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Effect of Salinity on Biomass of *Avicennia Marina and Rhizophora Mucronata* That Grown at Reed Bed System Reactor With Continuous Flow

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Abstract: Salinity is one of the environmental factors having an important role in controlling mangrove growth. Each type of mangrove has different adaptability. This condition causes the differences of structure and composition of mangrove with distinctive boundaries, ranging from zones close to land to zones close to the ocean. This research was aimed to examine the ability to grow mangrove (*Avicennia marina* and *Rhizophora mucronata*) at various levels of salinity, with addition bacteria *Vibrio alginolyticus* using reed bed system reactor with continuous flow. The reactors were arranged in series system, namely reactor with *Avicennia marina* (AM), reactor with *Rhizophora mucronata* (RM), reactor with *Avicennia marina* and bacteria of *Vibrio alginolyticus* (AMVA), and reactor with *Rhizophora mucronata* and *Vibrio alginolyticus* (RMVA). The artificial salinity that was used i.e 20 ‰ and 25 ‰.The indicator of mangrove plant growth was conducted by physical observation during the exposure time. The fresh weight (FW) and dry weight (DW) were measured at day 0 and the last day of experiment. The monitoring parameters such as pH and temperature were also measured. The results showed the FW and DW increased in all reactors. *Avicennia marina* with added bacteria has the greatest growth at concentration salinity of 25‰ with DW was 69,27 g. Salinity of 25‰ showed a greater growth result than salinity with a concentration of 20‰. This inceasing of DW of *Avicennia marina* and *Rhizophora mucronata*.

1 INTRODUCTION

Salinity was the level of salinity or dissolved salt contained in water in grams per litre of seawater (Chimayati and Titah, 2019). Salinity is one of the defining environmental features of mangrove habitats and ranges from seasonally freshwater to hypersaline conditions (Flowers and Colmer, 2008). Salinity can interpret as a condition where salt dissolves excessive and caused bad conditions for plant growth (Syakir *et al*., 2008). According to Bengen (2003) salinity greatly determines the development of mangrove area, this can occur because of the influence of salinity which can divide mangrove into several zonations, from the nearest zonation or bordering the sea (proximal zone) to the farthest zonation from the sea (distal zone). According to Purwanti *et al*. (2006), classification of the sample water for salinity parameters was divided into freshwater with a value of <0.5‰, brine water with the salinity ranging from 0,5–30‰, salty water 30–50‰ and very salty water or sea water has a salinity of more than 50‰.

Mangrove has the ability to tolerate the sea salinity and grow at above average levels (Ananthakrishnan, 1982; Flowers *et al*., 1977). Mangrove forest ecosystems are often called brackish forests because they are located in brackish areas, which are areas with salinity or salinity between 0,5‰ and 30‰. Another name is the tidal ecosystem because it is located in areas affected by tides (Indriyanto, 2006). They adapt themselves to fluctuating environment in several ways such as salt exclusion from roots (Hegemayer, 1997), salt secretion (Fitzgerald *et al*., 1992) and accumulating organic acids as osmotica to counter toxic effects of salinity (Popp, 1984). Mangrove plants comprise a heterogeneous group of independently derived lineages that are defined ecologically by their location in upper intertidal zones of tropical and sub-tropical climates and physiologically by their ability

to withstand high concentrations of salt or low levels of soil aeration (Basyuni *et al*., 2007).

The flora community found in mangrove forests has undergone adaptation and specialization as a mechanism for living in an environment with high levels of salt (Kustanti, 2011). Mangrove can adapt to low oxygen levels, tolerate high salt levels and can adapt to unstable soils and the influence of tides (Bengen, 2003). According to Saparinto (2007), mangroves depend on seawater (tides), freshwater, and sediment as a source of nutrients. In high salt conditions, plants will face two problems are obtain water from negative potential groundwater and overcome the high ion concentration of sodium, carbonate, and chloride which may be toxic (Salisbury and Ross, 1995). One indicator of mangrove growth is influenced by sediment where it lives, which contains macro and micronutrients, oxygen, and fresh water to maintain a balanced salt content in the physical (Chrisyariati *et al*., 2014). Limiting factors for mangrove production and growth include temperature and sunlight, salinity, anoxia and tides, bioturbation, and nutrient availability (Alongi, 1998). The growth and physiological mechanisms of mangroves differ in nature due to their complexity of structure and differences flooding regime, tidal inundation, a rapid influx of extra nutrients as well as the type of soil (Naidoo, 1987).

