Removal of Chemical Oxygen Demand in Brackish Water by Rhizophora mucronata using Reed Bed System Batch Reactor

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Abstract. River pollution can cause coastal pollution due to many pollutants can not remove during water flow from upstream to downstream. River has a capability to do self purification to remediate many pollutans, but wastewater disposal occurred at along the river. One of pollution parameter that caused by organic pollutant was Chemical Oxygen Demand (COD). In this research, the design of reactor was adapted from reed bed system commonly used in constructed wetlands. The purpose of the study was to determine the COD removal by Rhizophora mucronata using reed bed system reactor. There were 8 reactors, with duplo replicates, namely RM15 and RM 25 for treatment with Rhizophora mucronata at 15 ‰ and 25 ‰, respectively, and RMVA15 and RMVA 25 for treatment with Rhizophora mucronata and addition of Vibrio alginolyticus, respectively. Parameter of COD was determined using digestion reactor and spectrophotometer. Based on the results, the highest removal of COD reached 82.06% after 14 days at reactor of RM15. The highest of removal COD with addition of Vibrio alginolyticus was 80.89% after Day 2 at reactor of RMVA15. In conclusion, the Rhizophora mucronata that was grown at reed bed system reactor demonstrated can be used in removing organic matter.

Keywords: **bacteria; coastal pollution; mangrove; organic pollutant; reed bed system; salinity.**

1 Introduction

The freshwater volume in the world were just around 0.5% of the total volume of water or it reached 2.84x105 Km^3 [1]. It meant that seawater has the biggest volume in the world. River pollution can cause coastal pollution and sea pollution due to many pollutants can not remove during water flow from upstream to downstream. Although river has a capability to do self-purification to remediate many pollutants, wastewater disposal occurred along the river. Organic and inorganic pollutant was founded in rivers. Organic pollutants such us organophosphate and organochloride, inorganic pollutants such as heavy metals and nutrients. Generally, some organic pollutants that were removed by aquatic plants in wetlands system were chlorinated solvents, petroleum hydrocarbons, and explosives. However, some studies also considered other potential plants to treat other organic contaminants, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCB) [2].

Mangroves are perennial woody plants grown in tropical and sub-tropical inter-tidal zones [3]. Mangrove can survive in a high range of salinity. Based on our previous study [4], *Rhizophora mucronata* can survive until salinity of 30 ‰. Mangrove plants that were grown in natural and constructed wetlands have highly efficient for adsorbing process and absorbing wastewater-borne pollutants, including inorganic compounds such as nitrogen, phosphorus, heavy metals and toxic organic pollutants [5-9]. Based on Ke and Tam [10], the removal efficiencies of nutrients from the wastewater in constructed mangrove wetlands ranged from 75 to 98% and this percentages of removal of metals reached 88-96%. According to Tam and Wong [2], the percentage of reduction of Chemical Oxygen Demand (COD) was over 90%, removal of ammonium nitrate was over 95%, phosphorus removal percentages between 40 to 65% and very high percentage of heavy metals removal at a pilot-scale of constructed wetlands using system of sub-surface flow wetland with mixed of mangrove plants and without mangrove plant species in China. The parameter of COD usually was used to measure organic pollutant in water as the rapid indicator, The parameter of COD was a useful measurement of water quality. Generally, it was used in municipal and industrial wastewater treatment and it can indicate the efficiency of removal of organic pollutants or the treatment process performance.

The aim of this research was was to determine the Chemical Oxygen Demand (COD) removal in brackish water by *Rhizophora mucronata* using a reed bed system reactor with and without the addition of *Vibrio alginolyticus*. The design of reactor was

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adapted from a reed bed system commonly used in constructed wetlands. The basic components employed in the construction of constructed wetlands were containers, plant species, and sand and gravel media in certain ratios. The other invertebrates and microbes can develop naturally during the process [3]. Vibrio is a genus of bacteria indigenous to the aquatic environment. This genus bacteria was also as a contaminant of raw or undercooked seafood. They have Gram-negative, curved colonies, rod-shaped, halophilic, and non-spore forming bacteria. These bacteria can grow in saline aquatic environments through free of living in the water and attach to animate and inanimate surfaces [11].

