

Turbidity and Photoperiod Effect toward Bleaching Condition of *Sargassum* sp.

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Abstract: This study was conducted to determine the different turbidity and photoperiod effect toward bleaching condition of *Sargassum* sp. The study contained three studies (turbidity and photoperiod effect) using complete randomized design with 3 treatments and 6 replications in each study. Turbidity levels used were 10cm, 30cm and 50cm while photoperiod levels were 16 hours bright 8 hours dark, 12 hours bright dark and 8 hours bright 16 hours dark. The parameter observed were external and cytological anatomy observation. Each turbidity and photoperiod treatment significantly affected thallus color gradation and chlorophyll-a content of *Sargassum* sp. ($p < 0.05$). The lowest thallus color gradation and chlorophyll-a content in different turbidity were found at 10 cm (38.167% and 0.02305 μmol), while highest were observed at 50 cm (45.095% and 0.03280 μmol). The lowest color gradation and chlorophyll-a content in different photoperiod were obtained from 8 hours bright 16 hours dark (41.446% and 0.02723 μmol) while highest levels were observed at 16 hours bright 8 hours dark (49.934% and 0.03385 μmol).

I. INTRODUCTION

Sargassum sp. is one seaweed type from Phaeophyceae that spread around Indonesia, yet still not much cultivated. *Sargassum* sp. had potential to be developed as contained alginate and iodine which used for food industry, pharmaceutical, cosmetics and textiles [1].

The seaweed growth in waters was affected by several factors such as turbidity and photoperiod. High turbidity could lead to the hampered sunlight penetration into waters [2]. Photoperiod also affects the seaweed activity. Light penetration is limitation factor for seaweed growth, whereas light below need level would decrease photosynthesis process and cause seaweed death [3]. Imbalance turbidity and photoperiod will cause *Sargassum* sp. undergoes bleaching condition.

Bleaching was a process of color loss on the seaweed due to oxidized and degraded pigment inside the seaweed [4]. *Sargassum* sp. had carotenoid color substance containing chromophore group or color carrier group which was very unstable due to easily oxidized. Oxidized chromophore force would lose light absorption function, thus seemingly colorless or losing color [5]. Bleaching in *Sargassum* sp. was characterized by onset of the freckles/spots in some thallus that gradually became pale yellow and finally turned into white [6].

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The thallus texture in bleached *Sargassum* sp. would be slimy with exfoliated and flabby epidermis when exposed to the water current until ultimately broken. Thallus would also experience depigmentation and eventually lead to damage [7].

Further study regarding to the influence of turbidity and photoperiod on different level conditions toward *Sargassum* sp. bleaching was necessary conducted to determine optimum condition of stable environment for seaweed culture as well as improving survival range and production of *Sargassum* sp. in Indonesia.

II. METHODOLOGY

Materials

The equipment used were aquarium sized 30x20x100 cm^3 , seawater source tank, air pump, aerator, aeration hose, aerator stone, 40 watt fluorescence lamp, 1000 ml measuring glass, digital scale, volume pipette, bulb, drop pipette, object glass, cover glass, Secchidisk, thermometer, pH paper, lux meter, DO meter, refractometer, reaction tube, round cuvet, spectrophotometer (Human X-ma 1200, China), Charge Coupled Device (CCD) microscope and SEM (Scanning Electron Microscope) (Zeiss EVO MA 10, Germany).

The material used were 1.800 g of fresh *Sargassum* sp. seaweed retrieved from Saronggi Beach, Sumenep, Madura and 40 l of seawater for each aquarium. Material used for aquarium sterilization was chlorine, while for setting up the turbidity level was chemical food coloration. Another material used was methanol 96% (Merck CGaA, Darmstadt, Germany).

This study used Competed Randomized Design (CRD) experimental method divided into two parts of observation which were turbidity and photoperiod effect. Each effect comprised three treatment and six replications. Turbidity effect treatment contained 10cm, 30 cm and 50 cm level of turbidity which photoperiod effect treatment contained 16 hours bright 8 hours dark, 12 hours bright 12 hours dark and 8 hours bright 16 hours dark.

Sargassum sp. in the aquarium were reared for 14 days. The weight of *Sargassum* sp. used for one treatment and replication was 100gm from total seaweed needs were 1,800g. Seaweed pigmentation was observed by comparing thallus color gradation of *Sargassum* sp. used color analysis software application. Thallus parts observed were stipe, frond and bladder. Color gradation data was presented on percentage range.

Turbidity and Photoperiod Effect toward Bleaching Condition of *Sargassum sp.*

Total chlorophyll- α of *Sargassum sp.* was calculated used spectrophotometer. Total chlorophyll- α was measured on the first and last day of rearing. There were 10mg of seaweed powder sample was extracted into 5 ml of methanol 96% in the reaction tube and incubated for 24 hours at room temperature.

Cytological condition was observed under CCD microscope with 100x and 400x magnification. Tissue observed were obtained from holdfast, frond and bladder of seaweed were cut horizontally and vertically. Color

gradation and total chlorophyll- α were analyzed used ANOVA with CRD statistical analysis method and SPSS 16.0 computer software.