Avicennia has the ability to tolerate a wide range of salinity. The species is able to grow well in salinity that is close to bargain up to 90 ‰ but at extreme salinity, the tree grows stunted and the ability to produce fruit is lost (Noor *et al*., 2006). *Avicennia marina* collects the highest ion concentration from *Rhizophora mucronata* (Scholander *et al*., 1962), which means that the ability of *Rhizophora mucronata* to accumulate inorganic ions was lower than that of *Avicennia marina* (Titah *et al*., 2019).

The influence of salinity on mangrove growth was reported by Clough (1984) who states that the highest number of *Avicennia marina* and *Rhizophora stylosa* dry weight was obtained when grown at 25‰ seawater content. He also reported that CI^- and Na^+ ion levels were greater than K^+, Ca^{2+}, and Mg^{2+} ions in mangrove plant roots, stems and leaves which grown in five different concentrations of seawater that he tried. Stem and Voigt (1959) in Tomlinson (1986) argue that it was better to use low level of seawater for breeding *Rhizophora*. Connor (1969) in Tomlinson (1986) found the optimum conditions for *Avicennia marina* growth was in a solution containing 50‰ Na^+ ions and Na from seawater.

Bacteria can increase plant tolerance to environmental conditions that might reduce plant growth or development (Sulastri, 2018). *Vibrio alginolyticus* as helobacterium bacteria, where these bacteria can live in areas with high salt levels, are resistant to radiation and live in salt crystals. It functions in the process of the nutrition cycle and supports the life buffer of the ecosystem environment (Thompson *et al*., 2004). *Vibrio alginolyticus* bacteria was indeed found in saline water. These bacteria can grow and live in the area of plant roots which were in water that has a high level of salinity. *Vibrio* bacteria grows at pH 4–9 and optimally at pH 6,5–8,5 or under alkaline conditions with pH 9,0 (Chimayati and Titah, 2019). *Vibrio* bacteria could die under the acidic conditions. Kurniawan *et al*., (2018) reported that *Vibrio alginolyticus* need time of 2 h at pH 8 to grow, meanwhile it need time of 48 h at pH 5. It indicated that the bacteria did not develop at pH below 5, it showed by the value Optical Density (OD) was 0.

Plant growth can be defined as the enhancement size process and number of plant cells followed by the growth of plant dry weight, while the development of plants can be interpreted as a process towards achieving maturity (Kolinug *et al*., 2014). Plant growth and development is divided into two phases, they are vegetative growth phase and the generative growth phase (Prayunita, 2012). According to Popp (1994), mangroves collect high concentrations of inorganic ions like most other salt tolerant plants that function in leaf and other tissue osmoregulation. This form of osmoregulation involves the synthesis and accumulation of organic compounds sufficient to decrease the osmotic potential of cells and increase turgor pressure (Kusumiyati *et al*., 2017). Flowers *et al*. (1977) argue that in the early stages of adaptation to high salinity or the increase of salinity when the salt concentration in the liquid was increasing, the rate of ionic absorption was related to the growth rate of the plant. Mangrove plants take salt as nutrients for their growth needs.

The aim of this study was to determine the effect of salinity on the growth of mangrove *Avicennia marina* and *Rhizophora mucronata* with artificial salinity variation of 20‰ and 25‰ in a reed bed system reactor and combining with *Vibrio alginolyticus* bacteria.

