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Phytoremediation Capabilities of *Spirodela polyrhiza* and *Salvinia molesta* in Fish Farm Wastewater: A Preliminary Study

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Abstract. Fish farm wastewater needs to be treated as it contains considerably high loading of suspended solids and dissolved nutrients from accumulation of by-products e.g. fish excretions and uneaten feed. In this study, macrophytes, namely *Spirodela polyrhiza* and *Salvinia molesta* were examined for their phytoremediation efficiency in treating fish farm wastewater in a raceway pond rig. It was carried out indoor for 14 days under controlled environment. Water samples was collected once every 2 days for analysis of NO_3^-- N, PO_4^{3-}, NH_3-N, COD, turbidity, MLVSS and pH. The results showed that there was decrement of phosphate in fish farm wastewater using either *S. polyrhiza* or *S. molesta*. Interestingly, *S. polyrhiza* was found to be more efficient in phosphate uptake as it removed 72% phosphate at day 4 and up to 95% in the end of the experiment whereas 72% phosphate removal was only achieved by *S. molesta* at day 10. Similar ammonia decrement was observed for both plants and most of the ammonia were not detected in the wastewater by day 10 for *S. polyrhiza*, while by day 8 for *S. molesta*. Nitrate showed increment for both plants which could be due to nitrification. Both plants achieved highest COD removal on day 12, whereby 68% for *S. polyrhiza* and 63% for *S. molesta*. They were able to reduce turbidity and total suspended solids (TSS) to very low level and significantly increase clarity of wastewater. *S. polyrhiza* reduced up to 96% of initial turbidity value and 86% of TSS. 82% reduction of initial turbidity and 79% TSS decrement were observed for *S. molesta*. pH fluctuations were minimum for both plants, with a range between 7.62 to 7.77. Both plants demonstrated biomass increment for fresh weight in which 84% for *S. polyrhiza* while 85% for *S. molesta*. This study proved that the macrophytes were able to treat fish farm wastewater by significantly removing phosphate, ammonia, turbidity and TSS. It aids in minimizing pollutants released to receiving waters and producing biomass which can be utilized for agriculture sector.

1. Introduction

According to The State of World Fisheries and Aquaculture 2016, world total fish production had reached 167.2 million tonnes in 2014 while aquaculture production alone accounted for about 44% of the total fish production. Provided that aquaculture comprised only 7 percent of fish for human consumption in 1974, this share had rose up to 26 percent in 1994 and 39 percent in 2004 [1]. It is not surprisingly that the figure will soon overtake the wild-caught fish production after 2014. Therefore, aquaculture would play a major role in world fish production now and future to ensure food security and nutrition to ever-growing human population. Malaysia was listed as one of the top 25 major

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aquaculture producers in the world with total production of 521.0 thousand tonnes in 2014, ranked 15th among the countries [1]. Its inland aquaculture covered an area of about 794.2 thousand hectares [2]. However, improper management of the aquaculture site in term of effluent discharge would bring harm to the nearby water resources and environment.

In an enclosed, intensive inland aquaculture, the water used to culture the fish are generally easier to be concentrated with suspended solids and dissolved nutrients due to accumulation of by-products eg. fish excretions and uneaten feed [3]. In order to maintain the health and welfare of the fish, water exchange need to be done regularly [4]. However, this effluent is normally either directly discharged into the nearby waterways or undergone sedimentation pond before released. Sedimentation may help reduce suspended solids, but not to remove dissolved nutrient, so eventually fish farm wastewater still poses risk of harming the receiving water. This phenomenon is attributed to rural farmers who are characterised as low capital cultivator, making advanced treatment system is too expensive for them to be installed and operated whereas no clear provision made with regard to local aquaculture effluents [5] also cause no further treatment of the effluents since the issue is not prioritised. Therefore, an affordable, efficient yet easy to implement treatment system for the fish farm wastewater is needed to ensure successful of the system. The system will give the farmer a short in the arm if it can generate valuable products or side income.