2 Materials and Methods

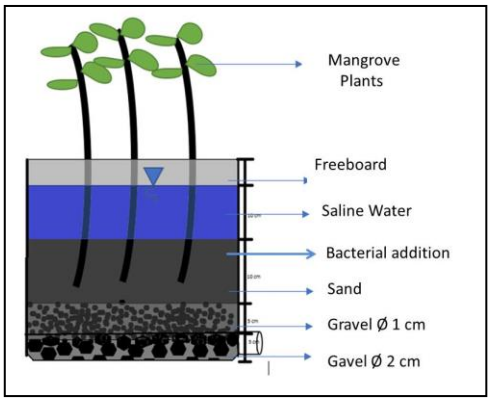
2.1 Mangrove Preparation

Rhizophora mucronata were collected from nursery of mangrove at Wonorejo, Surabaya. The age of plants was about 3 months. Figure 1 showed the mangrove acclimatization at greenhouse before mangrove was used in research.

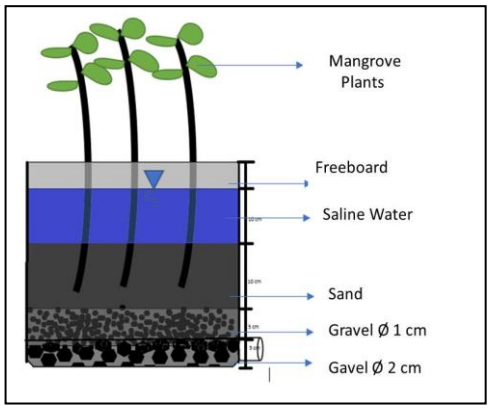
Fig. 1. Mangrove acclimatization.

2.2 Reactor Preparation

The plastic reactors were used with dimensions of 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 inial salinity of 15 ‰ and 25 ‰. as high as 10 cm with 3 L of water. Figure 2 described the reed bed system reactor. There were 8 reactors, with duplo replicates, namely RM15 and RM 25 for treatment with *Rhizophora mucronata* at 15 ‰ and 25 ‰, respectively, and RMVA15 and RMVA 25 for treatment with *Rhizophora mucronata* and addition of *Vibrio alginolyticus* bacteria at 15 ‰ and 25 ‰, respectively.



(a)



(b)

Fig. 2. A reed bed system reactor (a) with bacterial addition and (b) without bacterial addition

2.3 Bacterial Preparation

The bacterial requirement (*Vibrio alginolyticus)* was 5% (v/v), then bacteria required 0.15 L / reactor. This preparation of bacteria was conducted based on our earlier research [12].

2.4 Artificial Salinity Preparation

The preparation of artificial salinity was conducted based on our earlier research[12].

2.5 Parameter Analysis

The analysis of COD parameter was conducted using a digestion reactor or COD thermo reactor, and spectrophotometer (Nova 60, Germany). The 2 mL of sample was put in COD vial with holding the vial at 45^{o} angle. After that, the vial was cap tightly and it was rinsed and wipe clean with paper towel. The vial was placed in the preheated COD reactor or a COD thermo reactor. The blank was prepared by repeating the steps using 2.0 mL distilled water. All vials were heated for 2 hours. After that, the reactor was turned off and it was waited around 20 minutes to become cool. Each of the vials was inverted several times while still warm. All vials were put into a rack and kept in the room temperature. After all, vials were

cool, the reading process of COD concentration was conducted using a spectrophotometer.

Monitoring parameters also were determined. Those parameters were pH and temperature. The value of pH was measured using a waterproof pocket pH-meter model HI-98107 (Hanna, USA). The temperature was determined using thermometer model HI-98501 (Hanna, USA).

Determination of bacteria population using Colony Form Unit method according to Harley and Prescott method (2002) [13]. The population bacteria determination was calculated by the number of bacteriaL colonies of sample with divide by the dilution factor. The final plates in the series should have between 25 and 250 colonies [13].

The fresh and dry weights (FW, DW) were measured for each part of the sampled plants (roots, stems, and leaves). The fresh weight 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 FW and DW of whole plants can be calculated.