2 MATERIALS AND METHODS

2.1 Place of Research

This research was conducted at the greenhouse of the Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember, ITS. ,or the implementation of a reed bed system reactor and the Environmental Remediation Laboratory in same department for bacterial propagation and analysis parameters

2.2 Material and Method

2.2.1 Bacterial Preparation

The inoculation stage used NA *(Nutrient Agar*) (Merck, USA) and TCBS (*Thiosulfate Citrate Bile Salt Sucrose*) (Merck, USA) for selective media. NA media was used as the initial inoculation media. TCBS media was a special selective media for *Vibrio alginolyticus* bacteria. The selective media was used for the breeding of bacteria. This stage was conducted to confirm that the growing bacteria on the media was *Vibrio alginolyticus*. In this study, the addition of inoculum *Vibrio alginolyticus* was 5% (v/v) in each reactors or it reached 300 mL/reactor. The preparation of TCBS media was conducted by dissolving of 22,25 g of TCBS media in 250 mL sterile aquadest and put it in a 250 mL of erlenmeyer. Around 8 g of NB media was used for preparation media. Before the media was dissolved with the aquadest, the aquadest must be sterilized using a autoclave (Hirayama, Japan). The dissolving media process was conducted using a stirring rod on a heating stove until the media boiled. After that,tThe media was poured into an aseptic sterile petri dish. After the media thickens, the petri dish was turned, then the media was stored in the refrigerator. The regrowth of *Vibrio alginolyticus* was conducted by inoculating those bacteria on new TCBS media using ose All inoculation activities must be sterile by working near the Bunsen and ose needles must also always be sterile. After that, the inoculting media was put in an incubator for 24 h at 37^{o}C. After the bacterial growing, the bacteria was transferred into sterilized NB (*Nutrient Broth*) media (Merck, USA) and put it in the orbital shaker KIA Japan for 2 days to get the value OD=1. OD measurements were carried out using a spectrophotometer GENESYS^{TM} 30 Visible Thermo Scientific USA. . Bacteria that have a value of OD=1, the bacteria was ready to be pour into the reactor.

2.2.2 Plant Preparation

This research used two species of mangrove *Avicennia marina* and *Rhizophora mucronata*. All plants were collected from nursery of mangrove at Wonorejo, Surabaya. The age of plants were about 3 months. The second stage was to prepare mangrove plants by separating each type of mangrove and then cleaning it by washing the remaining sludge that was attached to the roots. Before all plants were used for research, mangrove plants were acclimatized for 2 weeks to determine the ability of plants on concentrate of saline water that be used.

2.2.3 The Artificial Saline Preparation

This research was carried out by an experimental method and by observation the mangrove condition during the operation of reactor. The saline water that be used in this study was an artificial salinity. The artificial salinity was made using distillation water and pro-analysis NaCl powder (Merck, USA). The pro-analysis NaCl was dissolved in distilled water. Around 5,370 g of pro-analysis NaCl was needed to make salinity of 20‰, and it needed 6,712.5 g to make salinity of 25 ‰.

2.2.4 Reactor Preparation

The reed bed reactors were made from fiberglass with dimension of 70 x 50 x 40 cm. The reactor in this study used a reactor made from fiberglass. Fiberglass is a strong and anti-rust material (Sunyoto *et al*., 2016). There were 8 reactors, as 4 reactors with the addition of bacteria, and 4 reactors without addition bacteria This research was conducted using a reed bed system reactor with a series arrangement in a continuous with debit 18 mL/minute. Preparation of reed bed system reactors in series was carried out based on the zoning of mangrove species growth ecosystems in nature.

Figure 1 described the reed bed system reactor. Figure 2 showed the reactors arrangement with the code of each reactor:

- AMVA 25-RMVA 25: *Avicennia marina* + *Vibrio alginolyticus* 25‰ - *Rhizophora mucronata* + *Vibrio alginolyticus* 25‰
- AM 25-RM 25: *Avicennia marina* 25‰ - *Rhizophora mucronata* 25‰
- AMVA 20 - RMVA 20: *Avicennia marina* + *Vibrio alginolyticus* 20‰ - *Rhizophora mucronata* + *Vibrio alginolyticus* 20‰
- AM 20 - RM 20: *Avicennia marina* 20‰ - *Rhizophora mucronata* 20‰

