



CO₂ application as growth stimulator of seagrass, *Thalassia hemprichii* under laboratory condition

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Abstract

Seagrass study needs to be continuously developed in order to know its potentiality of storing carbon. One of the studies uses carbon dioxide (CO₂) as growth rate indicator of the seagrass, *Thalassia hemprichii*, in a controlled laboratory scale. The seagrass used in this study was collected from Tongkaina waters, Sulawesi Utara. The study used Complete Randomized Design with treatments as follows: P₁ - CO₂ addition once per 3 days (5x100 ml/min for 25 minutes), P₂ - CO₂ addition once per 2 days (5x100 ml/min for 25 minutes), P₃ - CO₂ addition once a day (5x100 ml/min for 25 minutes), and control treatment (no carbon dioxide). Results showed that daily growth rate was influenced by carbon dioxide application and alkalinity. The highest wet weight occurred in P₃ media in day-30, 36.67 gr, while the lowest wet weight was recorded in P₁ in day-6, 27.60 gr. The highest growth rate was found in P₃ in day-6, 1.310%, while the lowest was recorded in P₁ in day-6, -1.772%. Carbon dioxide application significantly affected the growth rate of the seagrass. Tukey test indicated that seagrass growth rate in P₃ was significantly different from those of other treatments with P₃>L: 0.747>0.65, while P₁ and P₂ did not have significant difference in growth rate with P₁<L: 0.093<0.65 and P₂<L: 0.068<0.65, respectively. Alkalinity measurement showed that the highest was recorded in P₃, 80 mg/l and the lowest in P₁, 40 mg/l.

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Introduction

Seagrass bed is one of coastal vegetations that could become a solution in climate change prevention. Plants possess ability to absorb CO₂ from atmosphere to become energy useful for life through photosynthetic process.

Thalassia hemprichii is one of 13 seagrass species growing in tropical waters and sufficiently widely distributed in Indonesian waters (Thomascik *et al.*, 1997). According to Kiswara (1992), this species is very common and largely found in the reef flat, either growing as separate species (monospecific) or with other seagrass species or plants (mixed vegetation).

Kiswara (2010) studied the growth of *T. hemprichii* in the nature and provided information on the productivity and the biomass of the seagrass. However, further study has not been done yet on growth patterns, particularly the biomass under a controlled laboratory scale. One of the potential studies on seagrass growth rate in a controlled scale is the use of CO₂ as indicator of growth rate. This study was aimed at observing how far the effect of carbon dioxide application on the plant growth rate and the potentiality of absorbing CO₂.

The outcome is expected to be able to provide basic information on the effect of CO₂ on the growth rate of *Thalassia hemprichii*, and the use of CO₂ for seagrass culture activity could reduce CO₂ concentration in the ocean (carbon sink).

Materials and method

This study was carried out in the Hydrobioecological Laboratory, Fisheries Resources Management, Sam Ratulangi University, Manado. Materials used were seagrass, *Thalassia hemprichii*, collected from Tongkaina waters, North Sulawesi, sea water, and substrates, sand and mud, as planting medium, pure CO₂ gas, gravel, active carbon, sponge, and bioball. As culture medium, a 40 x 40 x 40 cm³ glass aquarium was used. Water temperature measurement used a thermometer, and temperature control used a heater. Water pH was measured with pH-meter, DO

with DO-meter, and salinity with refractometer.

Culture medium preparation used the closed running water system. The aquarium was filled with water of 130-150 liters and facilitated with filter. Each aquarium was planted with 30 individuals of seagrasses and cultivated in mud of 10 cm deep under laboratory conditions. The test *Thalassia hemprichii* selected in the study was young individuals, clean, fresh, same-aged, and had similar wet weight and condition (Atmadja *et al.*, 1996). The preparation started from weighing each individual of 20 gr and the culture period was 30 days.

The study used Complete Randomized Design with 3 treatments and 3 replications. The treatments applied were as follows: Treatment 1 = CO₂ application of 100 mL once in 3 days for 25 minutes, Treatment 2 = CO₂ application of 100 mL once in two days for 25 minutes, Treatment 3 = CO₂ application of 100 mL once a day for 25 minutes, and Control Treatment = no CO₂ application.

The growth rate of the seagrass was measured once in three days, and one individual of each treatment was weighed using a digital balance. The wet weight of the samples was recorded after placing them on the newspaper for 5 minutes to remove water on the seagrass.

