Phytoremediation in Sandpit Lake: Aquaponic

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Abstract Sandpit Lake in Sukabumi area is a suitable site for developing aquaculture. However due to rapid eutrophication only sustainable aquaculture should be applied. We evaluated the ability of lettuce in reducing nutrients from fish culture. This phytoremediation study was carried out using mesocosm, where lettuce as the phytoremediation agent were grown on vegetable floating raft (VFR) together with 25 fish which were grown underneath it and fed by fish feed. We built three sizes of VFR which were 20, 40 and 60% covered the mesocosm surface. The three sizes VFR were the treatment and the 0% covering served as control. Water quality sampling was carried out two times at day zero and day seven of the experiment. We collected temperature, oxygen, nitrogen, and phosphate data and analyzed them using ANOVA. Our result showed the nitrogen increased inside mesocosm meanwhile orthophosphat decreased. But the size of VFR did not give any effect to the water quality parameter change. However descriptively, we found that 40% of VFR was able to slowdown the nutrient TAN increasing and orthophosphat concentration found to be low. Moreover, it resulted in the highest lettuce survival. We recommend using 40% coverage in employing an aquaponic system in sandpit lake.

1. Introduction
The increase in human population growth leads to an increasing in food demand and aquaculture has been providing the demand by setting a rapid increasing production. Many lakes and reservoirs are used as aquaculture sites, and they receive the impact of aquaculture mostly from floating net cage systems [1, 2]. The aquaculture wastes, for example, livestock wastes, uneaten feed, and other chemical ingredients will be released into the environment and caused elevation of nutrient levels and may spread disease among culture fish to mass mortality [3]. Nitrogen and phosphorus are considered the major waste from aquaculture and their effect drives serious damage to the aquatic environment [2]. Eutrophication as a further result of the aquaculture wastes effect can reduce aquaculture productivity due to the low level of oxygen [4].

Nowadays, the purpose of aquaculture development is not only to get a high production but also sustainable and give less negative influence to the environment. In recent years many efforts have been done to reduce the impact of aquaculture waste on the environment. The use of low trophic organisms such as filter feeders, algae, and plants to decrease organic matter from aquaculture systems was investigated by many researchers [5-7]. Phytoremediation or using macrophytes in water quality problem treatment including aquaculture waste is commonly used. Aquatic macrophyte enhances water quality by absorbs nutrients [8], decline algae population [9], and provide refuge for zooplankton [10].
Floating macrophytes can be used in reducing nutrients for example phosphorous and nitrogen from fishpond [11]. Therefore introducing free-floating macrophytes can be a solution to prevent sandpit lake eutrophication from aquaculture waste. However, the introduction of floating aquatic macrophytes could be risky where there is a possibility of an uncontrolled population of floating macrophytes in the future and become a new problem. Introducing a non-aquatic macrophyte for instance vegetable in a controlled environment, which is designed to serve as a natural floating aquatic macrophytes could be an option.

There were several methods in introducing land plantation into the aquatic environment, where employing plant floating-bed was included. Plant floating-bed is also believed to enhance water quality in eutrophic lakes via nutrient reduction [12, 13]. Therefore, we try to develop land plants floating but we exclude soil in our plant system. Instead of using soil or clay, we tried a floating hydroponic. To make it float, we use a raft, hence we called the system vegetable floating raft (VFR).

Combining aquaculture and horticulture in one system or aquaponic is commonly used to reduce nitrogen and phosphorous from the aquaculture water. The by-product from aquaculture act as fertilizer to stimulate plant growth, hence both aquaculture and horticulture reached high production. Several methods of aquaponic are developed, specially aquaponic which is applied in the recirculating systems where fish tank and horticulture areas are separated. This system is proven as an effective method in treating wastewater from aquaculture systems [14]. Polyculture between fish and horticulture in open freshwater particularly in the fish cage was tested by several studies. Haque et al. [15] developed an integrated floating cage aquageoponic system (IFCAS) which gives Bangladesh farmers the opportunity in harvesting both vegetables and fish. They applied, floating hydroponic on the top fish cage. Other studies were conducted in the Cirata reservoir [16] or cage culture area in PLTA Koto Panjang Indonesia [17]. Specifically, Astuti et al. [16] pumped up deposit waste from fish floating cage and watering the hydroponic system located near the floating cage. However, there is still less information about the combination between floating fish cages and floating hydroponic in open water especially the VFR.