Phytoremediation is identified to be a treatment system which fulfilling those criteria. It is relatively low cost to maintain since it is solar-driven and no complex containment system needed. It is cheaper than conventional treatment methods that rely on electricity, pumping, aeration or chemicals additions and usually need large concrete or steel vessels. It is also the least harmful method as it uses naturally occurring organisms and preserves environment in more natural way, and it is some more aesthetically pleasing. Significant amounts of pollutants eg. phosphate, ammonia and etc. can be efficiently reduced by phytoremediation as shown in the available literature. Jesus et al. [6] demonstrated that phytoremediation of freshwater aquaculture using *Typha latifolia* in constructed wetland was able to reduce 61%, 78% and 98% of NH_4, NO_2 and PO_4 respectively. In Effendi et al. [7] study, they showed that ammonia, nitrate and orthophosphate can be reduced until 84.6%, 34.8% and 44.4% in freshwater crayfish culture wastewater by applying water spinach aquaponic system. Another phytoremediation work by Olguin et al. [8] indicated that *Salvinia minima* managed to decrease TKN, NH_4-N and P-PO_4 up to 97%, 99% and 88% respectively in anaerobic effluents of coffee wastewater. When the phytoremediation system is coupled with sedimentation pond, it will aid in removing the dissolved nutrients in the effluent as well the suspended solids.

The macrophytes are plants that have their own potential uses. Traditionally, *Wolffia arrhiza* has been eaten in Myanmar, Laos, and northern Thailand [9]. King et al. [10] showed that inclusion of *Salvinia molesta* in commercial fish feed diet will have higher fish weight on Nile tilapia (*Oreochromis niloticus*) compared to feeding with commercial feed alone and significant effect might be observed if feeding period is prolonged. The extracted lipid of *S. molesta* can be processed into biodiesel [11] and its biomass has the potential to be converted into organic fertilizer via vermiremediation [12]. Similarly, *Spirodela polyrhiza* can be promising substrate for biohydrogen production [13] and also be included in fish meals [14]. Hence, the aquaculture farmers can earn extra income out from the valuable plant stock harvested besides being applied to remediate the fish farm wastewater.

By taking the context mentioned earlier into consideration, the aim of this study is to examine the the water quality of fish farm wastewater after phytoremediation and its effect toward biomass of macrophytes. Since *S. molesta* and *S. polyrhiza* is the macrophytes species that locally available, they were employed for the study and their respective performances were being compared.

2. Materials and methods

2.1. Characterisation of fish farm wastewater

The fish farm wastewater was collected from a catfish pond near Sungai Udang, Nibong Tebal. It was then analysed and characterised according to standard protocol as stated in subsection 2.4. The water quality parameters taken for analysis included nitrate (NO_3^--N), phosphate (PO_4^{3-}), ammonia (NH_3-N), COD, turbidity, MLVSS and pH. The wastewater was later stored in cold room at around 4°C before used.

2.2. Plant stock establishment

Axenic *S. molesta* and *S. polyrhiza* were obtained from USM SCE's laboratory after previous successful isolation of the plants. The aquatic plants were subcultured and maintained in liquid Hoagland medium with 15g/L sucrose to provide needed stock for the study. All culture media were adjusted to pH 5.8 using NaOH before autoclaving at 121°C for 15 min. The explants of *S. molesta* were cut from the sterilized plant by stem splitting while healthy green fronds of *S. polyrhiza* were selected, for inoculation in the medium. Each 500ml Erlenmeyer flask contained 200ml liquid medium with five explants. All cultures were then incubated at 25±1°C under fluorescent tubes (1000 lux) with a 24h-photoperiod for 15 days.

2.3. Lab-scale phytoremediation on fish farm wastewater

This experiment was conducted to investigate the water quality of fish farm wastewater after phytoremediation and its effect toward biomass of macrophytes. *S. molesta* and *S. polyrhiza* were the floating macrophytes employed for phytoremediation and their treatment performances were compared. This study was carried out in bench scale raceway pond rig with its configuration as shown in [15] with dimensions of 50cm x 25cm x 9cm, inside the laboratory under controlled environment. 12 L of fish farm wastewater was poured into the storage tank and submerged pump was started to fill up and circulate the wastewater in the raceway pond rig system Healthy macrophytes were selected from axenic plant stock and undergone thorough rinsing and washing with water, so that the unutilised inorganic nutrients and sucrose remained on plant surfaces can be removed to avoid them from affecting the result of study. They were later dried by blotting with cloth and being weighed for 77 g before placing evenly on the surface of raceway pond. The circulation flow rate of wastewater in the rig was then maintained at 50 ml/min and the rig was closely monitored until equilibrium was achieved in the system whereby water level in storage tank reached steady state. The level was being marked. The phytoremediation study was carried out at 25±1°C under fluorescent tubes (1000 lux) with a 24h-photoperiod for 14 days. 100ml of water sample was collected each of every 2 days starting day 0 towards the end of experiment. The water level in the storage tank was ensured to maintain at the initial marked level before collection. Distilled water was added to the storage tank if the water level dropped below the marked level, which was due to evaporation. All water samples were contained with centrifuge tubes and kept in cold room at around 4°C before analysis. The water samples were tested for nitrate (NO_3^--N), phosphate (PO_4^{3-}), ammonia (NH_3-N), COD, turbidity, MLVSS and pH to evaluate the changes of water quality during phytoremediation period. At day 14, the macrophytes were harvested and dried carefully as the same way during preparation before being weighed for its final fresh weight.

