduction

Seaweed is known as macro-algae, the most substantial part of marine plants, which grow and develop in almost all waters and is one of the marine and fisheries commodities that have been used for a long time for food and non-food products as food ingredients, organic fertilizer industry, cosmetics industry, textile industry, and pharmaceutical industry. Then the largest utilization of seaweed caused in seaweeds containing various chemicals and other organic materials and vitamins needed to improve quality of products (Hidayat 1994).

Gracilaria sp. is one of the seaweed that has been used commercially for food and non-food products. Baghel et al (2014) stated that in the Gracilaria, thallus contained agarpectin, minerals, cellulose, hemicellulose, and lignin. Ruperez (2002), Santos et al (2004), Basmal (2010) Baghel et al (2014), Hapsari...
and Wahyu (2014) reported that seaweed contain macro elements (N, P, K, Ca, Mg and C) and micronutrients (Fe, Cu, Zn, Boron, Na, Cl and Mn). The filter aids/perlite was added during agar extraction to accelerate purification of agar from its solid waste. The addition of diatomaceous soil/filter aids to seaweed slurry caused the amount of solid waste to increase. The main chemical content in diatomaceous soil was silica (SiO$_2$) and a small amount of alumina, iron oxide, magnesium, sodium, potassium oxide, titanium oxide, phosphate, and calcium oxide. The SiO$_2$ is not soluble in water or oil so that diatomaceous soil has been used to refine water impurities, carbohydrates, and oil. Up to now, the utilization of mixed diatomaceous soil and solid waste from Gracilaria sp has not used optimally yet and only being dumped somewhere as a result will pollute the environment due to a distributing off odor, the soil becomes white and crumbling. The aim of identification and characterization of solid waste from the extraction process of Gracilaria sp. was to determine the composition of solid waste and the possibility of using it for other new products.

2. Material and methods

Solid waste from the agar extraction process obtained from 4 companies taken randomly. Each sample from the company taken as much as 30 kg then mixed. Samples brought to the Research Center for Marine Product Processing and Biotechnology Jakarta. After the solid waste was dry (4-5.73%), the size reduction was made using the milling machine. Dried solid waste moisture and ash content were analyzed (SNI 0442:2009), micro and macronutrients by using Atomic Absorption Spectrophotometer Merck Eligent AA400, growth hormone (auxin, cytokine, and gibberellin) using HPLC waters 2487 and detector UV-Vis (Linskens and Jackson 1987). Total of growth hormone was calculated wide graphics area of growth hormone and standard, cellulose (SNI TAPPI T 203), hemicellulose (ASTMD1104-56), alpha-cellulose (SNI TAPPI T 203) and lignin (SNI 8429:2017) silicate (SNI ISO 776:2010) and solubility in solvent cold and hot water (SNI 01-1305-1983), solubility in NaOH solution (SNI 04-1839-1990) capacity of cation engagement/KTK (direct distillation).

The amount of dried Gracilaria sp. required for each of processing plants collected from Directorate General of Strengthening the Competitiveness of Fisheries Product (DGSCFP) and directly discussed with the owners of the processing plants. Data collected from DGSCFPF included the amount of seaweed plant, installed capacity, and the requirement of dried seaweed Gracilaria sp. While data collected from the owner’s of the processing plant were the basis for calculating the total of solid waste produced by the extraction process of Gracilaria sp.

3. Results and discussion

3.1. Potential solid waste

The waste referred to in this paper is solid waste derived from the extraction process of Gracilaria sp. consisted of sand, coral, snails, filter aids, cellulose, hemicellulose, alpha-cellulose, holo-cellulose, lignin, filter aids, and other wastes. Based on the data collected from DGSCFP and the owner’s processing plants the total of dried seaweed Gracilaria sp. required to produce 3,509 MT/Y purified agar was 29,088 MT/Y (figure 1) for all Gracilaria processing plants (14 processing plants) in Indonesia. The solid waste produced an amount of 29,088 MT/Y came from solid waste of gracilaria and filter aids during the separation of filtrate from solid waste. Approximately total filter aids were added into gracilaria slurry for accelerating separation liquid agar from solid waste and the amount of 2%.
3.2. Micro and macro nutrient content

