THE EFFECT OF ACIDIFICATION ON GROWTH AND PHOTOSYNTHESIS RATE OF SEAGRASS Thalassia hemprichii (Ehrenberg.) Ascherson

PENGARUH ASIDIFIKASI TERHADAP LAJU PERTUMBUHAN DAN FOTOSINTESIS LAMUN Thalassia hemprichii (Ehrenberg.) Ascherson

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ABSTRACT

Seagrass is a water plant that has flowers and ability to adapt to live and grow in the sea like a terrestrial plant. The survival of seagrass is greatly influenced by physical and chemical parameters of waters, such as pH, temperature, and salinity. The Intergovernmental Panel on Climate Change (IPCC) report by the end of 21st century, CO₂ in the atmosphere has doubled along with the industrial development. The increase in CO₂ in the atmosphere causes ocean acidification, it can change the chemical structure and decrease the pH of sea water. The low pH of sea water influences plant physiology such as the inhibition of photosynthesis and growth. The purpose of this study is to examine the effect of pH on the growth and photosynthesis rate of seagrass Thalassia hemprichii. The study used Completely Randomized Design with 3 treatments control (8.10-8.50), medium pH (7.76-8.00) and low pH (7.50-7.75) in 5 replicates. The results showed that growth rate, photosynthetic rate and chlorophyll content has a bigger value on control treatment than the low pH treatment. The ANOVA test results were not significant for all treatment variables and had a negative impact on the survival of seagrass.

Keywords: acidification, chlorophyll, photosynthesis rate, Thalassia hemprichii

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ABSTRAK

Lamun merupakan tanaman air yang memiliki bunga dan kemampuan beradaptasi untuk hidup dan tumbuh di laut seperti tanaman terestrial. Kelangsungan hidup lamun sangat dipengaruhi oleh parameter fisik dan kimia perairan, seperti pH, suhu, dan salinitas. Intergovernmental Panel on Climate Change (IPCC) melaporkan pada akhir abad 21, CO₂ di atmosfer selalu meningkat seiring dengan perkembangan industri. Peningkatan CO₂ di atmosfer menyebabkan terjadinya asidifikasi laut sehingga dapat mengubah struktur kimia dan pH air laut. Rendahnya pH air laut berpengaruh terhadap fisiologi tumbuhan seperti terhambatnya proses fotosintesis dan pertumbuhan. Tujuan penelitian ini adalah untuk menguji pengaruh pH terhadap pertumbuhan dan laju fotosintesis lamun Thalassia hemprichii. Penelitian ini menggunakan Rancangan Acak Lengkap dengan 3 perlakuan yaitu kontrol (8.10-8.50), pH sedang (7.76-8.00) dan pH rendah (7.50-7.75) dalam 5 ulangan. Hasil penelitian menunjukkan bahwa laju pertumbuhan, laju fotosintesis dan kandungan klorofil memiliki nilai lebih besar pada kontrol dibandingkan dengan perlakuan pada pH sedang dan rendah. Hasil uji ANOVA tidak signifikan untuk semua variabel perlakuan dan memiliki dampak negatif pada kelangsungan hidup lamun.

Kata kunci: asidifikasi, klorofil, laju fotosintesis, Thalassia hemprichii
I. INTRODUCTION

By the end of the 21st century it is estimated that carbon dioxide (CO₂) in the atmosphere has doubled along with industry development and increased usage of fossil energy such as coal, oil and gas (Chen & Miller, 1979; IPCC, 2007; Revelle & Suess, 1957). This condition can increase CO₂ in the atmosphere by nearly 40% and decrease the ocean pH by 0.3-0.4 units (Brouns, 1985; Caldeira & Wickett, 2003; Feely et al., 2004). Orr et al. (2005) modeled a decrease in pH by 7.949 in 2050 and 7.824 in 2100. The impact that can be generated from the increase of CO₂ in atmosphere is sea acidification (Turley, 2008). Acidification may cause chemical changes of carbonate in the relative proportions of CO₂, bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) to shift the total dissolved inorganic carbon from CO₂ to HCO₃⁻ (carbonic acid) and CO₂ (Ciais et al., 2014). Water conditions at low pH can inhibit photosynthesis, inhibit growth, decrease immune response, witness fertilization and calcification of organisms (Kurihara et al., 2004; Brothers et al., 2016). Ow et al. (2015) revealed that acidification has an impact on photosynthesis and seagrass productivity.

