Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 5, No. 6, p. 153-159, 2014
http://www.innspub.net

RESEARCH PAPER **OPEN ACCESS**

CO_2 application as growth stimulator of sea grass, *Thalassia hemprichii* under laboratory conditions

Khristin I.F. Kondoy[1], Endang Yuli Herawati[2], Mohammad Mahmudi[2], Rodliyati Azrianingsih[3]

[1]*Faculty of Fisheries and Marine Science, Sam Ratulangi University. Manado. Indonesia*

[2]*Faculty of Fisheries and Marine Science, Brawijaya University, Malang. Indonesia*

[3]*Faculty of Mathematics and Natural Science, Brawijaya University, Malang. Indonesia*

Article published on December 06, 2014

Key words: CO2, Seagrass, *Thalassia hemprichii*, Growth.

Abstract

Seagrass study needs to be continuously developed to know its potential ability of storing carbon and absorbing CO2 (*carbon sink*) or called blue carbon used for photosynthesis. One of the studies uses carbondioxide (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 carbondioxide). Results showed that daily growth rate was influenced by carbondioxide 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%. Carbondioxide 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.

***Corresponding Author:** Khristin I.F Kondoy ✉ khristin.kondoy@gmail.com

Introduction

Seagrass bed is one of coastal vegetations that could become a solution in climate change prevention. In the last several years, the role of seagrass bed as one of the carbon emision sinks in the ocean started being discussed (Duarte, *et al.*, 2005, 2011). Before that, the expert's attention was focused on terrestrial vegetations as carbon sinks, such as forest and plantation, but neglected the role of coastal ecosystem. This carelessness was pobrably due to limited costal vegetation areas, less than 2% of the sea surface (Duarte, *et al.*, 2005).

One of the expert's concerns was the introduction of the blue carbon concept issued in collaboration between UNEP, FAO and UNESCO in the last of 2009. The concept is based upon their belief in the presence of three-marine ecosystem ability (mangrove, seagrass, and salt marsh) in maintaining the absorption and the reduction of carbon emision (Nellemann, *et al.*,2009). As one of the costal ecosystem components, the occurrence of seagrass ecosystem is highly important in carbon cycle (Kennedy *et al.*, 2010).

Plants, in this case seagrasses, possess ability to absorb CO2 from atmosphere to become energy useful for life through phosynthetic process. The carbon absorbed is then stored or passed to several compartments in the form of biomass (Kiswara and Ulumuddin, 2009).

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 carbondioxide application on the plant growth rate and the potentiality of absorbing CO2.

Based on information above, a study was conducted to provide basic information on the effect of CO2 on the growth rate of seagrass, *Thalassia hemprichii*, so that this study is expected to be able to provide information on how far the use of CO2 for seagrass, *Thalassia hemprichii*, culture activity could reduce CO2 concentration in the ocean.

Materials and method

Place and Time

This study was carried out in the Hydrobioecological Laboratory, Fisheries Resources Management, Sam Ratulangi University, Manado, from August 2013 to September 2013.

Materials

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.

Equipment

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.

Working Proceedures

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 *Thallasia 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 dan the culture period was 30 days.

Experimental Design

The study used Complete Randomized Design with 3 treatments and 3 replications. The treatments applied were as follows: Treament 1 = CO_2 application of 100 mL once in 3 days for 25 minutes, Treatment 2 = CO_2 application of 100 mL once in two days for 25 minutes, Treatment 3 = CO_2 application of 100 mL once a day for 25 minutes, and Control Treatment = no CO_2 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°-30°C. 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. Carbondioxide 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 CO_2 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.

Data Analyses

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 analyized using ANOVA at the confidence level of 95 % (Steel and Torrie, 1989). To see the effect of CO_2 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.

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 weigth 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 *Thallasia hemprichii*. Sufficient availability of important components in photosynthetic activity could result in better biomass. Carbondioxiide (CO_2), water, and solar energy are major components needed by the seagrass for phosynthesis 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 availabity 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).

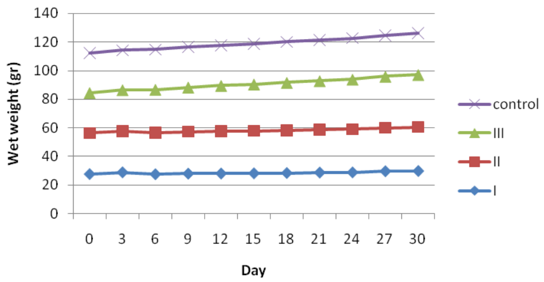

Fig. 1. Wet weight (gr) growth of *Thalassia hemprchii* under different treatments, CO_2 application of 100 mL for 25 minutes once in 3 days (P1), once in two days (P2), once a day (P3), and Control (C).

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%.

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 CO_2 application once a day had significant effect on the seagrass growth rate with $P3 > L = 0.747\% > 0.65\%$.

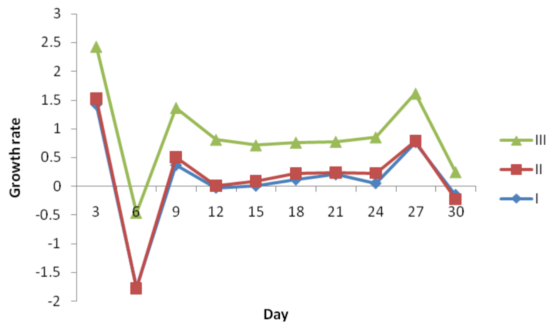

Fig. 2. Growth rate of *T. hemprichii*(%) with CO_2 application for 25 minutes once in 3 days (P1), once in 2 days (P2), and once a day (P3).