3.2.1. Macronutrients content. Micro and macronutrients are an essential element for growing and increasing the production of the plant. There are 16 elements (micro and macronutrients) needed in plant growth and 13 functional elements obtained by the plant in the soil including nitrogen (N), phosphor (P), Potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S). All these elements needed in large quantities, if there is a lack of micro and macro-nutrient intake will cause plant growth slows down. Micro-nutrients required by plant growth are iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B), molybdenum (Mo) and chlor (Cl). Deficiencies of those microelement caused by plant growth not normal. Others elements carbon (C) and oxygen (O) and hydrogen (H) obtained both from air and soil directly. The result analysis the waste composition of agar extraction was presented in figure 2.

The real organic in waste of agar extraction found 13.93 % and higher than other macro-nutrient. C-organic in waste of agar extraction came from mixing the Gracilaria sp, and small animals (snail) decomposition at the time being handling and drying of Gracilaria by seaweed farmers and delaying time (depend on how long the seaweeds was storage in the warehouse) extraction process of agar. The higher C-organic content found in dried Gracilaria sp., the lower quality of dried gracilaria due to the decay process of dried Gracilaria sp. during the delaying time extraction process. According to Kusuma (2012), moisture content and temperature affected the speed of microorganism to the decomposition of compost to produce C-organic. In Garcilaria sp., C-organic came from the decaying process that occurs during storage and does not occur when extracted Gracilaria used of the combination of hot alkaline solution (NaOH) that will kill
decayed microorganisms. The function of C-organic in the soil is to improve soil structure, as a soil aeration, enhance groundwater buffer, press the rate of soil erosion, to support and provide nutrients to the plants, improving the efficiency of fertilization, neutralizes the toxic properties of Al and Fe; C-organic is also an energy for the remains soil microorganism that was able to release nutrient for plants. Refers to the minister of agricultural regulation said that C-organic content in the fertilizer more than 5%. The result analysis of C-organic content in seaweed (Gracilaria sp.) solid waste 13.93% its mean solid waste from agar extraction can be used as a raw material for making fertilizer.

The total N value of dried seaweed Gracilaria sp. was 1.4% while the total N value found in solid wastes ranges from 0.15-0.23%. The extraction process carried out by the processor cannot separate 100% of the N content from Gracilaria. The N-total standard for growing plants in a range of 0.21-0.5%. Based on this statement, all the solid waste of agar extraction useful as a raw material for producing fertilizer. Nitrogen compounds in plants are needed to form amino acids into proteins, the formation of chlorophyll, nucleic acids, and enzymes. In general plant growth especially in the vegetative phase plays a role in shoot formation, development of stems and leaves.

Plants are in great need of nitrogen during the vegetative growth period in which high ratio C/N in the final product indicates that microorganism will actively utilize nitrogen to form protein for plant growth. The result of the C/N ratio in waste of agar extraction in a range of 33-76.4. According to previous stated that the best of the C/N ratio in the between 15-20. The present difference C/N amongst processing plants cause of the difference in the type of equipment used. According to Meyers (1994) reported that the C/N ratio in alga/seaweed is between 4-10.

The cation exchange capacity (CEC) is a chemical property closely related to the minerals contents in planting medium or fertilizer material. The fertilizer material with high CEC can trap and provide better nutrient than low fertilizer with CEC. The primary basic cations in Fertilizer was Ca, Mg, K, Na (high base saturation) can increase soil fertility, but by an acidic cation, Al, H+ (low base saturation) can reduce soil fertility. Because the nutrients are present in the colloid trapping complex, the nutrients are not easily washed away by water. CEC in the type of raw material of the existing fertilizer varies, influenced by the composition of raw material fertilizer. CEC generally used as a distinguishing indicator in the soil classification process. The CEC value of solid waste of agar extraction from different processing plants ranged from 2.58-22.57 me/100g.

