The Effect of Salinity on the Biomass of AVICENNIA MARINA and RHIZOPHORA MUCRONATE 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, using reed bed system reactor with continuous flow and the addition of the bacteria *Vibrio alginolyticus*. 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 ‰. Physical observation of the mangrove growth indicators was conducted 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 had the greatest growth at the salinity concentration of 25‰ with 69,27 g of DW. Salinity of 25‰ showed a greater growth result than salinity with a concentration of 20‰.

1 INTRODUCTION

Salinity is 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 be interpreted as a condition where salt dissolves excessively and causes 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 growth areas into several zones, 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 is 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, which 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).

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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: obtaining water from negative potential groundwater and overcoming the high ion concentration of sodium, carbonate, and chloride which may be toxic (Salisbury and Ross, 1995). One indicator of mangrove growth is it is physically influenced by the sediment where it lives, which contains macro and micronutrients, oxygen, and fresh water to maintain a balanced salt content (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 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 is lower than that of *Avicennia marina* (Titah et al., 2019).

The influence of salinity on mangrove growth was reported by Clough (1984) who stated 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+, Ca2+, and Mg2+ 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*, a helobacterium bacteria, can live in areas with high salt levels and 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 is 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* needed 2 h at pH 8 to grow, meanwhile it needed 48 h at pH 5. He indicated that the bacteria did not develop at pH below 5, shown by an Optical Density (OD) value of 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: 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 combined with *Vibrio alginolyticus* bacteria.

2 MATERIALSAND METHODS

2.1 Location of Research

This research was conducted at the greenhouse of the Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember, ITS, for the implementation of the reed bed system reactor and at the Environmental Remediation Laboratory in same

department for bacterial propagation and analysis of the growth 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 is 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 the 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 of sterile aquadest and then it was put in a 250 mL of erlenmeyer. Around 8 g of NB was used for preparation media. Before the media was dissolved with the aquadest, the aquadest must be sterilized using a autoclave (Hirayama, Japan). The media dissolving was conducted using a stirring rod on a heating stove until the media boiled. After that, the media was poured into an aseptic sterile petri dish. After the media thickened, 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 a 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 inoculating media was put in an incubator for 24 h at 37oC. After the growing process, the bacteria was transferred into a sterilized NB (Nutrient Broth) media (Merck, USA) and put in the orbital shaker KIA Japan for 2 days to get the OD value of 1. OD measurements were carried out using a spectrophotometer GENESYSTM 30 Visible Thermo Scientific USA. Bacteria with an OD value of 1 meant that it was ready to be poured 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 the mangrove nursery in Wonorejo, Surabaya. The age of the plants was about 3 months old. The second stage was to prepare mangrove plants by separating each type of mangrove and then cleaning it by washing the sludge on the roots. Before all plants were used for research, the plants were acclimatized for 2 weeks to determine the ability of plants to grow on the concentrate of saline water to be used.

2.2.3 The Artificial Saline Preparation

This research was carried out by an experimental method and by the observation of the mangrove condition during the operation of reactor. The saline water used in this study had 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 a salinity of 20‰, and it needed 6,712.5 g to make a salinity of 25 ‰.

2.2.4 Reactor Preparation

The reed bed reactors in this study was constructed from fiberglass, measuring 70 x 50 x 40 cm. Fiberglass is a strong and anti-rust material (Sunyoto et al., 2016). There were 12 reactors: 4 reactors with the addition of bacteria, 4 reactors without bacteria and 4 reactors without plants as the control. The reed bed system reactors were then arranged in a series arrangement with a continuous water debit of 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 and 2 describe the reed bed system reactor.
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‰
- BK1 25 - BK2 25:
  Control Reactor 1 25‰ - Control Reactor 2 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‰
- BK1 20 - BK2 20:
  Control Reactor 120‰ - Control Reactor 2 20‰

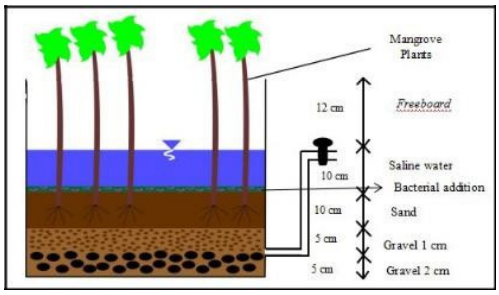

Figure 1: Reed bed system reactor with bacterial addition.