VFR is expected to serve as a water quality enhancer via reducing nutrient levels in aquaculture systems, particularly from fish floating cages. However, employing aquaponic in sandpit lakes is challenging due to the lack of information available regarding the plantation effectivity in reducing aquaculture waste. Hence our research aims were, first to test whether VFR is an effective phytoremediation tool in reducing nutrients from aquaculture sistem and second to test the possibility of employing VFR as aquoponic system in Sandpit Lake.

2. Materials and Methods

2.1. Study site
The study was conducted in the sand mining area in Kampung Awilarangan, Desa Cikahuripan, a border between Sukabumi Regency and Cianjur (Figure 1). There were six lakes in this area and named by number according to their position from the south to the nort. As a study station, we selected lake Nr. 6 which was recognized as a eutrophic lake [18].

2.2. Experimental floating cage (EFC) and Mesocosm
We build an experimental floating cage (EFC) and put it in the lake. We choose the location which had depth more than 10 m. The EFC frame was made from bamboo, and empty plastic barrels in volume 25 liters were used as a buoy. In each corner of the EFC, a cement ballast in weight 50 kg each was hanged. The EFC was separated into 12 rooms in size 1 m², where we attached plastic bags in each room which served as mesocosm (Figure 2).

Each mesocosm was a plastic bag in tube shape with diameter 1.3 m and 2.5 m length. The length was assumed as water depth where the light still existed. The mesocosm were filled with lakes water until the water surface inside the mesocosm had the same high as the water surface outside. Inside each mesocosm, a floating raft (VFR) was put.
2.3. Vegetable Floating raft (VFR)
Vegetable floating rafts (VFR) were used as a medium to cultivated vegetables in the aquaponic system. The general design of VFR where frames were made from bamboo sticks, and four empty mineral water bottles (600 ml) were used as a buoy for each raft (Figure 3). The VRF sizes were based on the floating rafts covering on experimental mesocosm area, whereas we had three sizes. We modified aquaponic shaded ponds methods which used by Haque et.al [15]. They applied moderated and highly shaded ponds. We developed this moderated and highly shaded ponds into covering size which had the same princip. We expanded with idea, less, moderate and highly shaded. The first size was the 20% coverage, which contained with 49 plastic pots with hydro tone as the medium for 49 lettuces. This size considered as less shaded. The second size was 40% coverage, contained 100 plastic pots and considered as moderate shaded. The last one was the 60% coverage filled, with 144 plastic pots considered as highly shaded.

2.4. Aquaponic system (fish and lettuce)
The aquaponic system contained fish and vegetable cultures. Where we used tilapia (planktivore fish) in size 10-12 cm. And we used lettuce with age 25 days from the see ding. In the beginning, we grew lettuce inside seedling try, where Rockwool and dacron were used as cultivation mediums. After reached study age, the lettuce was removed from seedling try to VFR. We arranged where the plantation body is still on the upper water surface while the roots are inside the water and do their role as the nutrient absorbers.

2.5. Data collection
2.5.1. Common garden experiment
We filled each mesocosm with 25 fingerling fish. Fish were fed using artificial fed twice a day (4% from fish biomass) [19]. Four coverages of VFR as treatment were used in randomized complete design, namely treatment A with covering size 0% (served as a control), treatment B was 20%, treatment C was 40%, and treatment D was 60% of experimental mesocosm plastic area. Each treatment was done in triplicate, hence we had 12 mesocosms.
2.5.2. Data analysis.
Water quality parameters such as, temperature, Dissolved Oxygen (DO), pH, and nutrients, as well as lettuce biomass were measured. And the number of surviving lettuce in the VFR was counted at the beginning and the end of observation. Due to shallow mesocosm, we assumed that there was no depth stratification, hence we collected water samples from one spot, which were 50 cm from the surface. We used a linear model to see a relationship between treatment, water quality, and lettuce biomass, where the change (delta) of water quality and survival rate as dependent variables and treatment as independent variables, and run the ANOVA. We used the means package to perform pairwise post hoc comparisons (Tukey test). The survival rate of lettuce was counted from the percentage of lettuce number at day seven (N7) on lettuce number at day zero (N0). All statistical analyses were performed in R v 4.02.0.