Seagrass is a water plant that has flowers and adaptability to live and grow in the sea like a terrestrial plant (Kawaroe et al., 2016). The seagrass ecosystem has many important roles, such as habitat and shelter of various biota, nursery ground, spawning ground and stabilizing aquatic sediments (Guinotte & Fabry, 2008; Koch et al., 2012; Christianen et al., 2013; Riniatsih & Endrawati, 2013). The seagrass ecosystem also plays an important role in the coastal carbon cycle (Oreska et al., 2017). One of seagrass species T. hemprichii known as dugong grass is a dominant species that can be found almost in all Indonesian waters and become key species of tropical seagrass in the Indo-Pacific region (Mukai, 1993).

Due to the important role of seagrass ecosystem makes several studies have been conducted to determine the response of acidification. Acidification can increase photosynthesis in Zostera marina and Phyllospadix torreyi (Beer et al., 2002; Koch et al., 2012), biomass changes and increased productivity of T. testudinum, Halophila uninervis, Cymodocea serrulata (Campbell & Fourqurean, 2013; Durako & Sackett, 1993; Ow et al., 2015). Based on several literature studies above, it is necessary for further study to determine the effect of acidification on growth rate and photosynthesis rate of T. hemprichii seagrass.

II. MATERIALS AND METHODS

The study was conducted from September to December 2017. T. hemprichii were taken in the Kepulauan Seribu while for transplantation and analysis of photosynthetic response was performed at the Marine Habitat Laboratory, Faculty of Fisheries and Marine Sciences, IPB University. Analysis of chlorophyll content was performed in the testing laboratory, Faculty of Agriculture, IPB University.

T. hemprichii was taken by digging the whole bud with the rhizome connected to the sediment. Afterwards the sample then was placed in a coolbox, then transferred to the aquarium at Marine Habitat Laboratory.

2.1. Research Design

The research was design using Completely Randomized Design (RAL). The aquarium was used as a container to grow 15 seagrasses for 3 treatments with 5 replications each. The treatment was pH in the range of 7.76-8.00 for medium level, low pH in the range of 7.50-7.75 and control. The position of the aquarium was placed randomly (Figure 1). The volume of sea water used is 29 L. The pH adjustment was performed by adding CO₂· 20.18 ppm for the low pH treatment and 19.79 ppm for the
mean pH, whereas the controls were added 19.20 ppm. LED light (Light Emitting Diode) P 800K was mounted 30 cm above the aquarium surface. Lighting time was 12 hours light and 12 hours dark. Sea water was replaced every 2 weeks while NASA fertilizer is added to support seagrass growth every 1 week as much as 29 mL. LED light (Light Emitting Diode) P 800K was mounted 30 cm above the aquarium surface. Lighting time was 12 hours light and 12 hours dark. Sea water was replaced every 2 weeks while NASA fertilizer is added to support seagrass growth every 1 week as much as 29 mL.

Physical chemical parameters are measured daily at 03.00 PM. Water quality parameters measured in situ that includes temperature using thermometer, salinity using refractometer, and pH using pH meter.

2.2. Growth Rate

Growth rate of seagrass were measured by leaves length at certain intervals, in the beginning and end of the observation. The formula for growth rate is as follows (Short & Coles, 2001):

\[ P = \frac{P_t - P_0}{\Delta t} \]

Information: 
- \( P \) = Leaf growth rate (mm/day);
- \( P_t \) = Leaf length at final measurement (mm);
- \( P_0 \) = Leaf length at initial measurement (mm);
- \( \Delta t \) = Time measurement interval (days).

2.3. Chlorophyll Content Analysis

Determination of chlorophyll content was administered on young adult leaves of each seagrass samples. The seagrass leaves was dried and then weighed. The sample then was added 90% acetone solution. Each tube was filled with solvent up to 6 ml then centrifuged at 4000 rpm for 12 minutes. All samples were frozen to prevent heat and light degradation. A total of 1 ml of supernatant was transferred into cuvette for spectrophotometry analysis at the wavelengths of 664.0 and 647.0 nm. Calculations of chlorophyll content (mg/g) are as follows (Granger & Izumi, 2001).

Chlorophyll a = 11.93 \( E_{664} \) - 1.93 \( E_{647} \) .... (2)

Chlorophyll b = 20.36 \( E_{647} \) - 4.68 \( E_{664} \) .... (3)

Information: \( E_{664} \) = Correction of absorbance, (absorbance at wavelength of 664 nm - absorbance at 750 nm).