Potassium is a macro element such as nitrogen and phosphorus, potassium plays an essential role in photosynthesis, as it directly increases the growth and leaf area. Besides, potassium can increase carbon dioxide uptake. Potassium also can transfer sugar to starch formation and protein, help the process of opening and closing stomata, storing water capacity, expanding root growth, increasing plant resistance against pests and diseases, strengthening the plant body so that the leaves are not easy loss. Improve the size and quality of fruit in the generative period / add sweetness to the fruit, supply a lot of carbohydrates, especially in tuber plants. Result identification and characterization of potassium content in the solid waste of agar extraction showed that in a range of 0.265%. According to Chen and Gabelman (2000) reported tomato plants to absorb K elements in large quantities ranging from 1-5% of the dry weight of plants.

Plants absorb P from the soil in the form of phosphate ions, especially H₂PO₄⁻ and HPO₄²⁻ which are present in soil solutions (Havlin et al 1999). H₂PO₄⁻ ions are more commonly found in more acidic soils, while at higher pH (<7) the HPO₄²⁻ form is more dominant. Besides these ions, plants can absorb P in the way of nucleic acids, fitins, and fosfohumat. Analysis of phosphorus levels in solid seaweed waste was found in
the range of 0.161%. Phosphorus is a macro element that makes up the components of every living cell. According to Tisdale et al. (1985) found that the P element in all living cells of plants that comprise plant tissues such as nucleic acid, fitins, and phospholipids and then also reported that the P concentration in plants generally ranged from 0.1% to 0.4%. Its lower P-value in solid waste of agar extraction, it is suspected that the processing techniques provided by the producers are different from each other. It is known that when alkali-treated gracilaria sp. was added with either acetic acid or phosphoric acid the alkali-treated gracilaria sp. turn into gracilaria slurry. The P element in plants much helps the formation of proteins and minerals that are very important for plants to stimulate the formation of flowers, fruits, and seeds. Even able to accelerate fruit ripening and make the seeds heavier. Duty to circulate energy throughout the plant parts, stimulating root growth and development.

The content of Na in seaweed solid waste was between 1.01% (figure 2). The inhibition of plant growth strongly influenced by the concentration of salinity the higher the salinity of plant growth the more inhibited. Tarigan (1996) says the process of inhibiting plant growth begins at a salinity concentration of 1500 ppm. There was a change of leaf number, stem diameter and dry weight of canopy, while for plant height change, dry root weight and leaf growth inhibition area started at salinity concentration of 3000 ppm. Mapegau (2006) gave a soil salinity of 4.5 mmhos/cm, significantly decreasing the weight of dry beans of corn crops. High levels of Na in the soil can poison the growth of plants. In this study Na content in solid waste of agar between 2,100–10,500 ppm. If referring to the statement of Tarigan (1996) the solid waste of agar if used as fertilizer must be mixed with other fertilizer materials. The high Na content in seaweed solid waste is due to the use of NaOH solution in the agar extraction process. The difference in Na content in seaweed solid waste is likely to be the different concentration of NaOH solution used by the processor.

The content of Mg in seaweed solid waste was 0.086% (figure 2). Mg concentrations in soil 125 μmolL−1 is enough to support plant growth (Karley and White 2009). Based on this statement, Mg in solid waste from agar extraction was higher compared to Karley and White (2009). Mg in plants serve as a regulator to absorb P and K, stimulate the formation of fatty compounds and oils, helps the translocation of starch and phosphorus distribution in plants, as well as the activator of various types of plant enzymes.