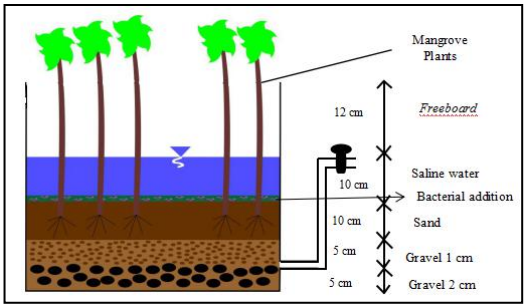

Fig. 1. Reed bed system reactor with bacterial addition

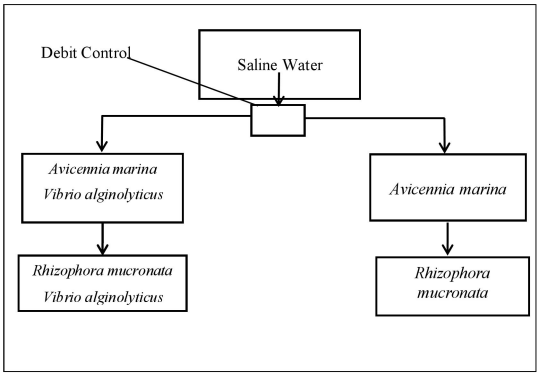

Fig. 2. The reactors arrangement

The media composition of each reactor was as follows: layer of gravel with diameter of 2 cm and height of 5 cm. The second layer of gravel with diameter of 1 cm and height 5 cm. The third layer was fine sand with height of 10 cm and artificial saline water was put on the top of the filter media. The high of artificial saline water was 10 cm and it needed 33,5 L for 1 reactor. .

Monitoring parameters also were measured. Those parameters were pH and temperature. The pH measurement was carried out using a portable pH meter digital Senz pH Singapore. Temperature measurement was conducted using. OHAUS Starter 3100C Conductivity Bench USA.

The fresh weights (FW) and dry weights (DW) were measured for each part of the sampled plants (roots, stems, and leaves). The FW was conducted as soon as possible after plants were cleaned using tissue. All of the plant parts were put in an oven at 105°C for 24 hours for the dry weight measurement. Lastly, the DW of whole plants can be calculated.

The calculation of Plant Water Concentration (PWC) was conducted by formula based on Penuelas *et al*. (1997).

$$PWC = ((FW - DW)/DW)\ 100$$

3 RESULTS AND DISCUSSION

Preliminary research conducted by acclimatization that aims that the test biota that is used in this study is that mangroves can adapt to the conditions or environmental media where the experiment and where plants can adjust to the conditions of the media used in the study.

Physical observations of mangrove plants were carried out during the acclimatization of mangrove plants at salinity concentrations of 20‰ and 25‰. Acclimatization was also aimed at making plants able to adjust to the growing environment in the treatment (Cahyani *et al*., 2016). Acclimatization results obtained *Avicennia marina* and *Rhizophora mucronata* can grow well at salinity concentrations of 20‰ and 25‰.

Based on observations for 2 weeks, there was no significant changes on *Avicennia marina* plants. The leaves and the stems of plants showed good conditions This indicated that *Avicennia marina* plants can survive at salinity concentrations of 20‰ and 25‰. The wilting plant did not occur during acclimatization. However, some leaves of *Avicennia marina* showed discoloration at a concentration of 25‰ without bacteria addition.

Rhizophora mucronata plants can survive with concentrations of 20‰ and 25‰, although some withering leaves occurred. Based on Titah *et al* (2018), that the salinity concentration of 30‰ can be toxic to *Rhizophora mucronata*.