3 Results and Discussions

Figure 3 showed the pH at each reactor during the test. Based on the data, the range of pH showed 5.63 – 7.45. The pH fluctuation occurred at all reactors. The increasing and decreasing pH can be occurred due to activities by a microorganism. However, mangrove can growth at those pH. According to Wantasen (2013) [14], the optimal pH for mangrove growth was about 7.0-8.5. Based on the data, the number of *Vibrio alginolyticus* at reactor RMVA was higher than at reactor without *Vibrio alginolyticus* addition. The number of *Vibrio alginolyticus* population was 1.42 x 10^3 CFU/mL and 3.1 x 10^3 CFU/mL at RMVA15 and RMVA25, respectively. However, the bacterial population of *Vibrio alginolyticus* at the reactors without bacterial addition reached 3 x 10^2 CFU/mL, it indicated that *Vibrio alginolyticus* was as an indigenous bacteria that have been growing at rhizophera of mangrove. Based on Rojas et al. (2001), Toledo et al. (1995), [15,16], bacteria of *Vibrio campbelli*, *Vibrio aestuarianus*, and *Vibrio proteolyticu* was found in association with several mangrove species. Some studies reported that the diazotrophs bacteria such as the genera Azotobacter, genera Sphingomonas, genera Pseudomonas, genera Desulfuromonas, genera Derxia, and genera Vibrio have been isolated and identified in mangrove rhizophere [17,18].

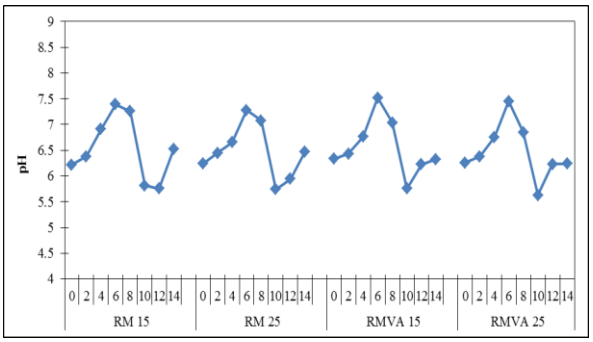


Fig. 3. Value of pH at each reactor
Explanation, RM15 = treatment with *Rhizophora mucronata* at 15 ‰, RM25 = treatment with *Rhizophora mucronata* at 25 ‰, RMVA15 = treatment with *Rhizophora mucronata* and addition of *Vibrio alginolyticus* bacteria at 15 ‰, and RMVA25 = treatment with *Rhizophora mucronata* and addition of *Vibrio alginolyticus* bacteria at 25 ‰.

Figure 4 showed the temperature during the test. The water temperatures range was 27.10 - 30.25 °C. The temperature classified into normal because of the range of water temperature in the sea waters. This temperature is still reasonable for tropical waters that range between 25.6-32.3 °C [19]. Meanwhile, the optimum temperature range for mangrove growth is 18-30 °C [20].

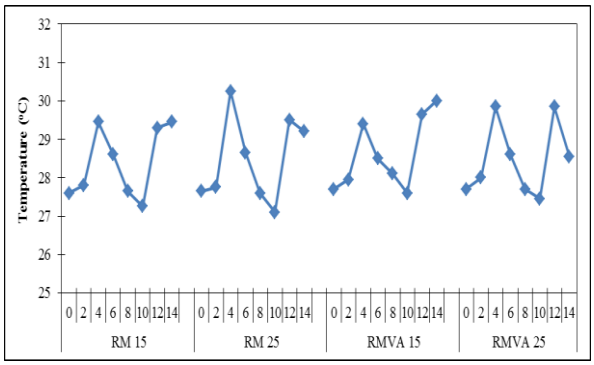


Fig. 4. Temperature at each reactor
Explanation, RM15 = treatment with *Rhizophora mucronata* at 15 ‰, RM25 = treatment with *Rhizophora mucronata* at 25 ‰, RMVA15 = treatment with *Rhizophora mucronata* and addition of *Vibrio alginolyticus* bacteria at 15 ‰, and RMVA25 = treatment with *Rhizophora mucronata* and addition of *Vibrio alginolyticus* bacteria at 25 ‰.

Figure 5 depicted the FW and DW of *Rhizophora mucronata* after the test. Based on the Figure, after 14 days of exposure, the DW on *Rhizophora mucronata* was higher at reactor of RMVA25 when compared with other reactors. The percentage of weight reduction were 50.2; 47.5; 46.5 and 38.0 % for RM15, RM25, RMVA15, AND RMVA25 respectively. It indicated that the water content in *Rhizophora mucronata* at reactor of RMVA25 was more stable.

