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Gracilaria sp. Waste, *Lactobacillus* sp. and *Chlorella* sp. Integration on Intensive Aquaculture with Aquaponic System

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Abstract
The aim of the research is to explore the utilization of *Gracilaria* sp. waste, *Lactobacillus* sp. and *Chlorella* sp. in intensive aquaculture with aquaponic system. Interestingly, fermented *Gracilaria* sp. waste liquid as much as 0.5% gives the best watercress *(Ipomoea aquatica)* growth (leaf length, leaf width, stem length, root length, and number of leaves) and nile fish *(Oreochromis niloticus)* growth (specific growth rate and absolute growth in the body length).

Keywords: Gracilaria sp. waste, Lactobacillus sp., Chlorella sp., aquaponic

1. Introduction

Seaweed as an alternative cultivation activity other than fishing has been mostly done by Indonesian fishermen this time, although the products are still in the form of dried seaweed *(raw material)*. Ilknur & Cirik (2004) state that the benefits of seaweed are not only as food. Its utilization as pharmaceutical ingredient and industrial raw materials also need to be explored further. Indonesian dried seaweed production since 2010 which is as much as 800,000 tons / year contribute 50% to the world production, of which 85% is exported. By the importers, the seaweed is then processed into food industrial materials as well as health and cosmetics products. Indonesia has 34 seaweed processing industries where the seaweed is processed into gelatin, alginate and carrageenan. However, the use of seaweed waste as a useful product has not been the focus of attention. Waste generated is usually only allowed to accumulate in the landfill. Although it is harmless, the waste dump could potentially cause pollution problems, especially if the landfill is no longer able to accommodate waste production.

The peculiar feature of seaweed as fertilizer is that it is rich of plant growth regulators (PGR) such as auxin, cytokinins and gibberellin. Not only increasing production, PGR also increases plant resistance to drought and insects. PGR in seaweed are abundant in stem (thallus) such as in *Kappaphycus* sp., *Sargassum* sp. and *Gracilaria* sp. Fertilizer made from seaweed waste has more value in form of macro-nutrients elements such as nitrogen (N), phosphorus (P), potassium (K), and in the form of micro-nutrients elements such as Fe, B, Ca, Cu, Mn and Mg which are expected to meet the needs of the plant.

As a source of nutrition, seaweed has a fairly complete nutrition. Chemically, seaweed is composed of water (27.8%), protein (5.4%), carbohydrate (33.3%), fat (8.6%), crude fiber (3%) and ash (22.25%). In addition to carbohydrate, protein, fat and fiber, seaweed also contains enzymes, nucleic acid, amino acid, vitamins (A, B, C, D, E and K). Amino acid, vitamins and minerals in seaweed reach 10-20 times more than in the land plants. Not only be used for food and pharmaceutical materials, seaweed can also be used as fertilizer or biofertilizer (Lovell 1989) which are useful to spur growth and increase plant production. The use of seaweed as a raw material of organic fertilizer in Indonesia has not been explored yet although the production potential is considerable and Indonesia is rich in seaweed variety which is estimated at 555 species (Anggadireja *et al.* 2006) such as *Kappaphycus* sp., *Sargassum* sp. and *Gracilaria* sp. Preliminary study also reveals that the content of nitrogen in seaweed 1.00%, phosphorus 0.05%, potassium 10.00%, calcium 1.20%, magnesium 0.80%, sulphur 3.70%, cooper 5 ppm, iron 1200 ppm, manganese 12 ppm, zinc 100 ppm, boron 80 ppm, organic compounds 50-55%, and ash content 45-50%.

The combination of the relatively complex nutrients is very possible to be used as bacteria enrichment media and probiotic techniques. Sutejo (2002) states that fertilization for the plants that live in the waters is still rare. Aquatic plants planting media combined with fish and even shrimp intensive cultivation in a single cultivation container is known as aquaponic system. The basic core of the aquaponic system is the optimum water supply for each commodity by utilizing recirculation system. This aquaponic technology system appears as the answer to the problems regarding the difficulty in finding a suitable water source for aquaculture, particularly in limited space (Nugroho & Sutrisno 2008). Through the aquaponic technology synergized in intensive aquaculture and aquatic plants cultivation as well as seaweed waste utilization and bacterial fermentation technology, it is expected to obtain a technology package that is easy to apply, inexpensive, environmentally friendly and have a more optimal added value, both in the growth of fish and aquatic plants cultivated.

2. Materials and Methods

The research conducted is a pilot of fermentation technology in order to produce probiotic enrichment media and

organic liquid fertilizer with reference to the results of the growth rate of fish and aquatic plants, identification of types and amount of probiotic bacteria, N and P uptake and its correlation with water quality parameters and ichtyotoxicity analysis on aquaponic system. This research uses experimental method. Experimental design used is a completely randomized design (CRD) with five treatments and four replications, namely: A: Control, B: Treatment with fermented seaweed harvest waste liquid as much as 0.25%, C: Treatment with fermented seaweed harvest waste liquid as much as 0.5%, D: Treatment with fermented seaweed harvest waste liquid as much as 0.75%; E: Treatment with fermented seaweed harvest waste liquid as much as 1%.

2.1 Preparing Research Equipment

Equipment used in the study are washed with detergent and rinsed with clean water, then washed again with 12 ppm of chlorine, then washed with clean water and dried in the sun. Each container is placed on a shelf where the position is in accordance with the study design.

2.2 Preparing Seaweed

Kappaphycus sp., *Sargassum* sp. and *Gracilaria* sp. used are the waste of seaweed harvest from aquaculture ponds or seaweed manufacturer. Then, the process of making fertilizer is undertaken by cutting the seaweed into small pieces and depressing them in order to take the juice. Then, the seaweed waste liquid is analyzed in the laboratory to determine the concentration levels of N and P.