3. Result and Discussion
We found the lettuce biomass for all treatments decreased along with the negative growth rate (Figure 3). Treatment did not give a significant effect on both lettuce growth rate and survival rate (Table 1) but affected the change of biomass (delta biomass). Treatment B was significantly different from treatment C and D (Tukey test, p< 0.05).
Table 1. Statistics of treatment (T) effects (ANOVA) in models of the change of lettuce biomass, lettuce survival rate and lettuce growth rate.

| Predictors | Lettuce delta biomass Estimates | p  | Lettuce survival rate Estimates | p  | Lettuce groth rate Estimates | p  |
|------------|-------------------------------|----|--------------------------------|----|-------------------------------|----|
| (Intercept)| -12.41                        | 0.400 | 87.75 | <0.001 | -0.05            | 0.089 |
| T [C]      | -74.73                        | **0.008** | 6.91  | 0.091 | -0.07             | 0.103 |
| T [D]      | -87.98                        | **0.004** | 0.67  | 0.851 | -0.06            | 0.153 |

| Observations | 9 | 9 | 9 |
|---------------|---|---|---|
| R2 / R2 adjusted | 0.799 / 0.733 | 0.450 / 0.266 | 0.417 / 0.223 |

Bold typing showed significance

Figure 4. Average of water quality, lettuce biomass and lettuce survival rate measured in day zero (the first day of experiment) and day seven for every treatment. Where treatment A (0%), B (20%), C(40%) and D(60)% were the VFR coverage.

We recorded stable temperature, and pH value during the experiment where the water temperature was 25°C on average and pH was 6 for all treatments (Figure 4). Dissolved Oxygen was found to be low in all treatments after seven days (Figure 4) whereas we found 4.40 mg/l on the first day and decreased. There was no treatment effect to DO (Table 2) concentration.

We measured nitrogen in the water in the form of nitrate (NO₃), nitrite (NO₂), and total inorganic nitrogen (TAN). In general, we found increasing in nitrogen concentration in all treatments (Figure 4). Statistically, we could not detect any significant differences in nitrogen concentration in all forms between treatments after seven days experiment. However, we saw a relationship between VFR covering size and NO₂ concentration (p< 0.05) where NO₂ will increase in a larger VFR size (Table 2, Figure. 5). Different from nitrogen concentration, we recorded decreasing in orthophosphate (PO₄) from all treatments (Figure 4). Where under 40% coverage treatment, PO₄ was being reduced more than other treatments. However once again we could not see any significant effect of VFR covering size on PO₄ concentration (Table 2).

Several researchers have tried the ability of lettuce as a bio filter in aquaculture systems. Their result showed the potential of using lettuce in a close recirculation aquaponics system [16, 20-22]. We used lettuce to test the VFR system as phytoremediation tools and expected a better water quality in the floating cage aquaculture. However, the lettuce biomass in the VFR system decreased after seven days.
Even though the survival rate of each treatment ranged between 87.76-94.67% (Figure 4) and the biomass was considered low due to the negative growth rate. A pairwise test showed that VFR covering size 20% has less biomass reduction compared to the 40% and 60% coverage. This might also be related to the less negative growth rate of 20% coverage (Figure 5) rather than the other two VFR sizes.

Table 2. Statistics of treatment (T) effects (Anova) in models of the change (delta) of dissolve oxygen (D_DO), Total anorganic nitrogen (D_TAN), nitrit (D_NO2), nitrat (D_NO3) and phosphorous (D_PO4)

| Predictors | D_DO | p | p | p | p | p | p | p |
|------------|------|---|---|---|---|---|---|---|
| (Intercept) | -0.92 | 0.137 | 0.10 | 0.161 | -0.00 | 0.283 | 0.11 | 0.086 |
| T [B] | -0.67 | 0.416 | 0.09 | 0.329 | -0.00 | 0.813 | 0.12 | 0.178 |
| T [C] | -0.12 | 0.880 | 0.10 | 0.299 | 0.00 | 0.321 | 0.07 | 0.396 |
| T [D] | -0.92 | 0.276 | 0.02 | 0.817 | 0.01 | 0.036 | 0.04 | 0.599 |
| Observations | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| R2 / R2 adjusted | 0.190 / -0.114 | 0.267 / -0.008 | 0.543 / 0.372 | 0.224 / -0.067 | 0.400 / 0.175 |

Bold typing showed significance

Figure 5. The change of water quality (delta), lettuce biomass and lettuce survival rate for every treatment (T). Where the treatments A (0%), B (20%), C (40%) and D (60%) were the VFR covering size.

