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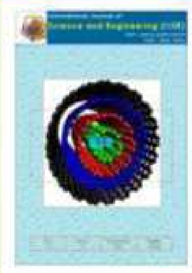

TREATMENTS OF PLASMA CORONA RADIATION ON SEAWEED *Gracilaria Verrucosa* (HUDSON) PAPENFUSS: Efforts to increase growth and biomass

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Abstract - *Gracilaria verrucosa (Hudson) Papenfuss has great potential to be farmed in the water resources in Indonesia. As natural resource, the weed has a major contribution in the field of industry both for human food and health. Efforts have been done intensively to increase the production capacity to meet the market demand especially gelatin, both national and international market. One of them is the application of plasma corona irradiation treatments on the weed to improve developmental pathways. The concept of plasma irradiation performed at atmospheric conditions may impact on nitrogen intrusion pathway that is important element in the growth of the weed. The aims of this study are to assess the potential impact of plasma irradiation in improving the growth of G. verrucosa and thus increase their biomass production. The treatments were done using five different duration of plasma irradiation, which were 2, 4, 6, 8, and 10 minutes at a 0,5mA stable source of voltage and 8kV of electrical current. Observations of growth rate include thallus length and biomass of G. verrucosa , that was observed every week for 28 days. The result showed that the growth of weed exhibited better than those without radiation. The best growth was reached in the group of treatment of 8 minutes irradiation, exhibited 65,91g of biomass and 9.5515% growth rate and length of thallus reached 22,33 cm and daily growth rate of 2.9759%. The lowest growth of the weed occurred in the treatment of 10 minutes irradiation, which was 44,82 g biomass, 8.123% growth rate, 17,13 cm thallus length with a daily growth rate of 1.9942%.*

Key words: G. verrucosa; plasma; growth; biomass; thallus length

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Introduction

Development of *G. verrucosa* cultivation in Indonesia provides a great opportunity and thus advantage because of the demand of gelatin has increased time to time at global market. In the development of seaweed cultivation, basic ecological factors associated with the growth and life needs to be known. *G. verrucosa* is a type of seaweed which has a fairly broad tolerance toward abiotic factors, can live in relatively slow water current on a muddy substrate, salinity range between 5- 43% and a pH range between 6-9 (Hoyle, 1975).

Plasma is a gas filled condition when a charged particle with the potential energy between the particles is smaller than the kinetic energy of the particles contained in the gas. One way of plasma generation is done through electrical discharges. Plasma is formed in the electrical discharge known as corona plasma radiation. Incandescent plasma corona discharge that occurs in the condition of atmospheric air spaces make nitrogen in the form of N_2 (dinitrogen) forced into the direct current to infiltrated into an irradiated medium (Azam *et al*, 2007).

Plasma technology is used as a generator of N^+ ions from the air. The amount of nitrogen composition in free air, up to 80%, causing the plasma radiation to the free air potentially large yield N^+ ions. Further infiltration of nitrogen ions into a material will change the microstructure of the material, so that the properties of the physical and chemical changes, resulting in the growth of the biota when irradiated (Muhlisin, 2005). Another study of the background of this research is the success of the corona plasma technology has been applied to *Chlorella vulgaris L.* with a more modest level (Putra, 2013).

Application of plasma in its treatment of *G. verrucosa* was expected to increase growth and the optimal length of time based on the variation of radiation exposure. Different radiation treatments long incandescent plasma corona on the weed is expected to provide significant growth difference compared to those without treatment.

Experimental Methods

Seedlings of *G. verrucosa* was obtained from Balai Besar Pengembangan Budidaya Air Payau (BBPBAP) Jepara, Central Java. Seedlings were then selected to have still young and fresh plants, then acclimatized to the room temperature conditions in the Laboratory of Aquatic Ecology, Integrated Laboratory Diponegoro University for 2 x 24 hours. The purpose of acclimation is give a chance for the plant to have physiological adaptation from *ex situ* to *in situ* in the laboratorium condition before the treatment. Biomass of G. verrucosa was measured in the form of thallus to have 5 gr and 10 cm long, then they were put into an area of 768 cm^2 aquarium filled by 13 L seawater (34 ppm). This initial measurement was obtained from the adjustment scale *in vivo* with 240 kg maximum biomass of seedlings farmed into 100 m^2, prepared for further treated using plasma corona irradiation. The exposure was carried out with 5 different treatment periods, ie 2 minutes, 4 minutes, and 6 minutes, 8 minutes and 10 minutes at a distance of 1-2 cm from the seedling exposure. Selection of different treatment duration was based on other studies that explain to higher plants with better cell structure, optimization radiation range 10-30 minutes long. Treatments using plasma radiation on *G. verrucosa* were done for 4 weeks from the time the seedling. Preparation and procedures for treatments of plasma radiation is shown in Figure 1.