2.4. Analytical analysis

2.4.1. Determination of nitrate and phosphate concentration for water samples.

The nitrate was determined by Cadmium Reduction Method (HACH method 8039) using NitraVer®5 Nitrate Reagent Powder Pillows by HACH DR2800 spectrophotometer at 500nm with allowable detection of high range (0.3-30.0mg/l NO_3^--N). The phosphate was determined by Ascorbic Acid Method (HACH method 8048) using PhosVer® 3 Phosphate Reagent Powder Pillows by HACH

DR2800 spectrophotometer at 880nm with detection range of (0.02-2.50mg/l PO_4^{3-}). This phosphate determination is in accordance to USEPA method 365.2 and Standard Method 4500-P-E for wastewater. Each method consumed 10ml of wastewater samples.

2.4.2. Determination of ammonia concentration and COD for water samples.
The ammonia was determined by Salicylate Method (Lovibond method 66) using VARIO Am tube test Reagent, Set HR, F5 (VARIO Ammonia Salicylate, F5 and VARIO Ammonia Cyanurate, F5 powder packs as well as VARIO Am Diluent Reagent High Range reaction tube) by LOVIBOND Maxidirect MD600 photometer at 660nm with detectable range of (0-50mg/l NH3-N). This method consumes 0.1ml water sample. The COD was determined by Dichromate/H2SO4 Method (Lovibond method 131) using COD VARIO 0 - 1500 mg/l tube test Reagent by LOVIBOND Maxidirect MD600 photometer at 610nm with detectable range of (0-1500mg/l COD/CSB). This method complies with Standard Methods for the Examination of Water and Wastewater.

2.4.3. Determination of turbidity of water samples.
The water samples were well-mixed by shaking the centrifuge tube vigorously. A clean cuvette was filled with water sample until the level mark. The bubbles in water samples were removed by placing cuvette in the degaser. The cuvette was wiped with lint-free tissue before inserting into measurement cell to measure its turbidity value. The turbidity value was determined by HANNA HI 93703 microprocessor turbidity meter peaking at 890nm with range of 0-1000NTU. The turbidity measurement was performed according to the ISO 7027 International Standard.

2.4.4. Determination of MLVSS.
Well-mixed water samples were prepared by shaking the centrifuge tube vigorously. The 30ml samples were filtered through a 47mm diameter weighed Whatman™ glass microfiber filters with mini air pump and the residues retained were dried in an oven to a constant weight at 105°C for 1 hour. Increase in weight of filter represents the total suspended solids. The filters with the residues were ignited in muffle furnace to constant weight at 550°C for 20 minutes. The remaining solids represent the fixed solids while weight lost on ignition was the volatile solids. This MLVSS test was carried out based on APHA 2540D and APHA 2540E.

2.4.5. Determination of pH.
pH measurement was performed using Hanna Edge® pH meter HI-2020. The water sample was continuously stirred with pH probe in order to obtain an accurate pH reading.

3. Results and discussion

3.1. Lab-scale phytoremediation on fish farm wastewater
This experiment was carried out to determine the water quality of fish farm wastewater during 14 days of phytoremediation and its effect toward biomass of macrophytes namely *S. molesta* and *S. polyrhiza*. Specifically, nutrient uptake by both macrophytes such as nitrate, phosphate and ammonia were accessed while COD level, turbidity, pH and MLVSS were also checked. Biomass in term fresh weight was being determined in the end of experiment. Table 1 shows the characteristics of fish farm effluent used for the experiment.

Table 1. Characteristics of fish farm wastewater.

Parameter	Units	Value
Nitrate (NO_3^- -N)	mg/L	-
Phosphate (PO_4^{3-})	mg/L	16.9
Ammonia (NH_3 - N)	mg/L	22.6
Chemical oxygen demand (COD)	mg/L	439
pH		7.76
Turbidity	NTU	315.3
Total Suspended Solids	mg/L	500.7
Volatile Solids	mg/L	334.0
Fixed Solids	mg/L	166.7

3.2. Nutrient uptake of the macrophytes

Nitrate, ammonia and phosphate are among the important nutrients which taken up by the aquatic plants from water body to sustain its growth.

