The Ability of *Avicennia marina* to Uptake Ions of Sodium and Chloride in Reed Bed System Reactor

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Abstract. Many technologies can be used to solve the clean water crisis. One of the technologies is desalination, but this technology is expensive. So that, it is necessary to find cheaper desalination technology and easier to operate. Bio-desalination is a technology that utilizes bacteria to remove sodium and chloride ions in seawater. In this research, the application of the phytotechnology concept for bio-desalination reactors was carried out. Bio-desalination technology used the uniqueness of mangrove plants (*Avicennia marina*) in a reed bed system reactor that adapted from a reed bed system commonly used in constructed wetlands (CWs). The aim of the research was to determine the uptake of sodium and chloride ions by *Avicennia marina* in a reed bed system bio-desalination reactor. The namely of reactor were AM15‰, AM25‰, AMVA15‰ and AMVA25‰. The VA code was shown that *Vibrio alginolyticus* addition in this reactor. The artificial saline water with initial salinity of 15‰ and 25‰ was chosen based on our previous study. Parameter of salinity was determined using salinometer. Concentration of sodium and chloride ions were analyses using ion chromatography. Based on the results, the concentration of sodium and chloride ions in *Avicennia marina* at AM15‰ were 843.18 mg/kg and 4959.96 mg/kg, respectively. Meanwhile, those were 1410.01 mg/kg and 5292.64 mg/kg at AM25 ‰, respectively. The concentration of sodium and chloride ions in AMVA15‰ were 1003.39 mg/kg and 3186.96 mg/kg, and it were 8036.43 mg/kg and 9783.91 mg/kg at AMVA25‰. The

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value of Bio-concentration Factor (BCF) and Translocation Factor (TF) were greater than 1, it indicated that *Avicennia marina* was as an hyperaccumulator plant for sodium and chloride ions. In conclusion, the *Avicennia marina* can be used in reed bed system bio-desalination reactor to reduce salinity.

Keywords. Bio-desalination, hyperaccumulator, mangrove, reed bed system, salinity

1 Introduction

Mangroves are perennial woody plants grown in tropical and sub-tropical inter-tidal zones [1]. Many species of mangrove plants can grow in high range of salinity. Based on our previous study [2,3], *Avicennia marina* and *Rhizophora mucronata* can survive until salinity of 30 ‰. The tolerance of mangrove species to salinity followed the sequence of *Avicennia alba*, *Avicennia marina*, *Rhizophora apiculata*, *Rhizophora stylosa*, *Ceriops tagal*, *Achantus ilicifolius*, this condition was supported their natural species distribution and habitat zones [4]. According to Saeni and Tanasale (1999) [5], mangrove can be used to reduce salinity. Based on our earlier study, the reduction of salinity reached up to 92% at a treatment using *Avicennia marina* in a batch reed bed reactor after 2 to 4 days of operation [6]. The other previous study reported the best percentage decline of salinity reached 90% at a treatment using *Avicennia marina* and *Rhizophora mucronata* with addition of *Vibrio alginolyticus* in a continues reed bed reactor after 6 day of running. The salinity reduction was 25‰ to 1.99‰ [7].

According to Scholander et al., (1962) [8], mangrove plants were divided into two groups, namely secretor and non secretor. Genus of *Aegialitas*, *Aegiaceras* and included in secretor plant. The concentration of sodium chloride in xylem was relatively high, and the ratio was 11:10 part of the concentration of seawater. The salinity absorption occurred through the salt gland metabolism. The released salinity can be crystallized through evaporation processes. The second group were *Bruguiera*, *Lumnitzera*, *Rhizophora*, and *Sonnerata*. The ration of sodium chloride and seawater was 11: 100 in xylem. However, it was 10 fold higher than non-mangrove plants. Although the non-secretor plants have a no special mechanism to actively extract salt, this plant released salt through the leaf surface (possibly via cuticular transpiration).

The aim of this research was to determine the uptake of sodium and chloride ions by *Avicennia marina* in a batch reed bed system bio-desalination reactor.