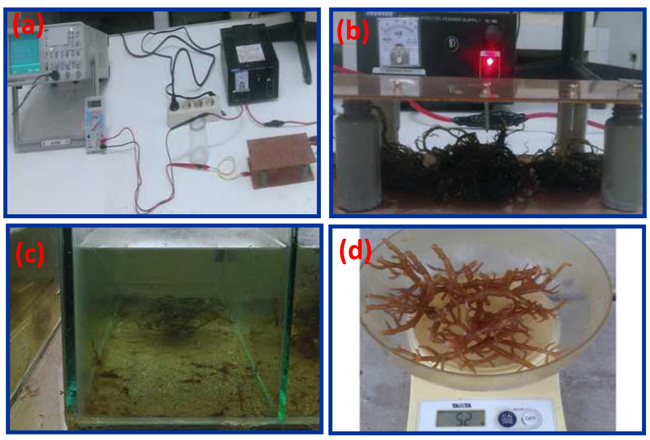

Figure 1. Preparation and procedures for treatments of plasma radiation: a). Generator of plasma corona radiation; b). Process of radiation of seaweed; c). In vitro culture in aquarium after various time of radiation exposure; d). Weight seaweed by using a balance every week for 28 days.

Observations on the growth of *G. verrucosa* were performed every 7 days for 28 days (4 weeks), a long with exposure using plasma treatment comparisons were performed and controls. Another treatment was done on a daily basis to monitor the conditions of abiotic seawater medium.

The rate of growth can be investigated through the formula according to Foster (2007):

$$G = Wt_1 - Wt_0$$

Remarks:

G = Growth rate of *Gracilaria* (gr)
Wt1 = Last observation biomass of *Gracilaria*
Wt0 = First observation of *Gracilaria*

Observations made in the early growth of seedlings and culture at the end of culture.

While the daily growth rate can be calculated as the following formula:

$$G = (\ln Wt1 - \ln Wt2) / (t1 - t2) \times 100\%$$

Remarks:

G = Growth rate of *Gracilaria* (%)
Wt1 = Last observation biomass of *Gracilaria*
Wt2 = First observation of *Gracilaria*

Thallus length measurement is performed to determine the growth of *G. verrucosa*. The differences in variant data obtained were analyzed using Anova. The graph performed as growth rate in thallus length and biomass for each week: 1st week (P1), 2nd week (P2), 3rd week (P3), and 4th week (P4), starting from the time of seedling.

Results and Discussion

The results showed that the plasma radiation treatment significantly affected the growth of *G. verrucosa*. Data resulting from the growth pattern of G. verrucosa were observed for 4 weeks in 28 days based on Duncan test results shown in Figure 2.