3.2.2. Micronutrients in solid waste of agar extraction. The solid waste from the agar extraction from gracilaria is not only contained macro but also micro-nutrients. The role of micronutrients is very important in the process of plant growth. The result's analysis of micronutrient content in solid waste from the gracilaria extraction found 5 types of micronutrients namely Cu, B, Fe, Mn, Zn and B. The micronutrient content of Mn was higher compared to other micronutrients, namely Mn was 57.58 ppm, then followed by B 32.32 ppm, Zn 8.42 ppm, Cu 4.85 ppm, and Fe 0.24 ppm. Mn plays an important role in the synthesis of chlorophyll, acts as a coenzyme, as an activator of several respiration enzymes, in the reaction of nitrogen metabolism and photosynthesis. The highest nutrient content after Mn was Boron (figure 3). Ahmad et al. (2009) reported that Boron (B) is one of the essential micronutrients for plants that play a role in the formation of breeding of cells, especially in shoots. Boron also plays a role in the growth of pollen, flowers, roots and legume plants play a role in the formation of root nodules. This boron can multiply the nodule branches to provide many bacteria and prevent parasitic bacteria. Loomis and Durst (1992) reported that boron is an essential micronutrient required for growth and development of vascular plants, diatoms, and species of marine algal flagellates, while bacteria, fungi, green algae, and animals apparently do not require B. Zn value in solid waste of agar extraction was 8.42 ppm, the Zn in plant grow to support producing chlorophyll. Zn deficiency causes plant stunting, reduces crop yields, affects water absorption capacity, transports nutrients and also reduces the adverse effects of heat and salt stress in a short time (Hafeel et al. 2013). Zinc deficiency is more often associated with flooded soil than dry soils (Kasim WA 2007, Disante
et al 2010, Peck and McDonald 2010 and Tavallali (2010). Landsay (1972) reported that the total Zn concentrations in soils vary between 10 to 300 μg/g with an average of 50 μg/g.

Cu content in solid waste of agar extraction was 4.85 ppm. Yruela (2005) states that Cu is a redox-active transition metal that is heavily involved in the physiological processes of plants. Furthermore, He said that the presence of Cu must be maintained in very small amounts given its high redox ability. Excess Cu can cause poisoning in plants. The average content of Cu in plants tissue is 10 μg.g-1 dry weight (Baker and Snef, 1995). Based on Baker and Snef (1995), Cu content in solid waste of agar extraction can be used to support raw material of fertilizer. Fe content in solid waste of agar extraction was 0.24 ppm, Iron (Fe) is one of the micronutrients in plants. Plants absorb iron in the form of Fe$^{3+}$ ions but are consumed more in the form of Fe$^{2+}$ ions. Iron can also be absorbed in the form of complex organic salts (chelate) and can also be absorbed by leaves in the form of Fe sulfate. High Fe content in soil and absorbed by plants caused necrosis which is characterized by the appearance of black spots on the leaves.

![Figure 3. Micronutrient in solid waste of agar extraction.](image)

### 3.3. Growth Hormones

The terms of growth regulators include plant hormones (natural) and artificial compounds that can affect the growth and development of plants. Basmal et al (2006) reported that seaweed containing growth hormones such as auxins (IAA), gibberellin, cytokinin-kinetin, and cytokines zeatin inside of thallus. Growth hormones present in thallus gracilaria are bound in cell tissue, and it is expected that during the process of separation pure agar does not mix with growth hormones. The agar extraction technique from gracilaria seaweed is a combination of chemical and heat processes for a specific time. The pulp of gracilaria that has been formed is added diatomaceous earth after that it was separated using a filter press. The separation of gracilaria pulp from the filter press produces a pure filtrate and solid waste. Basmal et al (2010) reported that the solid waste that was produced still contains micro & macronutrients and growth hormones. The result's analysis of gracilaria solid waste is indicated to contain still growth hormones such as auxins, gibberellins, cytokines (kinetin and zeatin) as seen in figure 4 below. The results of the analysis showed that the gibberellin growth hormone was the highest value among growth hormones which was 509.6 ppm then successively followed by cytokinin-kinetin was 244 ppm, auxins were 191 ppm, and the lowest cytokines-zeatin was 70.5 ppm.
Lijun (2005) found that the range of IAA concentrations in 16 types of seaweed from China was in the range 0.8-110.2 mg/g, with the highest values found in *Polysiphonia urceolata* seaweed, the lowest was found in *Hyalosiphonia caespitose* seaweed. Referring to the statement of Lijun (2005) it is proven that solid waste from *Gracilaria* extraction has higher IAA content than seaweed. IAA works to promote stem cell extension at concentrations of 0.9 g/L above that concentration IAA will inhibit stem cell extension. Synergies between auxin, gibberellins, and cytokines in plant growth are to assist in the process of accelerating the growth of roots, stems, germination, cell division, fruit ripening, reduced seed in the fruit. The existence of a complete growth hormone in solid waste extraction of *Gracilaria* is perfect for use as raw material for plant fertilizers.