Based on Figure 1, throughout the 14 days of experiment, the concentration of nitrate in fish farm wastewater for *S. molesta* macropyhtes was increasing gradually up to day 14. For *S. polyrhiza*, the nitrate concentration having similar trend with *S. molesta* macropyhtes. The reason both macropyhtes showed a higher nitrate level until day 14, was because nitrification process took place in phytoremediation system. Nitrification is a microbial process by which reduced nitrogen compounds (primarily ammonia) are sequentially oxidized to nitrite and nitrate. It occurred when the ammonia present was oxidized to nitrite through a bacteria group called Ammonia-Oxidising Bacteria (AOB) while Ammonia-Oxidising Archaea (AOA) oxidized ammonia to nitrite and produce nitrate [16]. For phytoremediation system, the wastewater was exposed to the direct light from lamp. Hence, great amount of oxygen was released by the macrophytes and filamentous algae in the wastewater through photosynthesis. This in turn promoted nitrification process since abundant oxygen was dissolved in the water column [17], leading to increasing nitrate concentration in the wastewater. The reason the plants did not remove nitrate was due to preference of plants in taking up ammonia over nitrate [18].

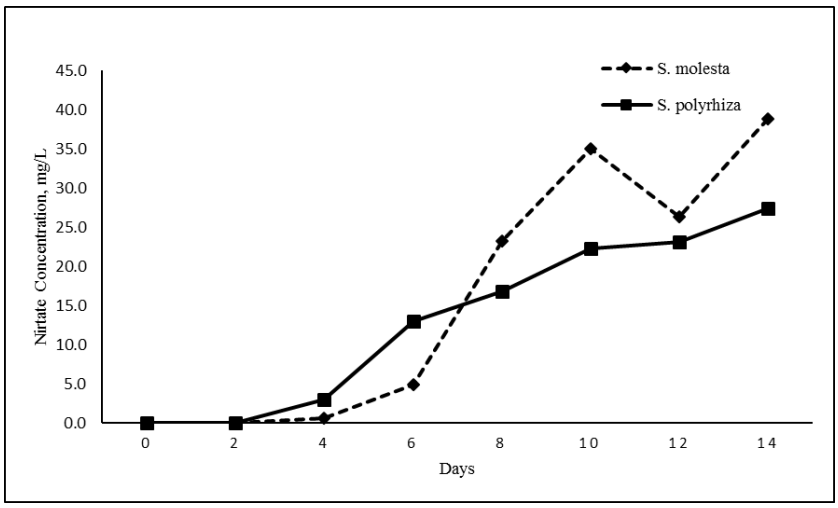

Figure 1. Concentration profile of nitrate in fish farm wastewater grown with *S. molesta* and *S. polyrhiza* species.

From Figure 2, it showed that there was decrement of phosphate in fish farm wastewater using either *S. polyrhiza* or *S. molesta*. Interestingly, *S. polyrhiza* was found to be more efficient in phosphate uptake as it removed 72% phosphate at day 4 and up to 95% in the end whereas 72% phosphate removal was only achieved by S. molesta at day 10. This indicated that *S. polyrhiza* removes phosphate more efficiently and in higher rate than *S. molesta* in the fish farm wastewater. This huge phosphate decrement trend was also shown in [15]. The phosphate can be taken up by plants roots, absorption through leaves and shoots in aquatic plants [17]. The concentration of phosphate or specifically called orthophosphate decreased throughout the experiment by plant uptake, was due to continuous need of metabolic activity by plant itself during growth which requires phosphorus for building ATP, ATP as well as other plant structural component. This phenomenon was also shown in the work of Ghaly et al. [19] in which the wastewater effluent contained less amount of phosphate at the end of the run and the plants gained weight significantly after phytoremediation.