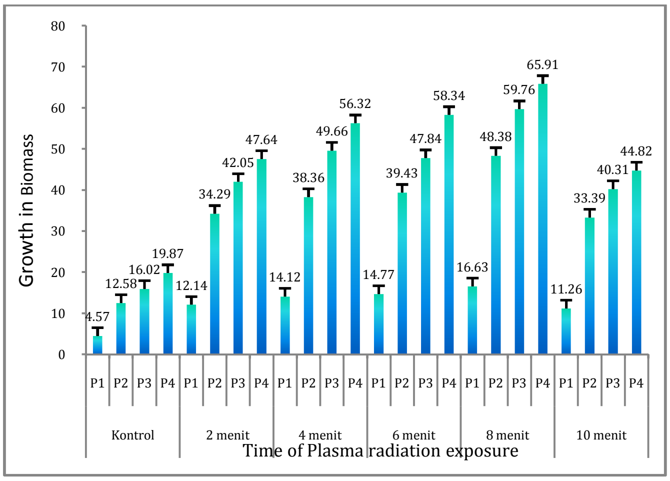

Figure 2. *G. verrucosa* growth rate of biomass

Based on the charts of the growth data after radiation plasma corona with different duration of radiation, showed that the best growth rate based on biomass occurred in the 8 minutes treatment of radiation exposure time. Treatment at this time exposure has the highest yield compared to the four other treatment periods, with the average value of the observation at 1st week which is 16.63 g, from the initial biomass cultivation on day 0.5g. At the 2nd week, the increase of biomass growth rate reached 48,38g biomass which is 2 times compared to the previous week. In the 3rd week, the growth rate reached 59,76g. The biomass increased from the previous week, but not as high as the percentage of growth acceleration in the increase in the 2nd week. In the 4th week, growth rate of weed still occurred with total value of biomass 69,91g. Increase in biomass after radiation has indicated a better growth rate compared to no treatment (control), owing to nitrogen utilization by cells of *G. verrucosa*. It is predicted that nitrogen absorption into cells of *G. verrucosa* increased as a result of plasma radiation treatment. Plasma corona discharge occurs in the condition of atmospheric air make nitrogen in the form of N_2 (dinitrogen) is forced into the direct current to infiltrated into the *G. verrucosa*. Furthermore, Azam (2007) stated that plasma corona discharge releases a nitrogen ion of radiation from atmospheric air (80%) into a material and can alter the microstructure of materials that alter the physical properties and chemical materials. Thus, in the presence of the addition of nitrogen may cause rapid growth and optimal metabolism of the weed. Nitrogen is an essential element in supporting growth of plants. Nitrogen is the element-forming protein, amino acids, and nucleic acids is an important component in the process of cell metabolism in growth. This is in line with the statement of Agus (2010), that nitrogen is one of the essential elements too construct protein as a component in the growth of plants. Furthermore, according to Triadyaksa (2007), nitrogen is the most common element found in the body parts of plants as essential compounds such as proteins, nucleic acids, DNA and many constituent vitamin content. In addition, the nitrogen also plays a important role in many biochemical reactions that structure the plant. In plants, nitrogen can be obtained through electrical discharges such as lightning in the form of oxidation of nitrogen. In this regard, the role of plasma technology serves to supply the needs of plant nitrogen directly from the air infiltration through N^+ ions.

The results of biomass growth rate on 2 minutes radiation exposure treatment for 4 weeks showed significant results compared to the controls. Treatment of 4 minutes radiation exposure has a higher biomass values than 2 minutes radiation for the same period. The average value of biomass for the treatment of 6 minutes radiation exposure has almost similar values of biomass of 8 minutes treatment. The treatment of plasma radiation corona in 8 minutes is the best treatment for the seaweed growth in the form of biomass compared to the overall growth of *G. verrucosa* treatments. The last treatment using 10 minutes radiation showed the lowest growth in the overall outcome of treatment. These results point to the excess nitrogen from *G. verrucosa* after plasma radiation treatment for 10 minutes that may led to the decrease in the use of nitrogen as a component to support their growth. According to Triadiati (2012), nitrogen is an very important element needed higher than other nutrients. Nitrogen deficiency will lead to sub-optimal plant growth, but the excess N will cause growth inhibition in plants.

Increased N excess in plants exceeds the optimal point, the majority of N assimilated breakaway as amides. Excessive N application will not only increase the levels of N in plants, but it reduces the synthesis of carbohydrates, even Nitrite accumulation can occur (NO_2) which are toxic if the levels are excessive. If carbohydrate synthesis reduced, the process of regeneration in the plant will be reduced. Energy is essential for plant growth and development, and is used for metabolic processes in plants. Disruption of metabolic processes of plants will inhibit the growth and development of plants (Putro, 2014). Based on test of Duncan, treatments of plasma radiation significantly affect the growth of *G. verrucosa* as thallus length. Data resulting from the growth pattern of *G. verrucosa* observed for 4 weeks in 28 days is shown in Figure 3 as follow :