Determination of N-Total is performed through semi modified micro-Kjeldahl, i.e. by weighing 10 mL of solution and putting it into a 100 mL measuring flask and diluting it with distilled water to the limit. 10 mL of the solution formed is taken and put into a 500 mL Kjeldahl flask and added with 10 ml of H_2SO_4(93-98% N-free). 5 g of a mixture of Na_2SO_4-HgO (20:1) is also added for a catalyst. The sample then is boiled until it is clear and the boiling process is continued for 30 min. After the sample is cool, the process is followed by washing the walls in the Kjeldahl flask using distilled water and boiling it again for 30 min. Once it is cool, 140 mL of distilled water is added as well as 35 mL of a solution of NaOH-$Na_2S_2O_3$ and zinc granules. The process is followed by distillation in which 100 mL of distillate is collected in an Erlenmeyer containing 25 mL of a saturated solution of boric acid and a few drops of blue methylene indicators. In the next step, the solution obtained is titrated with 0.02 HCl and the N total of the sample is calculated. N Total mg / mL = [(mL HCl x N HCl) / mL of sample solution] x 14.008 x f; where f is the dilution factor.

Determination of P is carried out by weighing 2 g of sample and putting it into a beaker and adding 7.5 mL of Mg-nitrate solution. The solution is then heated on top of an electric heater at $180^{\circ}C$ until it becomes thick. Then, the sample is moved into a muffle at $300^{\circ}C$ until no black residue. Furthermore, the sample is chilled and added with 15 mL of concentrated HCl and diluted with distilled water. The sample is then moved into a 250mL measuring flask and diluted to the limit. The next step is taking 100 mL of the sample solution resulted and moving it into a 250 mL beaker. Concentrated NH_4OH is added to the sample until sediment is resulted and then the sample is dissolved again by adding concentrated HNO_3 until the solution turns clear. The process is followed by adding 15 g of ammonium-nitrate to the sample and heating it using a water heater until the temperature reaches $65^{\circ}C$ and adding 70 ml of molybdate solution and settling it for 60 min. The next step is examining the sedimentation by taking 5 mL of supernatant and adding 5 mL of molybdate solution. It is then stirred until no more sediment formed. As the sedimentation is completed, it is necessary to filter out and wash it with distilled water. Furthermore the sediment is resolved using filter paper with the addition of NH_4OH (1:1) and hot water until the filter paper becomes clean. The volume of filtrate and the product of recent washing should not be more than 100 mL. The filtrate and the product of recent washing with concentrated HCl are then neutralized and it is then settled and added with 15 mL of magnesia mixture from the burette with a speed of 1 drop per second while shaking it. After being settled for 15 min, 12 mL of concentrated NH_4OH is added and then it is settled for 2 h. Supernatant is then poured through ash-free filter paper and the sediment is washed in the beaker with dilute ammonia until it is chloride free. The next step is drying the sedimentation and filter paper in a crucible which has been turned on to obtain a white residue. It is then chilled in exycator. The residue is then weighed as $Mg_2P_2O_7$. Weight of P_2O_5 is calculated by the formula of the weight of P_2O_5 (g in 100 mL of solution) = 0.6377 x weight of $Mg_2P_2O_7$ (g).

2.3 Fermented Seaweed Waste Technology

Fluid resulted from the process of preparing seaweed is fermented using culture dominated by fermentation bacteria *Lactobacillus* sp. Fermentation is the conversion of organic matter into another form with the help of microbe (Judoamijoyo 2003). Microbe performs fermentation by converting complex organic materials into simpler organic materials. Based on the results of the preliminary test, the composition of bacteria is *Lactobacillus* 8.7 $x10^5$ CFU/mL. This process begins by performing the waste of *Sargassum* sp., *Kappaphycus* sp. and *Gracilaria* sp. in amount of 500 g each with a solution of fermentation bacteria *Lactobacillus* sp. as much as 5 mL and then stirred evenly. The liquid is then put into a sealed container and fermented for 1 week. This is in accordance with the opinion of Inckle *et al.* (2005) who explains that the fermentation period is between one to two weeks. Fermentation period is adjusted to the desired treatment. Laboratory analysis is

performed after the fermentation process to determine levels of N and P.

2.4 Aquaponic System Planting Media

Planting media used in this study is combined with intensive aquaculture with aquaponic system (Thimen *et al.* 2005; Kohar *et al.* 2004). Fertilizer made from seaweed liquid waste resulted from the fermentation is put in each reservoir (0% concentration or control; 0.25%; 0.5%; 0.75% and 1%).

2.5 Nile Fish (Oreochromis niloticus) Biomass Weight Calculation

Biomass is the total weight of a population at a certain period and is expressed in weight unit (Gideon *et al.* 2007). Nile fish *(Oreochromis niloticus)* is measured regarding its body length and body weight and then placed in an aquarium with a density of intensive nile fish aquaculture. The fish is acclimatized and not fed for two days before given treatment. The feed to be used in this study is commercial feed. The amount of feed for each treatment is as much as 3% of the average body weight of fish weighed using analytical balance (SNI 01-6141-1999).

2.6 Preparing Aquatic Plants

The method applied refers to recommendation of Prasad *et al.* (2008) with modifications. Aquatic plants used are local and Ampenan from Lombok (West Nusa Tenggara) watercress with 25 cm stem length and 1 cm shoots which then placed in a container that had been prepared. Next, the reservoir containing fermentation product will flow according to the force of gravity towards the aquarium below. The water flow is then pumped towards the reservoir. Recirculation of the water flow will rotate until the study ends. At the end of the study, the length and width of leaves, stems, roots, number of leaves and the body length and body weight of the fish as well as the changes that occur are measured.