2 Materials and Methods

2.1 Mangrove Preparation

Plants of *Avicennia marina* were collected from nursery of mangrove at Mangrove Forest, Wonorejo, Surabaya. The age of *Avicennia marina* was around 3 months. Fig. 1 described the *Avicennia marina* acclimatization at greenhouse at Remediation Laboratory, Department of Environmental Engineering, ITS before those plants were applied in research.

Fig. 1. *Avicennia marina* acclimatization.

2.2 Reactor Preparation

The description of the reed bed system reactor was showed at Fig.2. Based on the figure, the dimensions of the reactors were 31.5 x 31.5 x 65.5 cm. The bottom layer was a double layer of gravel with 2 cm and 1 cm of diameter. The height of gravel layer was 5 cm. After then, the layer filled with 2 L of water. After that, a sand layer was put with 10 cm of high. The saturated water in second layer was 1.8 L. And the top layer was artificial saline water with initial salinity of 15 ‰ and 25 ‰. Fig. 2 described the reed bed system reactor. Fig. 2 (a) showed the treatment without bacteria addition, meanwhile Fig. 2 (b) showed bacteria of *Vibrio alginolyticus* addition for treatment. There were 8 reactors, with duplo replicates, namely were AM15‰, AM25‰, AMVA15‰ and AMVA25‰. Fig. 3 depicted the photograph during treatment running.

Fig. 2. A reed bed system bio-desalination reactor

Fig. 3. *Avicennia marina* was planted in a reed bed system bio-desalination reactor

2.3 Artificial Salinity Preparation

The artificial saline water with initial salinity of 15‰ and 25‰ was chosen based on our previous study [2,3]. The preparation of artificial salinity was conducted based on our previous study [2].

2.4 Preparation of Bacteria

This research was carried out using species bacteria of *Vibrio alginolyticus*. The pure culture of bacteria was be inoculated onto nutrient agar (NA) media using streak plate technique [9]. The age of bacteria that was ready to be used was 24 h. After that, one colony of bacteria was tranferred to nurient borth (NB), and kept in shaker of Innova 2000 (New Brunswick-Eppendorf, Jerman) at 150 rpm and room temperature, 33 °C for 24 h [10]. After that, the value of Optical Density (OD) of bacterial suspension was measured using spectrophotometer model Genesys 20 (Thermo, USA). If the value OD was 1, it was ready to be used in bio-desalination red bed system reactor.

2.5 Parameter Analysis

Parameter of salinity was determined using salinometer. All samples of root, stem and leave were dried at 105°C for 24 h. After that, each sample was extracted using extraction process for anions based on Stanisis et al. 2011a [11] and it used method based on Stanisis et al. 2011b [12] for cations. All extraction used de-ionized water. A total of 5 g of dried samples was added to 10 mL of de-ionized water (1:10) and extracted using a rotary agitator at 60 rpm for 1 hour at room temperature. The samples were centrifuged using a centrifuge model Jouan E82 (Thermo, USA) at 3000 rpm for 10 minutes and then filtered using a filter paper (Whatman, Germany). After that, samples were placed in bottle sample and it were kept in a refrigerator (-4°C). The analysis of sodium and chloride concentration in part of *Avicennia marina* were conducted using ion chromatography (IC), Metrohm, type of 882 Compact plus (Switzerland).

3 Results and Discussions

Fig.4 showed the concentration of sodium and chloride in part of *Avicennia marina* i.e roots, stems, and leaves after two weeks exposure with sinitial alinity of 15‰ and 25‰. This indicated that *Avicennia marina* can uptake the sodium due to this plant included as a secretor group of mangrove [8]. Generally, the concentrations of sodium ion and chloride ion at all treatments were high in stems, except at code treatment of AMVA25‰. However, the sodium concentration in roots, stems and leaves at code treatment of AMVA25‰ showed the highest value among the others treatments. Those value were 1413.24 mg/kg. 1242.45 mg/kg and 531.27 mg/kg, respectively at roots, stems, and leaves.