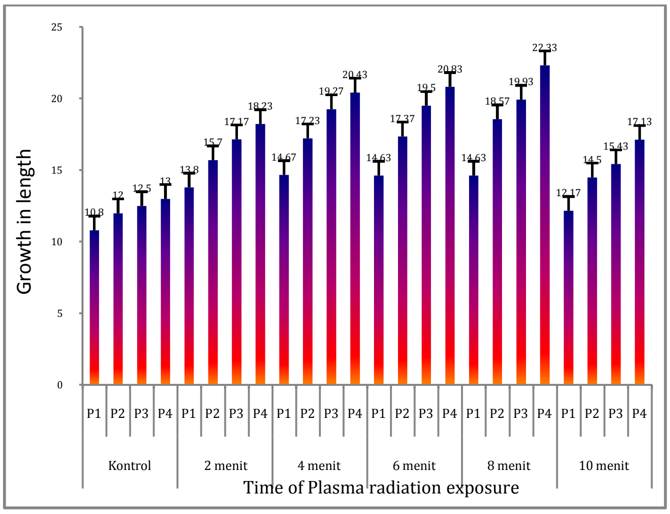

Figure 3. Average of growth rate of *G. verrucosa as* thallus length3

Growth in thallus length of *G. verrucosa* after plasma corona radiation in five different radiation duration is presented in Figure 3. The result of averaging between biomass growth and thallus length has a similar pattern. The graph shows that the best growth rates based on thallus length (cm) occurred in the treatment of radiation exposure time 8 minutes. Treatment at this time exposure has the highest yield of the other treatment periods, with the value of the average growth in thallus length on observations at week 1 which is 14,63 cm, from initial planting thallus length on day 0 with a length of 10 cm. At the 2nd week, the addition of thallus length of week 1 at a rate high enough to reach 18,57 cm. In the 3rd week, growth rate in thallus length is 19,93 cm. This growth has increased from the previous week, but the percentage of accelerated growth is not as high as the increase in 2nd week. In the 4th week, thallus still grew up to the average of 22,33 cm.

The rate of growth is reflected on outcomes related to average biomass growth of *G. verrucosa*. The highest growth rate occurred in treatment of 8 minutes plasma corona radiation with a percentage rate of 9.5515% per day. This growth rate was calculated from the initial biomass into 65,91g 5 g of *G. verrucosa*. Treatment in the 6 minutes radiation had growth rate of 9.0994%, from the initial biomass (5g) to be 58,34g. Subsequent treatment in the 4 minutes radiation had a growth rate of 8.9687%, from the initial biomass (5g) to be 56,32 g. Following treatment is in the 2 minutes radiation that had growth rate of 8.349%. The rate of this growth was calculated from the beginning of the biomass (5g) to be 47,64 g. Treatment with the lowest growth rate occurred in the 10 minutes radiation with a percentage rate of 8.123%. The rate of this growth was calculated from the beginning of the biomass (5g) to be 44,82 g after 28 days treatment. Through the percentage growth rate is known that plasma corona radiation has impact on the growth speed of G. verrucosa in form of biomass.

Similar to growth as biomass, Tte growth rate is also related to the results of average of thallus length of *G. verrucosa*. The highest growth rate occurred in 8 minutes treatment of plasma corona radiation with a percentage rate of 2.9759%. This growth rate was calculated from the beginning of thallus length (10 cm) *G. verrucosa* to be 22,33 cm. Treatment in the 6 minutes radiation had growth rate of 2.7184%. This growth rate was calculated from the beginning of thallus length (10 cm) of *G. verrucosa* to be 20,83g. Subsequent treatment in the 4 minutes radiation had a growth rate of 2.6466%, which was calculated from the beginning of thallus length (10 cm) to be 20,43cm. Following treatment in the 2 minutes radiation had growth rate of 2.2247%, which was calculated from the beginning of thallus length (10 cm) to be 18,23 cm. Treatment with the lowest growth rate occurred in the 10 minutes radiation with a percentage rate of 1.9942%, which was calculated from the beginning of thallus length (10cm) to be 17,13g. Through the percentage growth rate is known that plasma corona radiation has impact on the growth speed of G. verrucosa in the form of thallus length.

Conclusions

Treatments using plasma corona radiation on *G. verrucosa* impacted on the growth better compared to the group without radiation. The most effective plasma radiation had a significant effect on the growth of *G. verrucosa* was in the 8 minutesradiation, which was 65,91 g on the biomass with growth rate of 9.5515% and 22,33 cm on thallus length with growth rate of 2.9759%. Effect of plasma corona exhibited lowest in influencing the growth of *G. verrucosa* for 10 minutes, which was 44,82 g of biomass and growth rate of 8.123% and the thallus length of 17,13 cm with a growth rate of 1.9942%.

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