2.7 Fish Survival Rate

Survival rate is the percentage of fish that live in a cultivation period (Suyanto 2002). Effendie (1997) states that fish survival rate can be calculated by the formula:

$$SR = Nt / No \times 100\%$$

Description: SR: survival rate (%); Nt: Number of fish at the end of the test; No.: Number of fish at the beginning of the test

2.8 Nile Fish's Body Weight and Absolute Length Growth

Measuring growth including body weight growth (W) and total length growth (TL) is performed once every 7 days from the beginning to the end of the study. According to Anik (1989), the specific weight growth is the calculation of the difference of final weight and the initial weight divided by period of cultivation multiplied by 100%. Fish daily weight growth can be calculated using the following formula:

Specific Growth Rate (SGR)

$$SGR = \frac{(\ln Wt - \ln Wo) \times 100\%}{t}$$

Description: Wt: Average weight at certain time -t (g); Wo: Average weight at the beginning (t_0) (g), t: Period of cultivation (days)

2.9 Nile Fish Absolute Length Growth

According to Anik (1989), absolute length growth is the difference of nile fish body length at the beginning and the end of the cultivation. Fish absolute length growth can be calculated using the following formula:

$$Lm = TL_1 - TLo$$

Description: TL_1: total length at the end of cultivation (cm), TL_0: total length at the beginning of cultivation (cm); Lm: absolute length growth (cm)

2.10 Aquatic Plant Growth

Growth is associated with changes in number, size or cells level dimension, organ as well as individual that can be measured by the measurement of weight and length (Hidayat 1985). Leaf length is measured weekly by inverting leaf then measuring its length from the media top surface to the longest leaf using a ruler. Leaf width is measured weekly using a ruler to measure from the media top surface to the bottom surface. The stem length is measured weekly using a ruler to measure from the media top surface to the bottom surface. Stem length growth can be calculated using the following formula: stem absolute growth = final stem length–initial stem length. Root length is measured weekly using a ruler to measure from the media top surface to the bottom surface. Root length growth can be calculated using the following formula: Root absolute growth = final root length–initial root length.

2.11 Identifying the Type and Number of Probiotic Bacteria

Probiotic bacterial isolates in culture on gelatin GYP (Glucose-Yeast extract-Peptone) isolation media with the composition of 10 g of glucose, 10 g of yeast extract, 5 g of peptone, 2 g of beef extract, 1.4 g of Na-asetat.$3H_2O$, 5 mL of salt solution (0.1 g of $MgSO_4.7H_2O$, 0.1 g of $MNSO_4.4H_2O$, 0.1 g of $FeSO_4.7H_2O$; 0.1 g of NaCl; 50 mL of distilled water), 0.5 g of tween 80, 20 g of gelatin, 1 L of water. Once it is sterilized, 0.75 mg of $CaCO_3$/mL of media is added. The selection of *Lactobacillus* sp. is performed by analyzing its amount

committed towards isolates with the excellence character of tolerance to low pH. Tolerance to low pH testing is performed by replacing the isolates growth resulted from the enrichment of seaweed waste fermentation media with a broth MRS media whose pH is set to reach 2.5. Media turbidity is measured using a spectrophotometer at a wavelength of 600 nm, after the incubation for 0, 24, 24 and 72 h.

2.12 Ichtyotoxicity

10 ml of fermented seaweed waste is centrifuged at 1000 x g for 15 min. Furthermore, supernatant is concentrated using rotary evaporation. Bioassay for extract toxicity detection is carried out using nile fish *(Oreochromis niloticus)* larvae. Bioassay is performed in each 200 mL culture container with the addition of 1 mL of extract. Observation is performed for 24 h.

2.13 Water Quality

Supporting parameters in this study is the measurement of water quality parameters such as pH, performed weekly using pH universal indicator paper, whereas the temperature parameter is measured daily using a thermometer. Identification and plankton population calculation are performed based on observations using microscope with *sedgewich rafter* (50x20x1 mm^3) using the following formula:

$$N = \frac{1000}{3{,}14\,(d/2)^2} \times n$$

Description: N: plankton density (cells/mL), d: diameter of view surface (mm), n: the average number of plankton per view surface (units /mL).

Data analysis using Varian Analysis (ANOVA) with study design is Complete Randomized Design (CRD) to determine the differences in treatment. If there is any difference in treatment, Duncan range test is carried out with the confidence level of 0.05 to determine the differences among all treatments (Kusriningrum 2008).

3. Results and Discussion

Fermented *Gracilaria* sp., *Kappaphycus* sp. and *Sargassum* sp. waste using fermentation bacteria *Lactobacillus* sp. dominated culture shows different results. *Lactobacillus* sp. microbe performs fermentation process by converting complex organic matters into simpler molecules. It indicates that *Gracilaria* sp. waste is capable of supporting the growth of *Lactobacillus* sp. better than the use of *Lactobacillus* sp. bacterial growth media with *Kappaphycus* sp. and *Sargassum* sp. Waste (Table 1).

It is evidence that the inoculation of *Lactobacillus* sp. bacteria in *Gracilaria* sp., *Kappaphycus* sp. and *Sargassum* sp. waste fermentation media have the same trend where day-5 shows the highest growth of the amount of *Lactobacillus* sp. bacteria. So is the measurement of nitrogen and phosphorus levels in fermentation media, day-5 shows that the fermentation media with *Gracilaria* sp. waste is better than the levels of nitrogen and phosphorus in *Kappaphycus* sp. and *Sargassum* sp. waste fermentation media.