The physical and chemical parameters measured were water temperature, pH, dissolved oxygen, salinity, and alkalinity. Temperature measurement used thermometer attached inside the aquarium. To control the culture medium temperature, a heater was used and situated for the seagrass optimum growing temperature, between 27^o-30^oC. The pH, DO, and salinity measurements were carried out *in situ*, pH with pH-meter, DO with DO-meter, and salinity with refractometer. The use of DO-meter was carefully done, since dissolved oxygen in the aquarium was highly fluctuative due to aeration. Carbon dioxide and alkalinity were measured using titration method.

Water quality measurements done before application in culture media were, successively, 30, 30, 31, and 31 ‰ for salinity, 30, 30, 27, and 27°C for water temperature, 7.80, 7.90, 7.70, and 7.20 for pH, and 5.82, 5.95, 5.60, and 5.70 mg/l for DO, respectively. Based on these measurements, it is apparent that all values be in the range of water quality standard for seagrass culture, so that the study activity is expected to be able to run well and the seagrass could grow well. After CO₂ application in the culture media, the water quality measurements were done during the culture with time interval of one application in 3 days. Water temperature of all treatments was relatively homogenous, between 27 – 28° C, since the culture room is facilitated with air conditioner. Nevertheless, to maintain the water temperature in the tolerable range by the seagrass, the culture media was also provided with heater that the temperature could be set as desired.

The seagrass growth rate of all treatments was compared with that of control treatment. It was estimated on daily basis using Nelson *et al.*, (1980). Data were analyzed using ANOVA at the confidence level of 95 % (Steel and Torrie, 1989). To see the effect of CO₂ application on the seagrass growth rate and the best dose to obtain the optimum growth, Tukey test was used (Mattjik and Sumertajaya, 2002).

Results and discussion

Wet Weight of different treatments

Wet weight increment of *T. hemprichii* cultured for 30 days varied with treatment. In the first week of culture, day-1 to day-6, the seagrass did not show significant growth in all treatments.

The lowest wet weight in treatment 1 was 27.60 gr, while the highest was 29.75 gr. In treatment 2, the lowest wet weight was 28.70 gr and the highest was 30.54 g, while in treatment 3, the lowest was 27.99 g and the highest was 36.65 g, and in control treatment, the lowest was 27.60 g and the highest was 29.20 g. The seagrass of treatment 3 has the highest growth rate, and it could be seen from initial culture to the

end of culture period with increasing biomass increment.

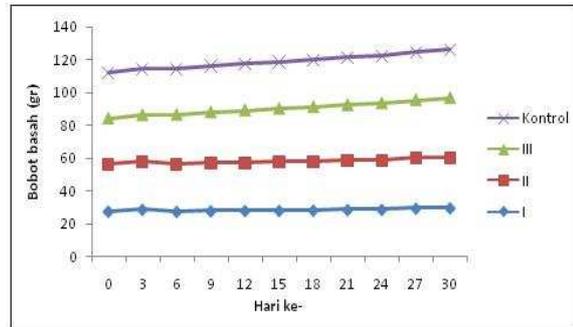


Fig. 1. Wet weight (gr) growth of *Thalassia hemprichii* under different treatments, CO₂ application of 100 mL for 25 minutes once in 3 days (P₁), once in two days (P₂), once a day (P₃), and Control (C).

The wet weight increment of treatment 1 decreases drastically in day-6, in which mean wet weight of the seagrass in treatment 1 declines from initial weight of 27.60 gr to 27.50 g caused by leaf falls that affects the mean growth value. Very significant seagrass growth increment of treatment 1 occurred successively in day-24, 27, and 30, 28.86 g, 29.65 g, and 29.82 g, respectively. The seagrass of control treatment has relatively slower wet weight growth rate than those of treatment applications. Mean wet weight of the seagrass in control media was 0.11 gr/3 days. Mean weight of each treatment is presented in Fig. 1.

Variation in wet weight of the present study could be described by photosynthetic activity of *Thalassia hemprichii*. Sufficient availability of important components in photosynthetic activity could result in better biomass. Carbondioxide (CO₂), water, and solar energy are major components needed by the seagrass for photosynthesis that will generate plant biomass and oxygen. The biomass was built by the producer and utilized by the consumers as food materials. Contradictorily, in respiration, the oxygen is required by the seagrass at night and produces the carbondioxide. Beside the availability of carbondioxide, water, and solar energy, the algae need some other important elements as well to support their growth, such as S, Ca, Na, K, Mg, Fe, Cu, Zn, Mn, CO, Mo, B,

and V (Siregar, 2005).