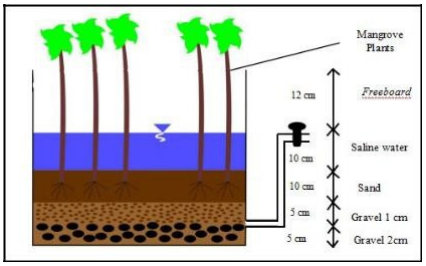

Figure 2: Reed bed system reactor without bacterial addition.

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

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 plant parts were put in an oven at 105°C for 24 hours for the dry weight measurement. After that, the total DW of all plants could be calculated.

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

$$PWC = ((FW - DW) / DW)\ 100 \qquad (1)$$

Preliminary research conducted by acclimatizing the test biota used in this study revealed that mangroves were able to adapt to the conditions or environmental media of the actual experiment and that the plants were able to 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 plantsable to adjust to the growing environment in thetreatment (Cahyani *et al*., 2016). Acclimatization results obtained showed that *Avicennia marina* and *Rhizophora mucronata* were able to grow well at salinity concentrations of 20‰ and 25‰.

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

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

The range of saline temperature was 29^{o}C - 32^{o}C (Figure 3). Mangrove and bacteria can live in this range of temperature. Bacteria can survive, grow and develop at certain temperature limits. *Vibrio alginolyticus* can survive at optimum temperatures between 30-35^{o}C, while the bacteria cannot grow below 4^{o}C and above 45^{o}C: *Vibrio alginolyticus* will die at 55^{o}C (Prajitno, 2005). During the experiment, the temperatures of several reactors were similar because the reactors were placed in same area and sunlight reached all reactors. Measurement showed that the water temperature was suitable for the growth of the planted mangroves, especially for *Rhizophora sp.* According to Saparinto (2007), mangrove species *Avicennia sp.* grows well at temperatures between 18-20ºC, *Rhizophora sp.*, *Ceriops sp*., *Excoecaria sp*., *Lumnitzera sp*. at 26- 28ºC, and *Bruguiera sp*. at a temperature 27ºC.

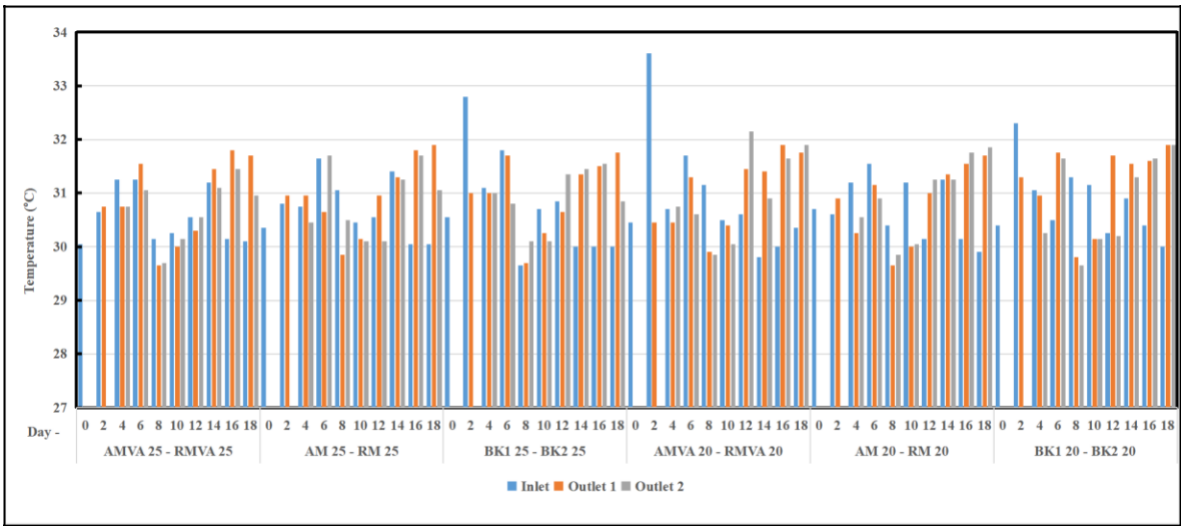

Figure 3: Temperature measurement.