High levels of nitrogen and phosphorus resulted from *Gracilaria* sp. waste fermentation media is expected to provide the best results in probiotic enrichment and biofertilizer products in intensive aquaculture with aquaponic system (Table 2). Although the levels of nitrogen and phosphorus resulted from *Gracilaria* sp. waste fermentation media is not significantly different from the levels of nitrogen and phosphorus resulted from *Kappaphycus* sp. and *Sargassum* sp. waste fermentation media, with the highest levels of nitrogen and phosphorus resulted from *Gracilaria* sp. waste fermentation media amounted to 0.3579% and 0.005%, the study focuses on exploring the results of *Gracilaria* sp. waste fermentation as probiotic enrichment and biofertilizer products in intensive aquaculture with aquaponic system.

Aquaponic systems tested in this study are the Nutrient Film Technique (NFT) and Floating Raft (FR) systems. In general, the NFT system needs water to flow continuously to the plants/vegetables cultivation media so that plants/vegetables continuously acquire nutrients. The use of the NFT system is expected to make plants/vegetables able to grow rapidly, as well as aquaculture which is synergized in the aquaponic system. However, the weakness of the NFT system is that the decay in the roots of plants/vegetables is inevitable if the water flow is not going well. In aquaponic system with FR model, plants/vegetables are placed on the media container/pot floated on aquaponic cultivation system, otherwise the fish metabolic residue products are usually difficult to be absorbed by the plants well and the possibility of damage plant roots as they were eaten by the fish. In this study, the improvement of the weakness of the NFT system as well as FR system is carried out in intensive aquaculture with aquaponic system (Figure 1). In NFT system, in order to avoid the occurrence of root decay process, the pump system to deliver water is equipped with automatic system capable of regulating the flow of excess water, while the weakness in the FR system is fixed by making multilevel aquaponic cultivation models in hopes of fish metabolic waste absorption occurs gradually and continuously as well as allows the fish not to eat the roots of plants / vegetables.

The test results of aquaponic cultivation system either with improved NFT model or improved FR model show that the occurrence of the weakness arising from each model can be avoided, such as no vegetables roots are decayed and no damage vegetables roots due to being eaten by fish. There are no significant differences between

aquaponic cultivation with NFT and FR model. The results for the leaf length growth of both local and Ampenan (Lombok, West Nusa Tenggara) watercress plants with different dose are obtained from the calculation of the difference in leaf length of watercress plants at the beginning and the end of the study (Figure 2).

Based on the ANOVA results, the average growth of watercress leaf length results in $p < 0.05$, which means that different dose leads to a real difference in the average growth rate of watercress leaf length. Duncan's multiple range test results show that treatment C, D and E are significantly different from treatment A and B, while treatment in E gives the best result although it is not significantly different from treatments C and D (Table 3). The average of leaf width of local and Ampenan watercress results in similar trends where treatment E gives the best result but it is not significantly different from treatment C and D (Table 4).

Based on the ANOVA results, the average growth of watercress leaf width results in $p < 0.05$, which means that different dose leads to a real difference in the average growth rate of watercress leaf length. Duncan's multiple range test results of local watercress show that treatment E is significantly different from treatment A and B, yet it is similar to treatments C and D, as for Ampenan watercress, treatment E is only significantly different from treatment A but it is slightly different from treatments B, C and D.

The average growth of stem length of local watercress as well as Ampenan watercress also result in $p < 0.05$ allowing a significant difference in the growth of stem length of local watercress as well as Ampenan watercress. Table 5 shows that treatment E gives the best result although it is not significantly different from treatments C and D. The average growth of root length of watercress with different doses is obtained from the calculation of the difference in roots length of watercress at the beginning and the end of the study. The highest average growth of roots length of water watercress is in treatment E (8.43 cm) followed by treatment D (8.25 cm), treatment C (7.98 cm), treatment B (7.63 cm) and the lowest average growth of roots length is in treatment A (7.41 cm). The average growth of roots length of Ampenan watercress with different doses during the study also shows a similar results trend (Table 6) with the best result in treatment E (11.53 cm) followed by treatment D (10.14 cm), treatment C (9.99 cm), treatment B (9.41 cm) and treatment A (6.91 cm). The average increase of the numbers of leaves of local watercress as well as Ampenan watercress results in $p<0.05$ allowing a significant difference in the average increase of the numbers of leaves of both local and Ampenan watercresss. Table 7 shows that treatment of E gives the best result although it is not significantly different from treatments C and D.

The main test parameters of this study in addition to measuring the increase of watercress growth (both local and Ampenan watercress) also measure fish growth and nile survival rate through ichtyotoxicity test. Fish growth includes the growth of the body weight (W) and the growth in total length (TL). The result of the nile fish body weight growth with different doses distribution are derived from the calculation of the difference in weight at the end and at the beginning of the study divided by the period of cultivation multiplied by 100%. The highest specific weight growth rate of nile fish is in treatment E (1.71%) followed by treatments D, C, B and A, while the cultivation treatment without aquaponic system only has a specific weight growth rate 0.69% (Table 8). It means that there is a 2 times increase in the specific growth rate of the nile fish cultivation with aquaponic.