Daily growth rate of *Thalassia hemprichii*

The seagrass growth rate of each treatment highly varied between -1.670% -1.295%. This variation could result from different response of each seagrass individual to internal factors, such as physiology and metabolism, and external factors, such as nutrients, fertility rate, substrate, and other environmental factors. Treatment 1 showed highly fluctuative growth, in which in day-6 the growth rate decreased up to negative, -1.670% due to leaf falls. It, however, rose up to 0.359% in day-9 due to new leaf growth. The decline in growth rate occurred also in day-2, -0.030%, day-24, 0.040%, and the end of culture period, -0.150%. Treatment 2 had fluctuative growth rate reaching negative growth. It could be seen in day-6 and 30 with growth rate of -0.006% and -0.065%. The highest seagrass growth rate in treatment 2 occurred in day-24, 0.177%, and the lowest in the end of culture, -0.065%. Increase in seagrass growth rate of treatment 3 is the highest, and the highest rate occurred in day-6, 1.298%, and the lowest growth rate occurred in day-30, 0.415%.

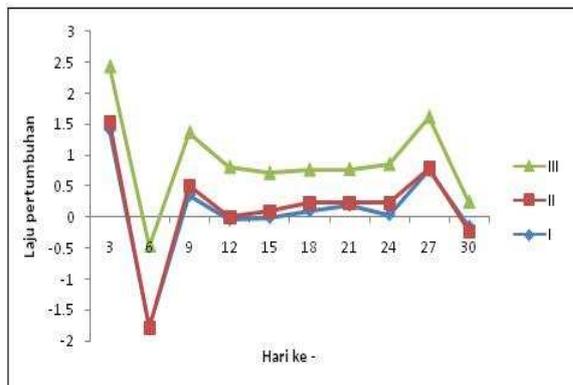


Fig. 2. Growth rate of *T. hemprichii*(%) with CO₂ application for 25 minutes once in 3 days (P1), once in 2 days (P2), and once a day (P3).

Statistical analysis at the confidence level of 95% exhibited that treatment 1 dan 2 did not significantly influence the seagrass growth rate, indicated with $P1 < L = 0.093\% < 0.65\%$, and $P2 < L = 0.068\% < 0.65\%$. Nevertheless, treatment 3 with CO₂ application once a day had significant effect on the seagrass growth rate with $P3 > L = 0.747\% > 0.65\%$.

Fig. 2 shows that growth rate occurs in the early week of culture, day-3 and 6 for all treatments, except treatment 1. However, the relative growth rate declines in the second and the third weeks of culture due to leaf falls. It is a natural process occurring in *Thalassia hemprichii* as aging process. Those were located across and far from the growth point where the the meristemathic cells continuously divided to produce new individual. The aging process in *Thalassia hemprichii* starts with root falls. The of root loss will fail absorbing nutrition from substrate. The nutrition could not also gained from the neighborhood individuals since the aginf also occurred in the tissues of the rhizome. The falling leaf has broken site at the leaf base. The aging leaf is brownish greener than the growing one. It could be caused by loweer chlorophyll content of the leaf. Decrease in chlorophyll content will also cause photosynthetic rate reduction. The absence of absorbed nutrients and photosynthetic rate decline bring about *Thalassia hemprichii* unable to survive. Salisbury and Ross (1995) stated that leaf aging is usually followed with immediate loss of chlorophyll, RNA and protein (including various enzymes). The aging *Thalassia hemprichii* will lack of nutrient supply since the nutrients tend to move to other organs, and in this case they will move to vegetative individual growth.

The seagrass, *T. Hemprichii*, growth rate is relatively slow. It could result from the environmental carrying capacity and the seagrass relocation from the sampling site to laboratory culture media affecting the seagrass adaptation to the new environment that could not only influence the growth rate, but also could cause mortality. According to Amiluddin (2007), good growth rate of seagrasses ranged from 3 to 5% per day during the planting time.