3 RESULTS AND DISCUSSION

The parameter of pH is a measure of acidity or basicity of a liquid. A normal pH is represented by the value range of 6-8. The pH of water depends on the type of the discharge of water (Fardiaz, 1993). The value of pHat all reactors showed a neutral pH when the reed bed reactor was run. Based on the data, the average pH range was 5.8-8.1 (Figure 4). The pH affected the growth rate of the bacteria. Each organism has a different optimum pH ranges: mangroves can survive at pH levels of 6-8. Mangroves aged 36 months are more resistive to large water pH range: older mangroves are known to have greater tolerance for pH and salinity ranges because they have a stronger root system compared to younger mangroves (Chrisyariati*et al*., 2014). The average pH value range of this experiment was 6 to 8. This showed that the pH level ofthe water was still in an acceptable range for both the mangrove and the aquatic biota growth. According to Koch (2001), pH level is closely related to decomposer activity: the more acidic the environment is for the decomposer, the slower the decomposing process of inorganics. The slow process of decomposition could greatly inhibit vegetation growth

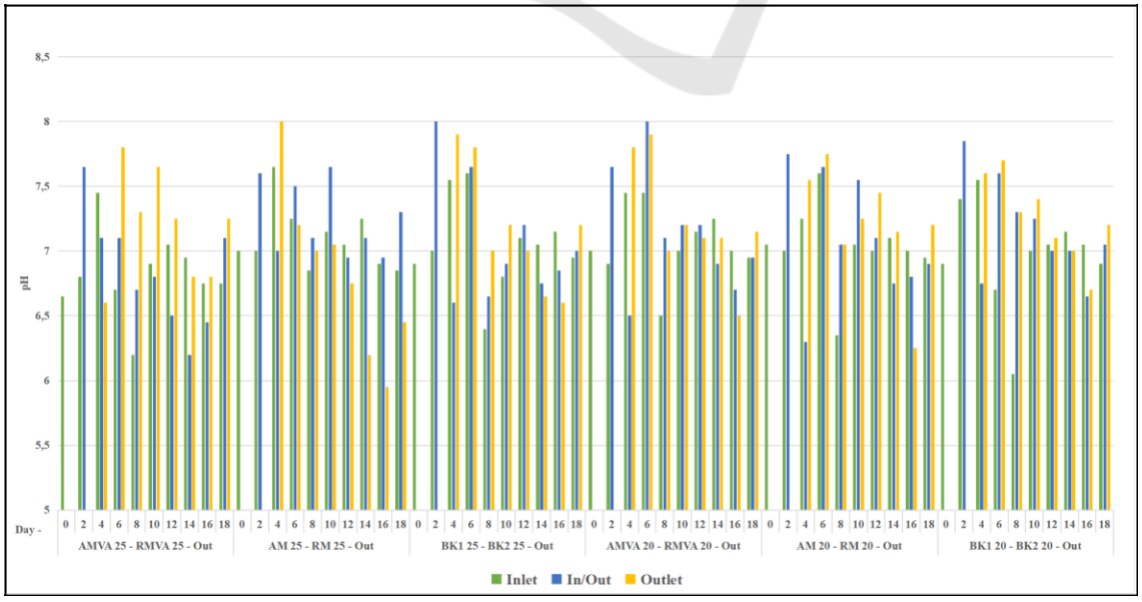

Figure 4: pH measurement.

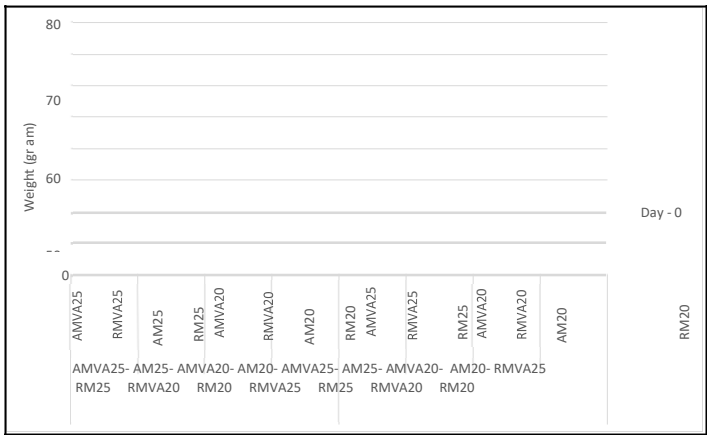

Figure 5: FW and DW measurement.

due to a lack of nutrient and mineral supply. In addition, a pH value range of 6.0 to 6.5 can reduce the diversity of plankton and benthic species (Effendi 2003).

The results of the growth and development process can be observed from the fresh weight and dry weight. Plant fresh weight is the result of the measurement of the fresh weight of plant biomass and the total accumulation of material produced during the growth. Therefore, the 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 the result of the net hoarding of CO_2 assimilation throughout the growing season which reflects the accumulation of organic compounds plants have successfully synthesized from inorganic compounds, especially water and CO_2.

Figure 5 shows the FW and DW of *Avicennia marina* and *Rhizophora mucronata* during the operation of the 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.