The highest absolute length growth of nile fish is in treatment E (1.43 cm) followed by treatments D, C, B and A, while the cultivation treatment without aquaponic system only reaches 0.39 cm (Table 9). This indicates that cultivation treatment with aquaponic system gives better results compared to treatment without aquaponic system because it is able to demonstrate aquaculture biointegration with fish and plants / vegetables productions at the same time. In aquaponic system, specific growth rate and absolute length of nile fish and vegetable growing optimally demonstrate the improvement of the mechanism of integrated cultivation compared to conventional cultivation system. Nile fish survival at the end of the study after being given different treatments shows 100% survival rate so that it can be assured that by a dose of 1% of the distribution of fermented *Gracilaria* sp. waste liquid is not pararel with the aquaponic cultivation system. It is also supported by the significant result of the ichtyotoxicity test where bioassay for the detection of extract toxicity carried outwith the larvae of nile fish (*O. niloticus*) shows 100% survival rate in 24 h observation. Parameters results of the average of water quality during this study indicate that the range of values of water quality remained within the range of optimal growth. Likewise, the identification of plankton during the study shows that the planktons identified are dominated by Chlorophyceae (*Chlorella* sp., *Volvox* sp., *Scenedesmus* sp., and *Chlamydomonas* sp.). The dominance of *Chlorella* sp. during the study is indicated by the average density per day as much as 2.2×10^5 cells/mL, followed by Volvox sp. (1.9×10^5 cells/mL), *Scenedesmus* sp. (1.6×10^5 cells/mL) and *Chlamydomonas* sp. (1.6×10^5 cells/mL).

In general, the increase of weight and fish dispersive density during the cultivation process will increase the value of FCR (*Feed Conversion Ratio*), levels of NH_3 and NO_2 and decrease the O_2 level. On the other hand, the lack of O_2 can inhibit growth and lower fish immune so that cultivation system often fail or does not achieve optimal cultivation. Factors affecting the fish growth are classified into two, internal factors and external factors. Internal factors that affect growth are offspring, sex, age and disease, while the external factors that affect growth are feed and water quality. Growth occurs if there is excess energy after the need for metabolism and

movement is fulfilled. Energy is obtained from chemical bonding conversion through a process of oxidation reaction against feed components, i.e. protein, fat and carbohydrate. During the metabolic process, these three components are converted into simpler compounds (amino acid, fatty acid and glucose) so that they can be absorbed by the body to be used or stored.

Aquaponic system is a form of synergy or bio integration that correlates aquaculture patterns using the principle of recirculation with hydroponic plants/vegetables production (Diver 2006). Aquaponic technology is also an alternative of aquaculture with hydroponic plants/vegetables using the principle of time and space saving cultivation and utilization of nutrients from feed and fish metabolism residue. Plants also serve as a filter of vegetation that decomposes the toxins into harmless substances to fish and increased supply of oxygen in the water is used to conserve the fish. This cycle will lead to mutual beneficial cycle (Pramono 2011). Ahmad *et al.* (2007) also states that aquaponic technology is proven as capable of successfully producing fish optimally with a limited space and water resources. In this case, aquaponic technology designed is attempted as an alternative to coastal community empowerment considering space and water use efficiency, the combination of aquaculture and plants/vegetables cultivation systems, easy, inexpensive and environmentally friendly aquaculture applications because it uses seaweed waste which is abundant and scattered in coastal areas.

The increase of the growth of leaf length, leaf width, stem length, root length, and number of leaves of watercress as well as specific weight growth rate and absolute length growth of nile fish with the distribution of a dose of probiotic enriched with *Gracilaria* sp. waste show that treatment E gives the best results although it is not significantly different from treatments C and D. This suggests that the optimal dose for the application of probiotic enriched with *Gracilaria* sp. waste in nile fish intensive aquaculture system with aquaponic model is treatment with the distribution of fermented *Gracilaria* sp. waste liquid as much as 0.5%. It means that the minimum dose of fermented *Gracilaria* sp. waste liquid as much as 0.5% can improve the growth of fish and plants/vegetables optimally. Choi *et al.* (2012) even suggests that natural antibacterial substances produced from seaweed have selective antibacterial effect by dismissing the activity of pathogenic bacteria so that beneficial bacteria easily breed. Seaweed has a long-chain polysaccharide that serves as a prebiotic (as probiotic nutrients). Rees (1969) and Murano (1995) state that *Gracilaria* sp. has a polysaccharide structure with its main structure 1,4-linked-3,6-anhydro-α-L-galactopyranose alternating with 1,3-linked-β-D-galactopyranose.The interesting thing of this study can also be seen from the cultivation treatment without aquaponic system which shows very different results compared to cultivation system with aquaponic model. Specific growth rate and the absolute length of nile fish in aquaculture without aquaponic system are only 0.69% and 0.39%, while in aquaculture with aquaponic system can reach more than 2-3 times. The success of the aquaponic system suggests that aquatic plants are able to function as biofilter, where toxin contained produced from the remaining feed and fish feces in the form of NH_3 can be neutralized by aquatic plants and reduced through recirculation system. Nugroho & Sutrisno (2008) states that the existence of the plants as biofilter can reduce the toxicity of NH_3 so that the water is still proper to be used as a medium to cultivate fish. Recirculation system used consists of compartment cultivation and water treatment allowing decantation, filtration, oxygenation and sterilization processes. Pramono (2011) even suggests that the use of aquatic plants as biofilter is capable of being the substrate of beneficial bacteria able to overcome and manage excess nitrogen compound which is harmful to fish in aquaponic system.