2.4. Photosynthesis Rate

The seagrass leaves at each treatment were taken and dried before the measurement of photosynthesis rate. The measurement of photosynthesis rate were performed by LI-COR 6400XT Portable Photosynthesis System equipped with CO\(_2\) injector and LED light source. The leaves of seagrass were put into the chamber for a few seconds. Observational data were analyzed by program arrangement in the form of light intensity response curve (PAR= Photosynthesis Active Radiation) from 1-1200
μmol CO₂ m² s⁻¹ as abscissa and net photosynthesis as its coordinates so it obtain the curve of seagrass photosynthetic growth rate on control and every treatment observed (Soleh, 2017).

ANOVA was applied to test significant differences between experimental treatment (carbonate addition (CO₃²⁻) to decrease pH) in all response variables (growth rate, chlorophyll and photosynthesis rate) at value of P = 0.05 (Suliyanto, 2012).

III. RESULT AND DISCUSSION

3.1. Physics and Chemical Water Parameters

Seagrass survival is influenced by physical and chemical factors of water directly or indirectly. Physical and chemical water parameters are presented in Table 1. The pH value of waters at control ranged from 8.07 to 8.48. The pH range at pH treatment (7.76) is 7.75-7.83. The pH range at the pH treatment (7.58) is 7.57-7.65. The increase in pH is thought to be due to reduced CO₂ due to photosynthetic activity.

Table 1. Physical-chemical water parameters.

| Parameters | Treatment of pH |
|------------|-----------------|
|            | Control | Medium | Low |
| pH         | 8.32    | 7.76   | 7.58 |
| Temperature (°C) | 28.68 | 28.66 | 28.70 |
| Salinity (psu) | 32.48 | 32.89 | 32.60 |

The temperature range of the controls are 27.5-29.6 °C, for the medium pH treatment are 27.5-29.9 °C and for the low pH treatment are 27.4-29.6 °C. Based on the temperature in Table 1, the temperature of waters on the control and treatment of pH (7.76) and pH (7.58) is still within the optimum range for growth and photosynthesis on T. hemprichii which is 27-30°C (Kondoy et al., 2014). Water temperatures that fall outside the normal range can still be tolerated by the seagrass plants but will disrupt the physiological process. The temperature range of 25-30°C will increase photosynthesis along with rising temperatures. If the temperature of the waters exceeds the optimum limit of seagrass growth it can lead to stress, death and loss of genetic biodiversity due to the high respiratory process compared to photosynthesis (Chefaoui et al., 2018; Staehr & Borum, 2011).

The salinity of T. hemprichii in the controls is in the range of 30.4-34.4 psu. The pH treatment (7.76) is in the range of 30.6-34.8 psu while the pH treatment (7.58) is in the range of 30-35.2 psu. Based on the salinity value in Table 1 it shows that the salinity range in the control and treatment is still within the optimum salinity of 20-40 psu (Touchette & Burkholder, 2000). Salinity that exceed the optimum limit causes stress on seagrass, it can reduce chlorophyll content, nutrient absorption, decreased leaf growth rate, morphological changes, photosystem function and death (Kuo & Lin, 2010; Marin-Guirao et al., 2013). Based on Table 1 shows that temperature and salinity in the control and treatment of medium and low pH are at the optimum range for seagrass growth.

3.2. Growth Rate of T. hemprichii

Leaf length growth is the difference in leaves that grow within specified time intervals (30 days). Based on Figure 2, the average length of seagrass leaves in the control was 3.76 mm/day with the growth rate ranged from 2.07 to 4.63 mm/day. The average length of seagrass leaves in pH treatment (7.76) is 2.32 mm/day and growth rate is in the range of 0.33-6.33 mm/day. The pH treatment (7.58) has an average length of 2.21 mm/day with a hose value of 0.83-4.1 mm/day. The ANOVA test showed that the average leaf growth rate was not significantly different (P> 0.05) among treatments.

The growth rate of seagrass leaves is
directly proportional to the content of chlorophyll and the rate of photosynthesis. The low rate of leaf growth in the treatment is suspected because that the seagrass is still adapting to the new environment. Seagrass growth is influenced by internal factors such as physiology, metabolism and external factors such as nutrient content of the substrate fertility and marine environmental parameters (Alexandre et al., 2012; Ow et al., 2015). These environmental factors affect the metabolism of carbon and nitrogen. Lack of nitrogen in the pH treatment (7.76) and pH (7.58) affects the formation of chlorophyll that interferes with the photosynthesis process and inhibits growth in seagrass leaves.

![Leaf growth rate in control and treatment for 30 days.](image)

Figure 2. *T. hemprichii* leaf growth rate in control and treatment for 30 days.