Alkalinity

Based on measurements during the study, the alkalinity of treatment 1 was 40 mg/l CaCO₃. It could result from that the water used as culture media had relatively same mineral content and carcondioxide

application did not significantly affect pH changes. The alkalinity of treatment 2 ranged from 40 to 60 mg/l CaCO₃ with slight pH variation ranging from 7 – 8.6. This variation was not followed with alkalinity difference. According to Effendi (2003), the alkalinity of CO₃²⁻ is stronger than the acidity of CO₂ at the equilibrium condition, OH⁻ ion the solution always exceeds H⁺ ion. The alkalinity of treatment 3 highly varied from 50 – 80 mg/l CaCO₃, but pH was relatively the same in the normal condition ranging from 7.00 – 7.10. The lowest alkalinity occurred in day-21 after CO₂ application, and it could be brought about by mineral reduction in the water from seagrass utilization for photosynthetic process. On the other hand, the highest alkalinity in treatment 3 occurred in day-12 and day-24 with a value of 80 mg/l CaCO₃. This high alkalinity could result from daily application of carbon dioxide in treatment 3 affecting pH decline down to 7, so that the alkalinity changed as well.

Based on the alkalinity content, present study found that it is still in a good range for the seagrass growth, in the range of the standard quality of 40 – 80 mg/liter CaCO₃. Good alkalinity ranges from 30 to 500 mg/liter CaCO₃. In nature, it ranges from 5 to hundreds mg/liter CaCO₃. The water alkalinity > 40 mg/liter CaCO₃ is called hard water and that with alkalinity < 40 is called soft water (Effendi, 2003).

Alkalinity is number of bases contained in the water determined by CO₃²⁻ and HCO₃⁻ in CaCO₃ unit (Dongoran, 2003). It is made of carbon dioxide and water that can dissolve the carbonate sedimentary rocks to become bicarbonates. The solubility of calcium carbonate decreases with increase in temperature and carbon dioxide. Calcium carbonate reacts with carbon dioxide to form calcium bicarbonate [Ca(HCO₃)₂] that has higher solubility than that of calcium carbonate (CaCO₃) (Cole, 1983).

Water Quality

Water quality is very important factor to determine the survivorship of aquatic organisms, especially

plants. According to Anggadiredja *et al.* (2007), water quality standard in seagrass culture is 28 – 33 ‰ for salinity and 26 – 30°C for water temperature. It is not so different from that of Mubarak *et al.* (1990), in which the water quality standard in seaweed, *Eucheuma spp.*, culture includes several environmental parameters, i.e water movement of about 20-40 cm/sec., visibility not less than 5 m, temperature of about 20-28°C, salinity of about 28-34 ‰ with an optimum of 25 ‰, pH of 7.3 - 8.2, and containing macronutrients, such as N, P, K, Ca, S, and Mg, highly needed for plant chlorophyll. The optimum dissolved oxygen (DO) for the seagrass growth is higher than 5 mg/l (Sulistijo and Atmajda, 1996).

Water salinity during the study ranged from 30 – 31 ‰ for all treatments. This range lies in the optimum range for *T. hemprichii* growth, between 28 – 33‰ (Anggadireja *et al.*, 2007). This value is relatively uniform due to high water evaporation in the aquarium, so that sea water and freshwater addition is needed to gain relatively homogeneous salinity. In the present study, water addition was usually conducted once in 3 days. High evaporation rate could result from low room temperature from AC operation.

Dissolved oxygen in all treatments varied. It ranged from 5.6 to 5.85 mg/l in treatment 1, 5.5 to 6 in treatment 2, and 5.7 to 6 in treatment 3, respectively. Mean DO concentration of the 3 treatments is in normal range categorized as good range based on Indonesian Living Environmental Minister Decision Numbered 51, 2004, concerning seawater quality standard (Sulistijo and Atmadja, 1996). Dissolved oxygen is number of oxygen dissolved in the water as major metabolism component used for growth, reproduction, and fertility in seagrass ecosystem (Odum, 1971). Dissolved oxygen variation in the present study could result from different diffusion in each.

The chemical parameter closely related to CO₂ is

acidity level (pH). The pH measurement resulted in a range between 7.00 – 8.10. Based on the pH values, it was found that the lowest pH had high CO₂ concentration. It was found in treatment 3 in day-12 and day-24, with pH of 7.00, and CO₂ concentration was 119.86 mg/l. In pH>8, no CO₂ concentration. It agrees with Mackereth *et al.*(1989) that pH is highly correlated with CO₂, in which the higher the CO₂ concentration, the lower the pH is and vice versa.

Conclusion

The best treatment on the growth rate of *T. hemprchii* was P₃ with CO₂ addition once a day (5x100 ml/min for 25 minutes). It is statistically significantly different at the level of 95%. Honest Significant difference test exhibited that *T. hemprchii* growth generated by P₁ and P₂ was not different from control with $P_1 < L = 0.093\% < 0.65\%$, and $P_2 < L = 0.068\% < 0.65\%$, while that of P₃ was significantly different from that of control.

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