Wijaya (2008) also states that the nitrogen in the plants encourages the growth of organs associated with photosynthesis, the leaf. Plant with enough nitrogen supply will grow leaves with wider blade with higher chlorophyll content, so that the plant is able to produce carbohydrate or assimilate in sufficient quantities to support vegetative growth. On the other hand, plant with nitrogen deficiency will grow smaller leaves, pale green young leaves due to lack of chlorophyll and old leaves experience chlorosis followed by necrosis and then fall. However, if nitrogen supply is excessive, plant tends to synthesize organic nitrogen compounds. Plant leaves tend to be floppy so that they decrease the efficiency of capturing sunlight and there is the possibility of the occurrence of necrosis at leaf margins as a result of NH_3 poisoning formed from nitrate reduction process. In addition, nitrogen is an essential component for the growth of the plant stem (Anggadireja *et al.* 2006). There are two forms of nitrogen absorbed by plant roots, the nitrate ion (NO_3^-) and ammonium ion (NH_4^+). Nitrogen absorbed in the form of nitrate ion will partially be stored directly in the cell vacuoles of root cells and organs cells on the ground. The rest will be reduced into nitrite (NO_2) by the reductase nitrate enzyme, subsequently the nitrite is reduced by reductase nitrite enzyme into NH_3. Furthermore, the ammonia formed reacts with H_2O to form NH_4 which is then assimilated into amide ($-(NH_2)_2$), and amine ($-NH_2$). Amide and amine are transported to the top of the plant and forming protein and amino acid. Ammonium assimilation in the root cells need carbohydrate in large quantities to prepare the carbon chain in the synthesis of amide and amine. Aquatic plants also require phosphorus for its growth. It is as expressed by Lingga & Marsono (2007), that phosphorus is an essential component to stimulate plant growth, accelerate and strengthen the growth of young plants to mature plants. This relates to the role of phosphorus as a nutrient source which is biodegradable and easily absorbed by

plants for the growth (Meyer *et al.* 2013). Phosphorus absorption by plant is carried out actively and controlled by the respiratory metabolism of carbohydrate. Plants with many roots have a chance to absorb phosphorus faster. Phosphorus is a limiting nutrient factor for plant growth. Root growth rate of Ampenan watercress also determines the speed of stem and leaf growth. Test data of nitrogen and phosphorus from fermented *Gracilaria* sp. waste show the best results of nitrogen as much as 0.3579% and phosphorus as much as 0.005% and thus the availability of nitrogen and phosphorus are able to stimulate the growth of cultivated plants. Genetically, local watercress is smaller and its growth is slower and smaller in size compared to Ampenan (Lombok, West Nusa Tenggara) watercress. Figure 2 shows the different growth of local watercress and Ampenan watercress, however treatments C, D and E given significantly show the best results compared to the control treatment.

The correlation of bioassay results for the toxicity detection of fermented *Gracilaria* sp. waste liquid extract using nile fish larvae *(O. niloticus)* shows 100% survival rate in accordance with the increase in the specific growth rate and the absolute length growth of nile fish. The active substance contained due to the distribution of the extract of fermented *Gracilaria* sp. waste liquid is not harm or interfering fish metabolism. Even Chojnacka *et al.* (2002) states that the active substance of seaweed extract in the form of polysaccharide, protein, polyunsaturated fatty acid, pigment, polyphenol, mineral and plant growth hormones, in which polysaccharide substance has the ability of prebiotic, growth and health-improving activity.

Utilization of *Lactobacillus* sp. as probiotic bacteria developed using fermented *Gracilaria* sp. waste liquid is able to make intensive aquaculture system with aquaponic system superior, in terms of the increase of cultivated fish and plants/vegetables growth. Verschuere *et al.* (2000) suggests that the probiotic bacteria are living microbes that have a beneficial effect on the host through the increase of feed use and nutrients value, host immune response to disease as well as environmental quality improvement. Irianto & Austin (2002) and Walker (2008) even suggest the three models of how probiotic works, namely suppressing bacterial populations through competition by producing antimicrobial compounds or through nutrient competition and attachment area in the intestinal wall, altering bacterial metabolism by increasing or decreasing the activity of the enzyme and stimulating immunity through increased antibody or macrophages activity. In its application stage, the probiotic bacteria perform life competition through nutrient competition and adhesion or attachment to the gastrointestinal tract thereby blocking multification, infection and invasion of pathogenic microbes. The next stage is the neutralization of the toxin and the production of antibacterial substances (e.g. propionic, butyric and acetic acid). The pH of the digestive tract environment is lowered so that pathogenic microbes cannot survive optimally and thus the resistance of pathogenic microbes becomes marginal and critical in the competition and the host immune-system stimulation. Pirarat *et al.* (2006) also suggests that the probiotic bacteria *Lactobacillus* is able to reduce the mortality rate of *O. niloticus* due to *Edwardsiella tarda* infection, where the bacteria boost the fish immune system into a better one through aggregation of phagocytic cells, increased phagocytic activity, further protecting the fish from *acute septicemic death* caused by *E.tarda* infection. Mortazavian *et al.* (2007) even states that *Lactobacillus* and *Bifidobacteria* are probiotic bacteria which are very important and widely used as a bacterial agent of probiotic products.

In aquaponic system, the nitrifying bacteria transforming toxic potential of ammonia compounds, especially the one which is not ionized into nitrate through the process of nitrification also synergize. The process requires oxygen. It lowers the acidity and affects alkalinity value. This is shown in the aquaponic treatment in which the recorded pH is about 8 to allow the nitrification process runs well. Nitrification is a natural process that returns to a normal condition carried out by bacteria by oxidizing and transforming ammonia compounds potentially toxic into non-toxic nitrate compounds. Woon (2007) reports on the nitrification process which is carried out in two stages, first is by bacteria of the genus *Nitrosomonas* which oxidize ammonia or ammonium into nitrite and the second is by bacteria of the genus *Nitrobacter* which oxidize nitrite into nitrate. Nitrification uses a small amount of energy that is released as ammonia is oxidized into nitrate and reduces inorganic carbon into CO_2 into organic carbon. Among the group of organisms, nitrification is known as *chemoautotrophic* bacteria that can oxidize organic matter with non-photosynthetic processes (Saidu 2009). It is clarified by the range of levels of NH_3 and NO_2which are <1 ppm so that nitrification process in the aquaponic system is believed to work well.