III. RESULT AND DISCUSSION

Color Gradation

Color gradation of *Sargassum sp.* thallus on turbidity and photoperiod effect were represented on Table 1.

Table. 1 Turbidity and photoperiod effect on color gradation of *Sargassum sp.*

Turbidity (cm)	Color Gradation(%) \pm SD	Photoperiod (hours bright/ hours dark)	Color Gradation (%) \pm SD
10	38.167 ^a \pm 3.45121	16/8	49.934 ^a \pm 2.4603896
30	43.937 ^b \pm 1.30394	12/12	46.251 ^a \pm 7.0958071
50	45.095 ^b \pm 1.21355	8/16	41.446 ^b \pm 1.9611213

Note : Different superscript letter on the column shows significant difference (p<0.05)

ANOVA analysis result showed that every treatment of turbidity and photoperiod level gave significant difference against the color gradation of *Sargassum sp.* (p<0.05). DMRT statistical analysis result showed that 10 cm turbidity level was significantly difference from 30 and 50 cm turbidity level (p<0.05), while 30 cm turbidity level had no significant difference with 50 cm turbidity level. Photoperiod treatment of 8 hours bright and 16 hours dark showed significant difference between other treatments while 16 hours bright and 8 hours dark showed no

significant difference against 12 hours light and 12 hours dark. Highest color gradation as different treatment result given was presented on 50 cm turbidity level with 16 hours light and 8 hours dark of photoperiod level while lowest was found at 10 cm turbidity level with 8 hours light and 16 hours dark photoperiod.

Chlorophyll- α Content

Chlorophyll- α content of *Sargassum sp.* after treatments given was represented on Table 2.

Table. 2 Turbidity and photoperiod effect on chlorophyll-a content of *Sargassum sp.*

Turbidity (cm)	Chlorophyll- α Content (μ mol) \pm SD	Photoperiod (hours light/hours dark)	Chlorophyll- α Content (μ mol) \pm SD
10	0.02305 ^a \pm 0.0028452	16/8	0.03385 ^a \pm 0.0023118
30	0.02823 ^b \pm 0.0007763	12/12	0.03015 ^b \pm 0.0022002
50	0.03280 ^c \pm 0.0003578	8/16	0.02723 ^c \pm 0.0020142

Note : Different superscript letter on the column shows significant difference (p<0.05)

ANOVA result showed that every turbidity and photoperiod level was significantly difference against the chlorophyll-a content of *Sargassum sp.* (p<0.05). DMRT test showed that significant difference was discovered among all treatments (p<0.05). Highest chlorophyll- α content on *Sargassum sp.* was observed at 50 cm turbidity level (0.03280 μ mol) with 16 hours light and 8 hours dark photoperiod (0.03385 μ mol) while lowest was at 10 cm turbidity level (0.2305 μ mol) with 8 hours light and 16 hours dark photoperiod (0.2723 μ mol).

Cytological Change

Cytological change of *Sargassum sp.* after turbidity and photoperiod treatments given are shown on Figure 1 and 2.

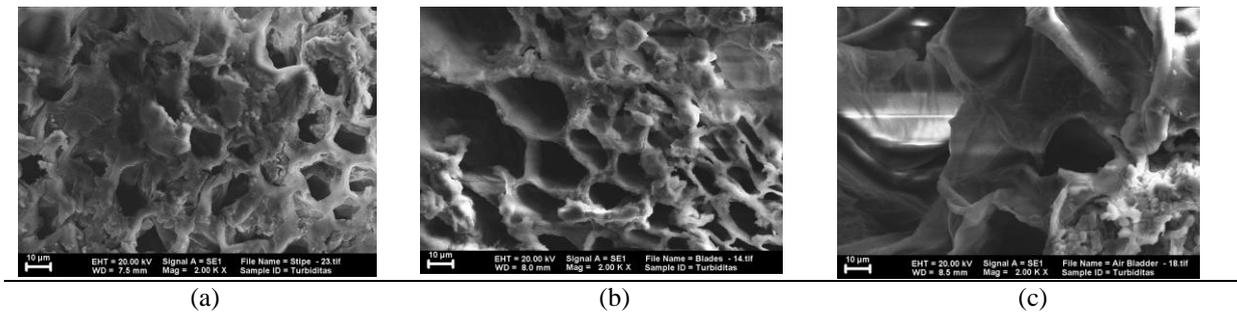


Fig. 1 SEM of 10 cm Turbidity Effect on Cytological Change of *Sargassum* sp. (a) Stipe, (b) Frond (c) Bladder