### 3.4. Cellulose in solid waste of agar

The main constituent of the *Gracilaria* sp. cell wall consists of cellulose, hemicellulose, and lignin. Cellulose is a linear polysaccharide from a glucose repeating unit that is associated with β-1,4 glycosidic bonds and is the most abundant renewable carbon source and mainly forms the main structural cell components in lower and higher plants. Siddantha et al (2009) mentioned that the highest yields of cellulose (crude) found in *Gelidiella acerosa* (13.65%), *Chamaedoris auriculata* (9.0%) and G. acerosa (3.10%) and also found in the G. acerosa α-cellulose (8.19%). Munifah (2018) reported that in the solid waste of agar extraction contained 2.08% lignin, 28.19% cellulose and 10.63% hemicellulose. Results analysis found that the silicates were 49.18%, holocellulose was 17.7%, alpha-cellulose was 14.26%, hemicellulose was 1.65%, and lignin was 1.12% in solid waste of agar extraction (figure 5). The high level of silicate content in extracted solid waste is caused by adding the diatomaceous soil into *Gracilaria* sp. slurry to separate filtrate agar from solid waste material. Cellulose, hemicellulose, and lignin and silicate in solid waste of agar extraction can be used as raw material for fertilizer but must be decomposed by using microbial decomposers. The difference in cellulose, hemicellulose and lignin content between the literature (Munifah 2017) and the results of the analysis is likely due to the different processing techniques and quality of seaweed used.
3.5. The level of solubility
The purpose of determining the solubility level of solid waste from Gracilaria sp. extraction is to determine its utilization. In the filtration process, there are a few of agar which is carried along in solid waste. Silica, cellulose, hemicellulose, lignin cannot dissolve in water, and only agar can dissolve. The presence of agar in solid waste will undoubtedly be beneficial for further processing because agar can function as adhesives, emulsions, and thickener in the process of mixing with other materials. The results of determining the solubility of solid waste in cold water, heat and 1% NaOH solution was done by adding as much as 1% w/v then stirring until homogeneous. The next process was separated the solid from the liquid. The solids obtained was dried in an oven after that calculating the weight of dry solids. The calculation results found the solubility level of solid waste in the cold water of 11.67%, 23.17% of hot water, and in 1% NaOH solution of 31.32% (figure 6).

4. Conclusion
The results of identification and characterization of solid waste from the extraction of Gracilaria sp. found micro & macronutrients, growth hormones, silicates, alpha-cellulose, hemicellulose, lignin, and some can dissolve in cold, warm water and NaOH solutions. The results showed that solid waste from agar extraction had the micronutrients Cu (4.80 ppm), Fe (0.24 ppm), Zn (8.42 ppm), Mn (57.58 ppm), B (32.32 ppm) as well as the macronutrients N (0.20%), P (0.12%), K (0.17%), C-organic (10.96%), Na (0.66%), Ca (0.61%),
Mg (0.09%), CEC (13.5 me/100g), and N/C ratio of 54.7. Solid waste also contained growth hormones consisting of auxins (191 ppm), gibberellin/GA3 (509.5 ppm), cytokinin-kinetin (244.5 ppm) and cytokinin-zeatin, (70.5 ppm). Furthermore, the solid waste had some residual amounts of holocellulose (17.7%), alpha-cellulose (14.26%), lignin (1.12%), pentose as hemicellulose (1.65%) and silicate (49.18%). The ash content was 60.19% and a slightly acidic pH of 6.3. This agar extraction solid waste was able to be diluted in cold water (11.67%) and hot water (23.17%) as well as in 1% sodium hydroxide solution (35.32%).

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