We recognize there were several possibilities of decreasing lettuce in the VFR system inside the mesocosm. The first was water pH, where the lettuce was found to decrease in fresh and dry weight under alkaline conditions [23]. Even though our mesocosm water has a pH 6 in average, but lettuce seemed to grow better at pH 5 [23]. Other possibilities were the insufficient nutrients, which were an important growth factor for plantations. Soundy et al. [24] investigated lettuce nutrients need in a hydroponic system and revealed that lettuce needs 15-30 mgl-1 P in the irrigation water every 2-3 days. Meanwhile, Mahlangu et al. [25] suggested 100-120 mgl-1 nitrogen in ammonium nitrate form to support lettuce growth in the hydroponic system. We recorded the PO4 concentration in our mesocosm ranged around 0.20 – 0.44 mgl-1 and ammonium nitrate ranged between 0.32-0.44 mgl-1 after 7 days of the experiment. Hence, we assumed that the nutrient level in the mesocosm did not support the growth of lettuce. This also could be the reason why 20% VFR covering size has less biomass decrease, due to less nutrition competition between lettuces in the VFR system.

The ability of floating lettuce to absorb nutrients from the water might be limited too. We used small and young lettuce; their roots might not reach the water column, which nutrients were available. Astuti et al. [16] used a pump to bring the water, which contains waste deposits from the bottom cage to the hydroponic system. Hence, the plantations were able to use the waste. In our case the VFR was floating
on the surface, therefore the organic matter, which still concentrated in the lower column, might not reachable to them. For those reasons, maybe the lettuce was resulting in less biomass after seven days of the experiment.

The VFR gave no effect on water quality and nutrient concentration moreover it covering size did not relate to the change of water quality except NO$_2$ concentration and lettuce survival rate. The water quality and nutrient concentration in treatment without VFR and with VFR mesocosm were not different. However, we wanted to get an advantage from the aquaponics system with two productions, which were fish and vegetables. Therefore, in the future the 40% VFR covering size can be applied. Data visualization was helping to understand that the treatment with coverage the 40% VFR seemed to give a better result than the other coverages (Figure 5). For example, under this treatment, there were more dissolve oxygen and less nitrogen (TAN) as well as phosphorous (PO4) concentration in the water on the last day of experiment (Figure 4). Additionally, it has more biomass of lettuce rather than 20% and 60% VFR covering size. And it tended to have a better survival rate of lettuce (Table 1). Similar suggestion offered by Haque et al. [15] where they result showed that moderate shade give a better result rather than high shade. In our study 40% coverage was considered as a moderate coverage.

Our result also gave us an overview of how to develop VFR in the future, especially in the application and the methods of experiment. Regarding the suitability of water quality and nutrients in supporting vegetable growth, other vegetable species could be studied in the VFR system. We used 25 days old vegetables after seedling, which might be too young, investigation about the suitable age of vegetables to put in the VFR also needs to be done. We used small mesocosm and assumed there will be no stratification in the water column or differences in water quality but due to the waste deposit might be concentrated in the bottom it might be helpful if we sampled the water from the bottom too. We experimented for seven days and put the VFR system on the same day we started the fish cage. This might be the reason that the nutrient from the aquaculture waste was not formed maximum. Waiting another week before installed VFR in the aquaculture system might give nutrients a chance to develop and give a better result visualization to see the effectivity of VFR.

4. Conclusion

In conclusion, our result could not find clear proove of VFR effectivity as phytoremediation tools where VFR size did not give a significant effect on water quality improvement. But to get benefit from VFR as aquaponics system we recommended the 40% coverage. It gave more lettuce production rather than 20% and contributed less NO$_2$ rather than the 60% coverage. More research is needed before applying the VFR in the sandpit lake especially regarding the vegetable species and the method in experimenting.

AuthorSs Contribution

All authors contributed during writing process. PO and NM designed the study and analysed the data. MR analysed and visualized the data. PO and NM contributed equally to this work and shared the first authorship.

Acknowledgement

We thank our field team Rensi Novianti, Neneng Nurbaueti, Arif Supendi, and Muhammad Karim for the excellent teamwork in collecting data for the project. This study was part of developing integrated multi trophic aquaculture (IMTA) in sand pit lake research funded by Ministry of Education and Culture in the contract number 1602/K4/KM/2017.

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