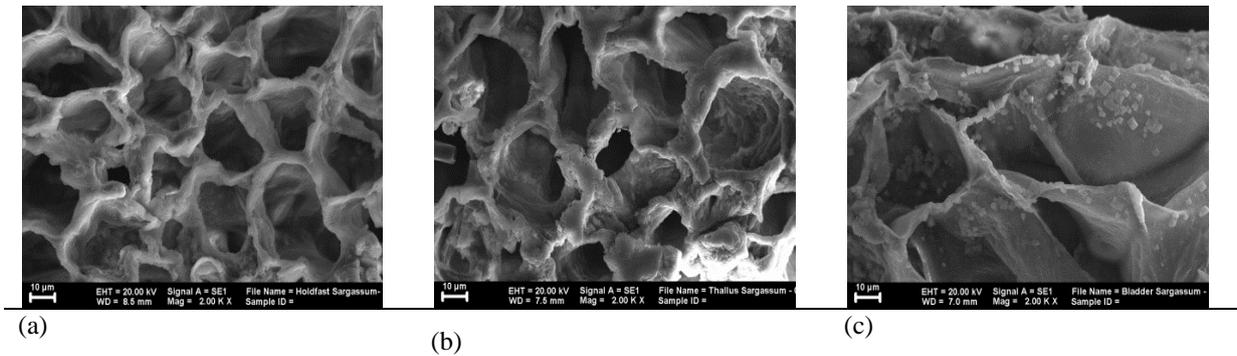


Fig. 2 SEM of Photoperiod Effect on Cytological Change of *Sargassum* sp. (a) Stipe, (b) Frond (c) Bladder

Discussion

ANOVA result on the color gradation of thallus *Sargassum* sp. showed that turbidity treatment gave significant difference against color gradation of thallus *Sargassum* sp. ($p < 0.05$). This result indicated that water turbidity condition influenced the bleaching condition of *Sargassum* sp. Color state on the seaweed thallus was influenced by environmental changed factors as modification process which was the impermanent shape and phenotype change due to environmental change such as climate and oceanographic condition that relatively high. When seaweed experienced stress due to low water turbidity would caused low light intensity [8]. Stress signs in seaweed would be represented on the onset of freckles/spots in some thallus that gradually became pale yellow until turning completely white lead the seaweed happened to be in color loss [6].

Based on Table 2, chlorophyll-*a* level sequence on *Sargassum* sp. was started from highest level was 50 cm followed by 30cm turbidity and 10cm. Chlorophyll-*a* was green pigment found in plants, algae, and cyanobacteria was used as a food supplement to help optimize metabolic function [9]. ANOVA result on chlorophyll-*a* level of *Sargassum* sp. showed significant difference against the turbidity level in the water ($p < 0.05$). This result meant that turbidity influenced the chlorophyll-*a* content of *Sargassum* sp.

Data of initial chlorophyll-*a* level was $0.01447 \mu\text{mol}$ and increased during the seaweed rearing. Increased chlorophyll-*a* happened due *Sargassum* sp. were kept for two weeks in the controlled environment. Age also affected the chlorophyll level possessed in a leaf [10].

High turbidity level affected the slimy structure and texture when hit the water currents with chipped epidermis, making the emerged network (cortex) and finally became broken (loss). High turbidity caused *Sargassum* sp. could not absorb light for photosynthesis.

Water with high turbidity contained abundant particles that covered entire thallus, inhibiting the nutrient absorption using diffusion through the thallus wall. This caused the *Sargassum* sp. to be lack of energy and resulting death. Seaweed changing condition of physiological and morphological condition could be caused by the nutritional deficiencies [11].

Data on the cell shape and size of *Sargassum* sp. showed there were different appearance among the treatments. *Sargassum* sp. on high turbidity water formed irregular cells with non uniform size while 30cm and 50cm turbidity showed cell shape and size that are relatively same as initial rearing.

At the beginning of seaweed growth, seaweed would be more focused on enlarging diameter and elongating main thallus caused number of cells would be less than new thallus.

Decreased amount of chlorophyll- in *Sargassum* sp. was due to light intensity effect also caused difference in morphology and carotenoid content. Seaweed on the water surface could absorb excessive light, yellow light would decrease the light absorption. The deeper seaweed placed, the lower light intensity they would absorb [12].

The rate of photosynthesis was also affected by the light quality and quantity that affected the alga reproduction. It also happened to the structural response of algae absorbing light intensity that included a change of size, morphology, and cytoplasmic inclusions [13].

The light energy was absorbed by the chlorophyll molecules from an antenna located in the thylakoid membrane and transferred to the chlorophyll reaction center.

Turbidity and Photoperiod Effect toward Bleaching Condition of *Sargassum* sp.

Electrochemical reaction began there, which produced two vital biological compounds that rich in energy, i.e. ATP (AdenosineTriphosphate) and NADPH (Nicotinamide Adenine DinucleotidePhosphate Hydrogen). Oxygen was produced as the byproduct process and released into the atmosphere. Meanwhile, products produced in the light phase were used inside the cell to the carbohydrate formation or sugar from the carbon dioxide on the dark phase. Sugar produced by plants through the photosynthesis process was stored within the plant cell as an energy. This energy will be transformed by the mitochondria in the plant cells into an energy form that could be used by the plant itself. When the gained light was less than the plant needed, a photosynthesis would not run that resulted in the plant did not take its own food.

IV. CONCLUSION

In conclusion, Different turbidity and photoperiod level during *Sargassum* sp. rearing which suffered from the bleaching condition showed significant difference, based on the color gradation, chlorophyll-*a* content and cytological change.

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