The range of saline temperature were 29.ºC - 32ºC (Figure 3), Mangrove and bacteria can live in this temperature. Bacteria can survive, grow and develop at certain temperature limits. *Vibrio alginolyticus* can survive at optimum temperatures between 30-35ºC, while at 4ºC and 45ºC the bacteria cannot grow. Bacteria *Vibrio alginolyticus* will die at 55ºC (Prajitno, 2005). The temperature in several reactors showed the similar value due to the reactors were placed in same area and sunlight can shine down to all reactors. Based on results of water temperature, temperature value was a good temperature for the growth of planted mangroves, especially *Rhizophora sp.* According to Saparinto (2007), a good temperature range for the growth of mangrove species *Avicennia sp.* at temperatures of 18 - 20ºC, *Rhizophora sp*., *Ceriops sp*., *Excoecaria sp*., *Lumnitzera sp*. good growth at temperatures 26 -

28ºC. Meanwhile *Bruguiera sp*. Can grow well at a temperature of 27ºC.

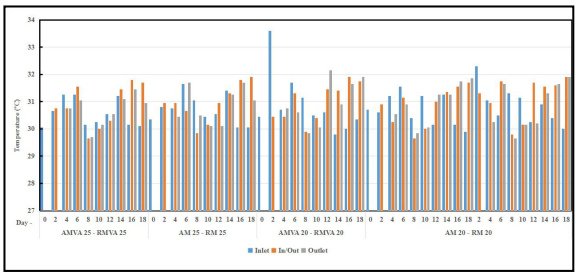

Figure 3. Temperature Measurement

The parameter of pH was a measure of acidity or basicity of liquid. Normal pH were 6-8. The pH of water depends on the type of discharge of water (Fardiaz, 1993). The value of pH at all reactors showed a neutral pH during running the reed bed reactor. Based on the pH data, the pH range shows 5.8 - 8.1 (Figure 4). The pH affected the growth rate of the bacteria. Each organism has a different optimum pH range. Mangrove can survie at pH of 6 – 8. Mangroves with age of 36 months old have a resistant on large water range of pH. It could also cause old mangroves increasingly have a greater tolerance for pH and salinity because it already has a strong root system compared to younger mangroves (Chrisyariati *et al*., 2014). The average pH is between 6 to 8. This showed that the pH of the waters is still in the range both for mangrove vegetation and aquatic biota. According to Koch (2001), pH was closely related to decomposer activity. The decomposer activity is so low that the overhaul of organic material becomes inorganic slow at acidic condition. The slow processes of decomposition could greatly inhibit vegetation growth due to a lack of nutrient and mineral supply. In addition, a pH value of 6.0 to 6.5 can reduce the diversity of plankton and benthic species (Effendi 2003).

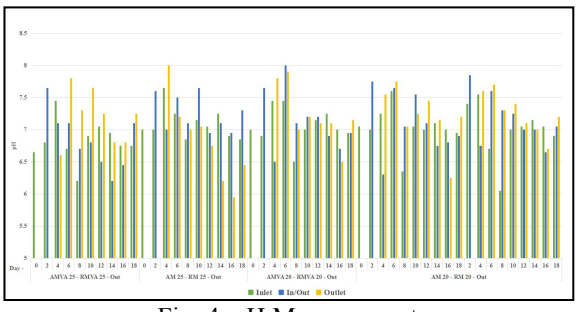

Fig. 4. pH Measurement

The results of the growth and development process can be observed from the fresh weight and dry weight. Fresh weight is the result of the measurement of the fresh weight of plant biomass as an accumulation of material produced during growth. Therefore observation of fresh plant weight and fresh weight is needed to determine the plant biomass (Buntoro *et al.*, 2014). Whereas dry weight according to Gardner *et al* (1991), is a result of the net hoarding of CO_2 assimilation throughout the growing season which reflects the accumulation of organic compounds that plants have successfully synthesized from inorganic compounds, especially water and CO_2.

Figure 5 showed the FW and DW of *Avicennia marina* and *Rhizophora mucronata* during the operation of a reed bed reactor for 18 days in salinity of 20‰ and 25‰. Based on the figure, the FW and DW of *Avicennia marina* and *Rhizophora mucronata* increased. It indicated that *Avicennia marina* and *Rhizophora mucronata* can grow normally during the operation of a reed bed reactor.