3.3. **Content of Chlorophyll**

*T. hemprichii*

Chlorophyll is a pigment owned by plants and plays a role in the process of photosynthesis. Chlorophyll absorbs and uses the energy of sunlight for the synthesis of oxygen (O₂) and carbohydrates of CO₂ and water. Chlorophyll content on seagrass tissue can affect the physiological response. Seagrass plants contain only 2 chlorophyll pigments namely chlorophyll a and chlorophyll b. The difference of the chlorophyll pigment lies in the absorbed wavelength. Based on Figure 3 the average content of chlorophyll a in the controls were 1.83 and 1.10 mg/g with a chlorophyll a in the range of 1.22-2.49 mg/g and chlorophyll b 0.76-1.47 mg/g. The pH treatment (7.76) average content of chlorophylls a and b are 1.36 and 0.83 mg/g with of each chlorophyll is in the range of 1.09-2.33 mg/g and 0.75-0.83 mg/g. The pH treatment (7.58) has an average of chlorophyll a and b of 0.91 and 0.56 mg/g with of each ranging from 0 to 1.66 mg/g and 0-1.06 mg/g. The result of ANOVA analysis showed that average yield of chlorophyll content is not significant (P>0.05).

The amount of chlorophyll in a plant will affect the photosynthesis and growth of seagrass. The low chlorophyll content of treatments especially in low and medium pH is seen from the color of the seagrass leaf form pale to black (Figure 4).

The amount of chlorophyll is determined by the availability of light, depth, type of seagrass, leaf age and nutrient (Wiginton & McMillan, 1979; Lee et al., 2007; Clores & Carandang, 2013). The color changes on the leaves of the seagrass are suspected caused by a stressful condition to the environment. Adaptation mechanisms on seagrass when stress will depend on the intensity, type and duration of the disturbance. When in a state of stress, the seagrass will lose the chlorophyll and carotenoid molecules so the leaf color will be different when the leaves are naturally aging (Duarte et al., 2012). The pheophytin reaction occurs when the plant is in an acidic pH. In acidic conditions, chlorophyll will be very easily degraded and will form pheophytin due to the shift of two hydrogen (H) atoms (Dwidjoseputro, 1994). The pressure from H atoms causes the loss of Mg²⁺ ions which is the main components of chlorophyll so that the color of the leaves in the medium and low pH treatment are brown.
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3.4. Photosynthetic Rate T. hemprichii

The mean value of the photosynthetic rate (Figure 5) in the control of 19.11 μmol CO₂ m⁻² s⁻¹ is in the range of 15.62-21.39 μmol CO₂ m⁻² s⁻¹. The mean value of photosynthesis rate in pH treatment (7.76) is 12.61 μmol CO₂ m⁻² s⁻¹ in the range 0-18.28 μmol CO₂ m⁻² s⁻¹ while at pH (7.58) the value is 12.31 μmol CO₂ m⁻² s⁻¹ and is in the range of 0-17.71 μmol CO₂ m⁻² s⁻¹. The ANOVA test showed an insignificant pH decrease (P> 0.05) against the mean rate of photosynthesis. Seagrass capacity in response to changes in aquatic environments primarily to pH depletion depends on the availability of nutrients and light.

Factors that influence the rate of photosynthesis in addition to light and nutrient composition are chlorophyll content and tissue age. The rate of photosynthesis in the control was higher when compared to the treatment at pH (7.76) and pH (7.58), this corresponds to the chlorophyll content in each treatment. The low chlorophyll content of medium and low pH treatments caused a low rate of photosynthesis in seagrass T. hemprichii. This is because chlorophyll plays an important role in the absorption of free CO₂ in the waters (Ariyati
The availability of CO$_2$ in the waters affects the process of photosynthesis. Addition of CO$_3^{2-}$ results in reduced CO$_2$ free in the waters and an increase in the amount of O$_2$ (Susana, 1988).

In the control treatment, seagrass will use HCO$_3^-$ during photosynthesis which is abundant so that when photosynthesis takes place, seagrasses will use carbon from HCO$_3^-$ to replace CO$_2$. The HCO$_3^-$ is used by extracellular dehydration of HCO$_3^-$ into CO$_2$ through a carbonic anhydrase (CA) enzyme bound to the membrane (Uku et al., 2005).

**IV. CONCLUSION**

The ANOVA test were not significantly different among treatments, however, growth rates, chlorophyll content, and photosynthetic rate of *T. hemprichii* seagrasses had greater value on the control when compared with lower pH treatment. Acidification has negative effect on growth rate, chlorophyll content and photosynthesis rate of *T. hemprichii*.

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