Symbiotic relation between bacteria and plankton also takes place, in which bacteria serving as organic compounds decomposer are able to support plankton to grow and proliferate. Inorganic substances such as nitrogen and phosphorus resulted from bacterial decomposition become a source of nutrients for plankton. Heinanen (1991) states that bacteria and plankton are always related, as bacteria decompose organic compounds into nutrients that will be utilized by the plankton and plankton provides organic matters for bacterial growth. Feed residue and fish feces will also be utilized by plankton. In the process of growth, plankton requires O_2 to be able to decompose the feed residue and nitrogen into useful compounds. This study shows that the aquaponic system with its recirculation is able to maintain DO content to be in the normal range (3-5 ppm). *Chlorella* sp. dominance in the water during the study brings a positive impact on fish, aquatic plants and probiotic content circulating in the aquaponic cultivation media. Beheshtipour *et al.* (2013) even states that *Chlorella* sp. and

Spirulina sp. are able to improve the viability of probiotic thereby increasing the functional character of probiotic bacteria because these microalgae contain nutrients and complete *nutraceutical* materials as functional food. Tokusoglu & Unal (2003) suggest that Chlorella consists of polyunsaturated fatty acids (PUFAs) (38.94%) and 13.32% of total lipids. Palmitic acid in Chlorella is as much as 15.41%, EPA (20:5 n-3) as much as 3.23% and docosapentaenoic acid as much as 3.11%. The docosahexaenoic acid (C22: 6 n-3) contained in Chlorella is very high up to 20.94%. Chlorella is also rich in P, Na, K, Ca, Mg and Fe minerals. The *Chlorella* sp. dominance in this study also shows mutual symbiosis that can improve optimal growth of cultivated fish and plants. The technology engineering needs to be explored further by uncovering the mechanisms in terms of biochemistry and molecular biology studies so as to encourage the use of *Gracilaria* sp. waste as a probiotic enrichment and biofertilizer products in intensive aquaculture with aquaponic system.

4. Conclusion

Proper cultivation model using seaweed waste engineering technology as probiotic enrichment and biofertilizer products in intensive aquaculture with aquaponic system can be obtained by using fermented *Gracilaria* sp. waste liquid and probiotic bacteria *Lactobacillus* sp. and the dominance of plankton *Chlorella* sp. in the water of cultivation, either with NFT or FR system. Optimal dose of probiotic enrichment and biofertilizer products from seaweed waste in intensive aquaculture with aquaponic system is treatment with the distribution of fermented *Gracilaria* sp. waste liquid as much as 0.5% with the probiotic bacteria *Lactobacillus* sp.

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Table 1. The amount of *Lactobacillus* sp. bacteria on *Gracilaria* sp., *Kappaphycus* sp. and *Sargassum* sp. waste fermentation media

Amount of *Lactobacillus* sp. (CFU/mL)	**Day-**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Fermentation with *Gracilaria* sp. media	0.5×10^4	1.7×10^4	2.3×10^4	8.7×10^4	3.7×10^5	3.1×10^5	5.7×10^4
Fermentation with *Kappaphycus* sp. media	0.4×10^4	0.9×10^4	1.6×10^4	6.1×10^4	2.7×10^5	2.9×10^4	1.5×10^4
Fermentation with *Sargassum* sp. media	0.1×10^4	0.8×10^4	1.1×10^4	4.1×10^4	0.9×10^5	1.7×10^4	0.5×10^4

Table 2. Results of nitrogen and phosphorous levels test on day-5 in *Gracilaria* sp., *Kappaphycus* sp. and *Sargassum* sp. waste fermentation media

Seaweed Waste Fermentation Media (Day-5)	**Nitrogen Level (%)**	**Phosphorus Level (%)**
Fermentation with *Gracilaria* sp. waste	0.3579	0.005
Fermentation with *Kappaphycus* sp. waste	0.2336	0.003
Fermentation with *Sargassum* sp. waste	0.2173	0.003

Table 3.The data of average leaf length growth of watercress with the distribution of a dose of probiotic enriched with *Gracilaria* sp. waste

Treatment	Leaf Length of Local Watercress ± SD (cm)	Leaf Length of AmpenanWatercress± SD (cm)
A	$2.30^{b} \pm 0.15$	$5.48^{b} \pm 0.52$
B	$2.49^{b} \pm 0.43$	$5.72^{b} \pm 0.69$
C	$2.92^{a} \pm 0.66$	$7.79^{a} \pm 0.61$
D	$3.04^{a} \pm 0.57$	$7.81^{a} \pm 0.53$
E	$3.05^{a} \pm 0.61$	$8.02^{a} \pm 0.25$

Table 4.The data of average leaf width growth of watercress with the distribution of a dose of probiotic enriched with *Gracilaria* sp. waste

Treatment	Local Watercress Leaf Width ± SD (cm)	Ampenan Watercress Leaf Width ± SD (cm)
A	$1.59^{b} \pm 0.27$	$3.24^{b} \pm 0.35$
B	$1.56^{b} \pm 0.28$	$3.49^{ab} \pm 0.23$
C	$1.78^{a} \pm 0.35$	$3.59^{ab} \pm 0.24$
D	$1.80^{a} \pm 0.40$	$3.74^{a} \pm 0.19$
E	$1.69^{a} \pm 0.25$	$3.82^{a} \pm 0.61$

Table 5.The data of average stem length growth of watercress with the distribution of a dose of probiotic enriched with *Gracilaria* sp. waste