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 of the experiment. The FW of *Avicennia marina* was 69.26 g, and the DW was 24.03 g. This DW value of *Avicennia marina* is the lowest of all plants used in this experiment: *Rhizophora mucronata* plants, in the same conditions, had the highest DW value of all plants with 34.16 g of DW and an FW value of 53.8 g. The addition of *Vibrio alginolyticus* is suspected to play a role in the uptake of nutrients such as Na dan Cl ions. According to Westrich *et al*. (2016), *Vibrio* bacteria does play a key role in the cycling of the essential micronutrient Fe.

This indicates that the absorption of salinity by *Rhizophora mucronata* with the addition of *Vibrio alginolyticus* bacteria is very good. Based on the results of the FW and DW calculations, the rather stable value of FW and DW would produce a stable water content. Figure 6 shows that the FW and DW

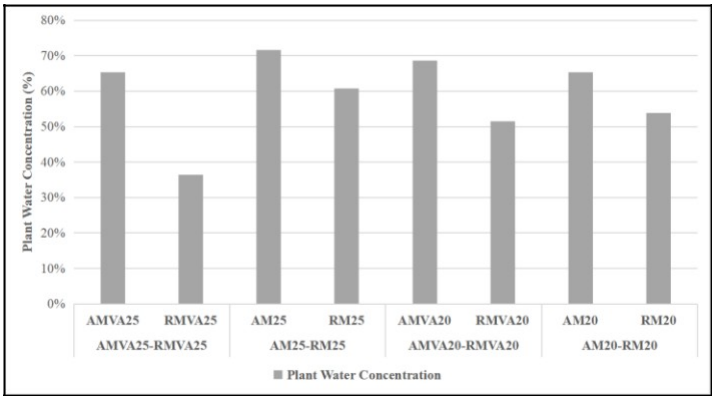

Figure 6: Plant water concentration.

Table 1: Plant water concentration calculation (Day – 18).

		Fresh Weight	Dry Weight	Plant Water Concentration
AMVA25-RMVA25	AMVA25	69.27	24.03	65%
	RMVA25	53.8	34.17	36%
AM25-RM25	AM25	44.1	12.53	72%
	RM25	52.7	20.67	61%
AMVA20-RMVA20	AMVA20	55.57	17.4	69%
	RMVA20	53.9	26.17	51%
AM20-RM20	AM20	39.23	13.6	65%
	RM20	52.07	24.02	54%

values of *Rhizophora mucronata* plant with the addition of *Vibrio algionolyticus* bacteria were not different. It indicates 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 Figure 6 and Table 1, the value of WTP in some reactors were in thatrange. However, in the reactor with *Rhizophora mucronata* and *Vibrio alginolyticus* at a salinity of 25‰, the WTP value was 36%.

These results are in accordance with the best WTP value for plants (50-70%). Based on the prior definitions of plant growth, it can be considered that there was growth as there was an increase in FW and accumulation of DW. A good growth ofmangrove plants is shown by the increase in DW values (Nurdin, 2008).

The difference in DW can be caused by the numberof leaves. The leaves are a place for the accumulationof plant photosynthesis. An increase in the process of photosynthesis can also increase the result of photosynthesis. The increase of photosynthesis activity can increase the amount of organic compounds in plant. The organic compound could then be transportedto all plant organs and affect the dry weight of plants.

4 CONCLUSIONS

Based on the results, the level of salinity affects the growth rate 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 a salinity concentration level of 25‰ was 69.27 g, which is highest FW value of all other plants. The highest value of DW was obtained in the *Rhizophora mucronata* plantwith the addition of *Vibrio alginolyticus* in a salinityconcentration of 25‰, reaching 34.16 g. The*Rhizophora mucronata* plant with the addition of*Vibrio alginolyticus* bacteria in a salinity concentration of 25‰ had the most stable water content value with an FW value of 53.8 g and DW of 34.16 g, resulting in a water content value of 57.46%. In conclusion, concentration of salinity and the addition of *Vibrio alginolyticus* can affect the FW and DW of mangrove plants in a reed bed system reactor with continous flow.

The results showed that all two mangrove species are highly salt tolerant and can survive in brackish water and perhaps even higher salinity although all the studied species were under rehabilitation condition in a mangrove conservation center. *Avicennia marina* had the best tolerance to highly saline conditions since this species maintains very negative water potential under saline conditions.

We hope that our research will provide the necessary groundwork for further researches, for example in the are of bio desalination with mangrove in reed bedsystem reactor with or without bacterial addition with continuous flow.

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