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 photysynthetic 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 $CaCO_3$. 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 $CaCO_3$ with slight pH variation ranging from 7 – 8.6. This variation was not followed with alkalinity difference. According to Effendi (2003), the alkalinity of CO_3^{2-} is stronger than the acidity of CO_2 at the equilibrial condition, OH^- ion the solution always exceeds H^+ ion. The alkalinity of treatment 3 highly varied from 50 – 80 mg/l $CaCO_3$, but pH was relatively the same in the normal condition ranging from 7.00 – 7.10. The lowest alkalinity occurred in day-21 after CO_2 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 $CaCO_3$. This high alkalinity could result from daily application of carbondioxide 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 $CaCO_3$. Good alkalinity ranges from 30 to 500 mg/liter $CaCO_3$. In nature, it ranges from 5 to hundreds mg/liter $CaCO_3$. The water alkalinity > 40 mg/liter $CaCO_3$ 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 CO_3^{2-} and HCO_3 in $CaCO_3$ unit (Dongoran,2003). It is made of carbondioxide and water that can dissolve the carbonate sedimentary rocks to become bicarbonates. The solubility of calsium carbonate decreases with increase in temperature and carbondioxide. Calsium carbonate reacts with carbondioxide to form calsium bicarbonate [$Ca(HCO_3)_2$] that has higher solubility than that of calsium carbonate ($CaCO_3$) (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 homogenuous 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 tretament 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 CO_2 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 CO_2 concentration. It was found in treatment 3 in day-12 and day-24, with pH of 7.00, and CO_2 concentration was 119.86 mg/l. In pH>8, no CO_2 concentration. It agrees with Mackereth *et al.*(1989) that pH is highly correlated with CO_2, in which the higher the CO_2 concentration, the lower the pH is and vice versa.

Conclusion

The best treatment on the growth rate of *T. hemprchii was* P3 with CO_2 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 P1<L= 0.093%<0.65%, and P2<L= 0.068%<0.65%, while that of P3 was significantly different from that of control.

References

Amiluddin, Masita N. 2007. Study on growth and carageenan content of seaweed, *Kappaphycus alvarezii,* infected with ice-ice disease in Pari waters, Seribu Islands. Thesis. Graduate School. Bogor Agriculture Institute, Bogor.

Anggadiredja A, Zatnika H, Purwoto, Istini S. 2007. Seaweeds Culture, processing, marketing of potential fisheries commodities. Penebar Swadaya, Jakarta. 146 p.

Cole GA. 1988. Textbook of Limnology. Third Edition. Waveland Press, Inc.,Illinois,USA. pp:401.

Duarte CM, Middelburg JJ, Caraco N. 2005. Major role of marine vegetation on the oceanic carbon cycle. Biogeosciences **2,** 1-8.

Duarte CM, Middelburg Aminudin. 2008. Study on carbon reserve in public forest cultivation (case study: public forest of Dengok, Playen Playen District, Gunung Kidul Regency). Thesis. Bogor: Postgraduate School, Bogor Agriculture Institute.

Duarte CM, Kennedy H, Marba N, Hendriks I. 2011. Assesing the capacity of seagrass meadows for carbon burial: current limitations and future strategis. Ocean Coast Manag. In press.

Effendi H. 2003. Study on water quality for aquatic living resources management. Kanisius Yogyakarta.

Kennedy H, Beggins J, Duarte CM, Fourqurean JW, Holmer M, Marba N, Middelburg JJ. 2010. Seagrass sediments as a global carbon sink: Isotopic constraints, Global Biogeochem. Cycles 24 GB4026, doi:10.1029/2010 GB003848.

Kiswara W. 1992. Seagrasses in the reef flat of Pari island, Seribu islands, Jakarta. Oseanology in Indonesia. No. **25,** 31-49.

Kiswara W, Ulumuddin YI. 2009. Role of coastal vegetation in global carbon cycle; mangrove and seagrass as carbon sinks. Workshop Ocean and climate change. Ocean as climate change control: the role of Indonesia sea in reducing the global warming process acceleration. Bogor, August 4th, 2009.

Kiswara W. 2010. Preliminary study: seagrass potential as carbon sink and absorption in Pari waters, Jakarta Bay. J. Indonesian Oseanology and Limnology di Indonesia.**36(3)**,361-376.

Mackereth, Heron J, Talling JF. 1989. Water Analysis. Freshwater Biological Association, Cumbria, UK.

Mattjik A, Sumertajaya M. 2002. Experimental designs with SAS Aplication and Minitab 1. 2nd ed. Bogor. IPB Press.

Mubarak H, Ilyas S, Ismail W, Wahyuni IS, Hartati ST, Pratiwi E, Jangkaru Z, Arifudin R. 1990. Technical guide to seaweed culture. Agricultural research and development board. Departement. Agriculture, Jakarta.

Nelleman, C. (ed.). 2009. Blue carbon: The role of healthy oceans in binding carbon. *A rapid response asseement*. United Nations Environment Programme. Norway.

Nelson DW, Sommers LE. 1996. Total carbon, organic carbon, and organic matter. In: Methods of Soil Analysis, Part 2, 2nd ed., A.L. Page *et al.*, Ed. Agronomy. 9:961-1010. Am. Soc. of Agron., Inc. Madison, WI

Odum EP. 1971. Fundamental of Ecology. W.E Saunders, Philidelphia. 574p.

Siregar SA. 2005. Disposal water processing Installation. Kanisius. Yogyakarta. Indonesia.

Sulistijo, Atmadja WS. 1996. Seaweed culture development in Indonesia. Puslitbang Oseanografi LIPI. Jakarta, Indonesia.

Tomascik JW, Mah AJ, Nontji A, MOOSA MK. 1997. The Ecology Of The Indonesian Seas, Part Two. Periplus Edition.Canada.