Treatment	Stem Length of Local Watercress ± SD (cm)	Stem Length of Ampenan Watercress ± SD (cm)
A	$3.59^{b} \pm 0.40$	$8.08^{c} \pm 1.59$
B	$3.91^{b} \pm 0.65$	$9.17^{bc} \pm 2.33$
C	$4.24^{ab} \pm 0.45$	$11.08^{abc} \pm 4.67$
D	$4.34^{ab} \pm 0.76$	$12.27^{ab} \pm 1.78$
E	$4.93^{a} \pm 0.17$	$13.83^{a} \pm 4.09$

Table 6.The data of average of roots length of watercress with the distribution of a dose of probiotic enriched with *Gracilaria* sp. waste

Treatment	Roots length of Local Watercress ± SD (cm)	Roots length of Ampenan Watercress ± SD (cm)
A	$7.41^{b} \pm 0.90$	$6.99^{c} \pm 1.19$
B	$7.63^{b} \pm 0.62$	$9.44^{b} \pm 0.59$
C	$7.98^{ab} \pm 0.60$	$9.94^{ab} \pm 0.95$
D	$8.25^{a} \pm 0.75$	$10.19^{ab} \pm 1.21$
E	$8.43^{a} \pm 1.07$	$12.34^{a} \pm 1.34$

Table 7. The data of the the average of increase numbers of leaves of watercress with the distribution of a dose of probiotic enriched with *Gracilaria* sp. waste

Treatment	Increase of the numbers of leaves of Local Watercress ± SD (cm)	Increase of the numbers of leaves of Ampenan Watercress ± SD (cm)
A	$5.32^{b} \pm 0.10$	$8.97^{c} \pm 1.01$
B	$5.69^{b} \pm 0.51$	$9.73^{c} \pm 0.17$
C	$7.93^{ab} \pm 0.37$	$14.98^{ab} \pm 1.15$
D	$11.84^{a} \pm 0.45$	$16.58^{a} \pm 1.17$
E	$12.53^{a} \pm 0.91$	$17.01^{a} \pm 1.41$

Table 8. Specific weight growth rate of nile fish with the distribution of a dose of probiotic enriched with *Gracilaria* sp. waste

Treatment	The Specific Weight Growth Rate of Nile Fish (%)
A	0.88
B	1.01
C	1.22
D	1.42
E	1.71
N	0.69

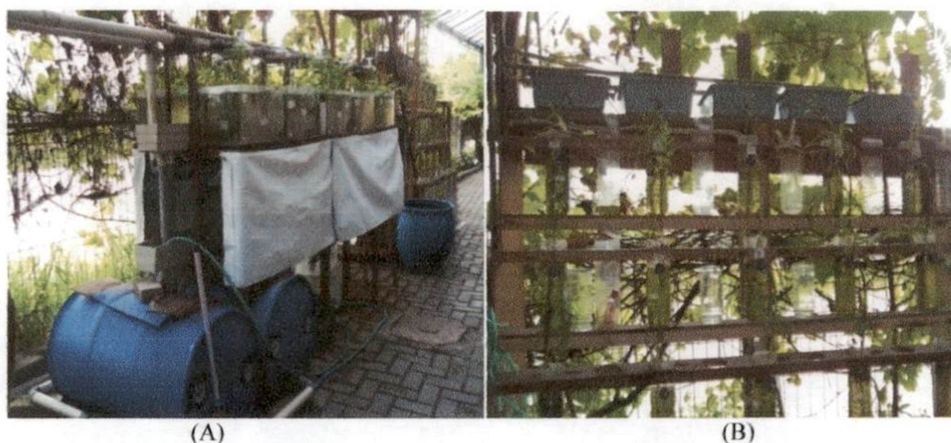
Description: N = cultivation treatment without aquaponic system

Table 9. Absolute length growth of nile fish with the distribution of a dose of probiotic enriched with *Gracilaria* sp. waste

Treatment	Absolute Length Growth of Nile Fish (cm)
A	0.68
B	0.77
C	1.09
D	1.12
E	1.43
N	0.39

Table 10. Water quality of aquaponic intensive cultivation system

Water Quality Parameters	Range
Temperature (°C)	28-30
pH	7-8
DO (ppm)	3-5
Salinity (‰)	0
NH_3 (ppm)	<1
NO_2 (ppm)	<1
NO_3 (ppm)	1-2



(A) (B)

Figure 1. Aquaponic cultivation system with Nutrient Film Technique Model (A) and Floating Raft System (B)

Local Watercress Ampenan Watercress

Figure 2. Local and Ampenan watercress growth. The scale indicates 10 cm.

Description: A = control; B = treatment with the distribution of fermented *Gracilaria* sp. waste liquid 0.25%; C = treatment with the distribution of fermented *Gracilaria* sp. waste liquid 0.5%; D = treatment with the distribution of fermented *Gracilaria* sp. waste liquid 0.75%; E = treatment with the distribution of fermented *Gracilaria* sp. waste liquid 1%; SD = standard deviation; Different superscripts in the same column indicate differences ($p < 0.05$).

(Units / mL)

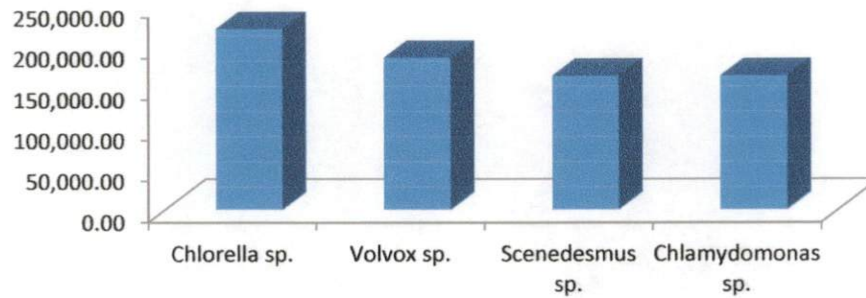


Figure 3. Average numbers of daily planktons domination

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