Fig. 4. Ions concentration of sodium and chloride in part of *Avicennia marina*
Explanation, AM15‰ = treatment with *Avicennia marina* at 15 ‰, AM25‰ = treatment with *Avicennia marina* at 25 ‰, AMVA15‰ = treatment with *Avicennia marina* and addition of *Vibrio alginolyticus* bacteria at 15 ‰, and AMVA25‰ = treatment with *Avicennia marina* and addition of *Vibrio alginolyticus* bacteria at 25 ‰.

Fig. 5 showed the sodium and chloride concentration in a whole plant of *Avicennia marina*. The highest of sodium and chloride concentration occurred at treatment of AMVA25‰. Based on our previous study, Titah et al. (2019a) and Titah et al. (2019b) [2,3], *Avicennia marina* and *Rhizophora mucronata* can take up sodium and chloride, and then, sodium and chloride are translocated to aerial part, stems and leaves. Based on scanning electron microscopy-energy dispersive X-ray (SEM-EDX) results in our previous study about phytotoxicity of salinity on *Avicennia marina*, the sodium and chloride weight concentration were founded in root, shoot and leaves, respectively [2]. Mangroves accumulate high concentrations of inorganic ions like most other salt-tolerant plants that function in the osmoregulation of leaves and other tissues [13]. *Avicennia marina* has the ability to regulate salt content by secreting it through the glands [14] due to *Avicennia marina* included in as a secretor group of mangrove. The mangrove can decrease salinity, chiorinity, and conductivity of water, except for water which did not contain sea water. The increasing of chloride ion in the tissues was influenced by the increase of chloride ion in root tissues [5].

Fig. 5. Ions concentration of sodium and chloride in whole plants of *Avicennia marina*
Explanation, AM15% = treatment with *Avicennia marina* at 15 %, AM25% = treatment with *Avicennia marina* at 25 %, AMVA15% = treatment with *Avicennia marina* and addition of *Vibrio alginolyticus* bacteria at 15 %, and AMVA25% = treatment with *Avicennia marina* and addition of *Vibrio alginolyticus* bacteria at 25 %.

Fig. 6 depicted the value of Bioconcentration Factor (BCF) and Translocation Factor (TF) for ion of sodium and chloride. BCF was the concentration of test substance in organisms or tissues (mg/kg) /concentration of the substance in media (mg/L), meanwhile the TF was the concentration of test substance in stem (mg/kg) /concentration of the substance in root (mg/kg).

Fig. 6. Value of BCF and TF for ion of sodium and chloride
Explanation, AM15% = treatment with *Avicennia marina* at 15 %, AM25% = treatment with *Avicennia marina* at 25 %, AMVA15% = treatment with *Avicennia marina* and addition of *Vibrio alginolyticus* bacteria at 15 %, and AMVA25% = treatment with *Avicennia marina* and addition of *Vibrio alginolyticus* bacteria at 25 %.

Based on the Fig. 6, the value of BCF were upper than 1 for sodium and chloride ions. Similar condition, the value of TF was upper than 1 for sodium and chloride ions. It indicated the *Avicennia marina* was a hyperaccumulator plant for sodium and chloride ions.

4 Conclusions

The concentration of sodium and chloride ions in *Avicennia marina* at AM15‰ were 843.18 mg/kg and 4959.96 mg/kg, respectively. Meanwhile, those were 1410.01 mg/kg and 5292.64 mg/kg at AM25 ‰, respectively. The concentration of sodium and chloride ions in AMVA15‰ were 1003.39 mg/kg and 3186.96 mg/kg, and it were 8036.43 mg/kg and 9783.91 mg/kg at AMVA25‰. The value of BCF and TF were greater than 1, it indicated that *Avicennia marina* was as an hyperaccumulator plant for sodium and chloride ions. In conclusion, the *Avicennia marina* can be used in reed bed system bio-desalination reactor to reduce salinity.

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