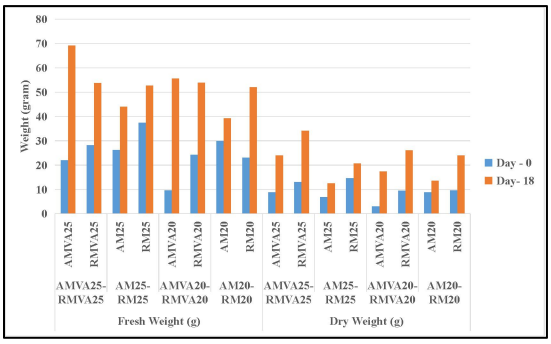

Fig. 5. FW and DW Measurement

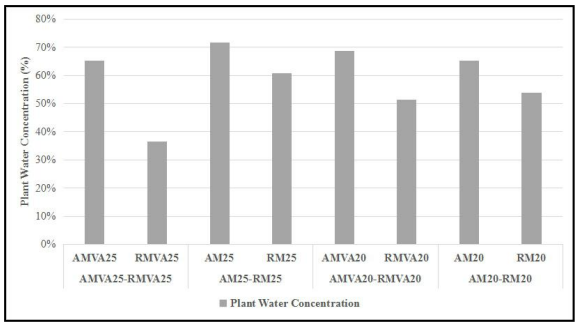

Fig. 6. Plant Water Concentration

.*Avicennia marina* plants with the addition of *Vibrio alginolyticus* at a salinity concentration of 25‰ showed the highest of FW compared to other plants on the last day. The FW of *Avicennia marina* was 69.26 g, and the DW was 24.03 g. This value of DW was less when compared to DW of *Rhizophora*

mucronata plants in the same conditions get the highest value of DW compared to other plants. It reached 34.16 g and FW value of 53.8 g. The addition of *Vibrio alginolyticus* may has a role to uptake the nutrient such as ion Na dan ion Cl. Based on Westrich *et al*. (2016), *Vibrio* play a key role in s an essential micronutrient of Fe cycling.

This indicated that the absorption of salinity by *Rhizophora mucronata* with the addition of *Vibrio alginolyticus bacteria* was very good, Based on Fig. 5 the *Rhizophora mucronata* plant with the addition of *Vibrio algionolyticus* bacteria showed the value of FW and DW values were not different. It indicated that the water content in the *Rhizophora mucronata* plant with the addition of *Vibrio algionolyticus* bacteria were stable.

Based on the calculation of water plant concentration (WTP), on the Fig. 6, the value of WTP in some reactors were in that range. However, the WTP value in the reactor with *Rhizophora mucronata* with the addition *Vibrio alginolyticus* at salinity concentration of 25‰ was 36%. These the best WTP value for plant were 50-70%.

Based on the statement on the growth of the plant itself can be considered as an increase in FW and accumulation of DW. The good growth of mangrove plants can be shown by the increasing of DW (Nurdin, 2008).

The difference in DW can be caused by by the number of leaves. The leaves were a place for the accumulation of plant photosynthesis. An increase in the process of photosynthesis can also increase the results of photosynthesis. The increasing of photosynthesis can increase the organic compounds in plant. The organic compound could be transplanted to all plant organs and affect the dry weight of plants.

4 CONCLUSIONS

Based on the results, the level of salinity affects the biomass of mangrove plants. Based on the calculation of FW and DW values, the FW of *Avicennia marina* with the addition of *Vibrio alginolyticus* bacteria in the salinity concentration level of 25‰ was 69.27 g. It was the highest FW value compared to other plants. The highest value of DW was obtained in the *Rhizophora mucronata* plant with the addition of *Vibrio alginolyticus* at a salinity concentration of 25‰, it reached to 34.16 g. In the *Rhizophora mucronata* plant with the addition of *Vibrio alginolyticus* bacteria at a salinity concentration of 25‰ was the most stable water content value with a FW value of 53.8 g and DW 34.16 g resulting in a water content of 57.46%. In conclusion, concentration of salinity and addition of *Vibrio alginolyticus* can affect the FW and DW of mangrove plants in a reed bed system reactor with continous flow.

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