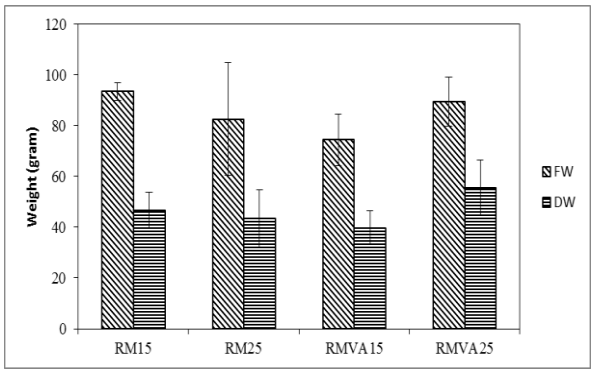


Fig. 5. FW and DW of whole plants at each reactor
Explanation, FW = fresh weight, DW = dry weight, RM15 = treatment with *Rhizophora mucronata* at 15 ‰, RM25 = treatment with *Rhizophora mucronata* at 25 ‰, RMVA15 = treatment with *Rhizophora mucronata* and addition of *Vibrio alginolyticus* bacteria at 15 ‰, and RMVA25 = treatment with *Rhizophora mucronata* and addition of *Vibrio alginolyticus* bacteria at 25 ‰.

Figure 6 showed the percentages removal of COD at each reactor. Based on the Figure, the salinity was high so the removal of COD decreased. The highest of COD removal occurred at salinity of 15‰. The highest percentage of COD removal was 82.06% at initial salinity of 15‰ for 14 days. According to Tam and Wong [2], the initial COD that ranged between 200 to 400 mg/L can be reduced until below 20 mg/L after treated by the constructed wetland using mixed mangrove plant and non-mangrove plant. It showed much lower than the discharge standard of 50 mg/L. The average removal percentage of COD was over 90%. So, the selection of wetland plants for constructed wetlands is an important thing due to the different species of plants that can show variation of pollutant removal efficiency [2].

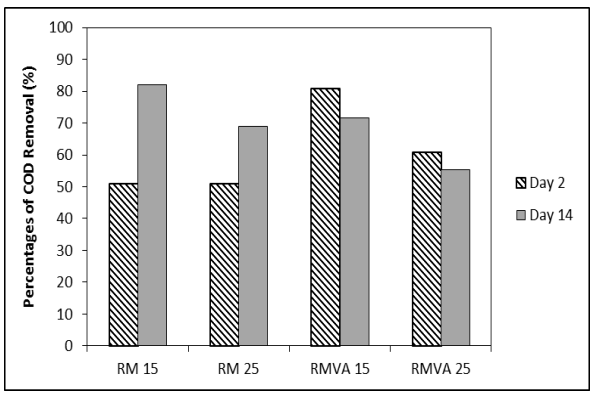


Fig. 6. Percentages removal of COD at each reactor
Explanation, RM15 = treatment with *Rhizophora mucronata* at 15 ‰, RM25 = treatment with *Rhizophora mucronata* at 25 ‰, RMVA15 = treatment with *Rhizophora mucronata* and addition of *Vibrio alginolyticus* bacteria at 15 ‰, and RMVA25 = treatment with *Rhizophora mucronata* and addition of *Vibrio alginolyticus* bacteria at 25 ‰.

The addition of *Vibrio alginolyticus* can increase the removal of COD on Day 2, however, it showed decreased of COD removal at Day 14. The highest of removal COD with addition of *Vibrio alginolyticus* was 80.89% on Day 2. It indicated that bacteria can enhanced the removal of COD within the make time of process was shorter.

According to Mahmood et al. [3], the process of organic pollutants by plants can be predicted through the four mechanisms, first, those organic pollutants were uptaken by plants directly, and then accumulation, and metabolism of pollutants in plant tissues or it was called as detoxification. Second mechanism was transpiration of volatile organic hydrocarbons through leaves or it was known as avoidance. Third mechanism was the exudates releasing from the plant roots that can stimulate microbial activity at the rhizhopera and biochemical transformations process or it was called as chelation. The last mechanism, the enhance of the mineralization process of pollutants in rhizosphere due to the presence of mycorrhizal fungi and microbial consortia associated with the plant root surfaces.

4 Conclusion

The highest removal of COD reached 82.06% after 14 days at reactor of RM15. However, the highest of removal COD with addition of *Vibrio alginolyticus* was 80.89% after Day 2 at reactor of RMVA15. The addition of *Vibrio alginolyticus* can increase the COD removal although the COD removal decreased on Day 14. In conclusion, the *Rhizophora mucronata* that was grown at a reed bed system reactor can be used in removing organic matter for organic pollution in brackish water.

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