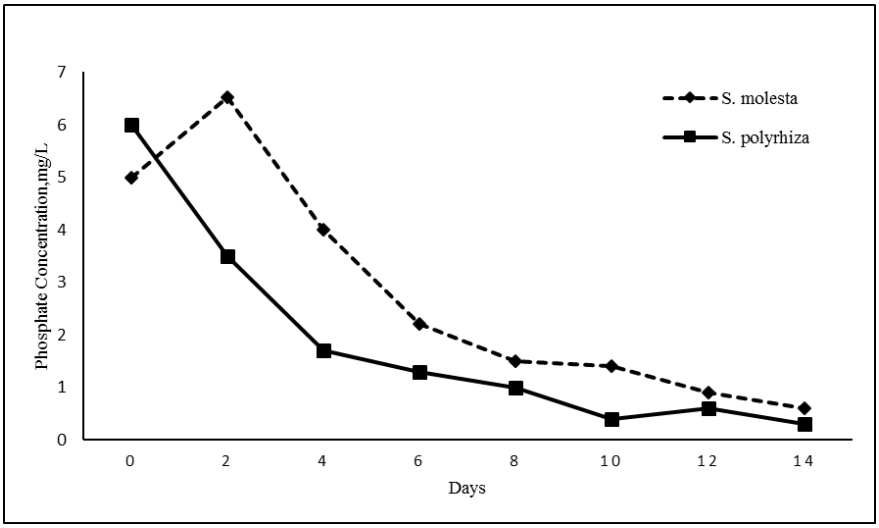

Figure 2. Concentration profile of phosphate in fish farm wastewater grown with *S. molesta* and *S. polyrhiza* species.

Figure 3 showed the trend for ammonia concentration for *S. molesta* and *S. polyrhiza* macropyhtes during 14 days. Similar ammonia decrement was observed for both plants and most of the ammonia were not detected in the wastewater by day 10 for *S. polyrhiza*, while by day 8 for *S. molesta.* The reason the ammonia level dropped significantly after going through phytoremediation was because of both direct plant uptake and the consumption of ammonia in nitrification [20]. Furthermore, similar study was conducted by Azim et al. [21] showed that ammonia concentration in a pond treated with bamboo was lower and the *periphyton* macropyhtes improved water quality in aquaculture system by nitrification. In addition, enhanced bacterial biofilms on the substrates might reduce ammonia level through nitrification [22].

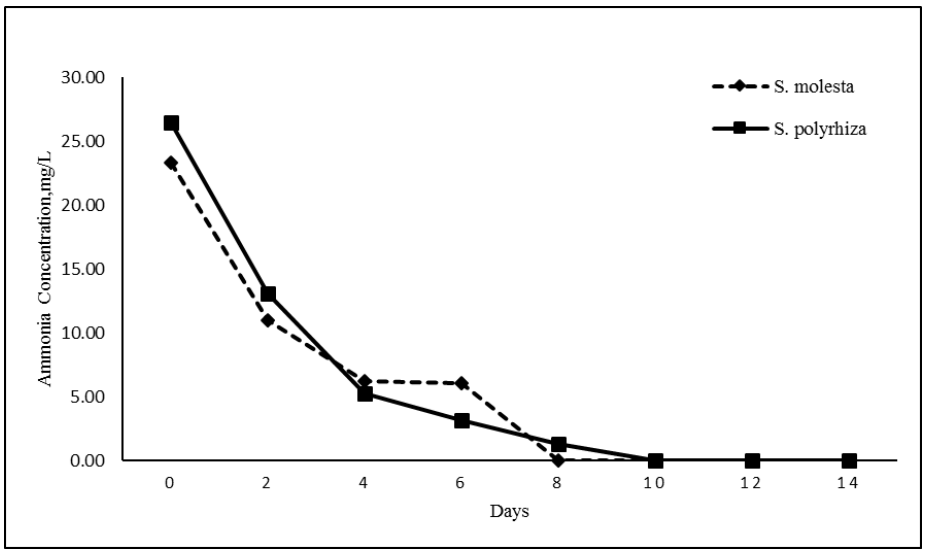

Figure 3. Concentration profile of ammonia in fish farm wastewater grown with *S. molesta* and *S. polyrhiza* species.

3.3. Water quality after phytoremediation

The water quality of fish farm wastewater which undergoes phytoremediation may either deteriorate or improve. Chemical Oxygen Demand (COD), Mixed Liquor Volatile Suspended Solids (MLVSS), turbidity and pH are among the analysis tests used to examine the water quality of the wastewater.

Chemical Oxygen Demand (COD) is a measure of the oxygen required to oxidize soluble and particulate organic matter in the water. It is an important water quality parameter as it provides an index to assess the effect of discharged wastewater will have on the receiving environment. Figure 4 showed the trend for COD concentration for *S. molesta* and *S. polyrhiza* macropyhtes during 14 days of phytoremediation period. Both plants showed reduction in COD level in their respective wastewater. COD level for *S. molesta* decreased from 186 mg/L at day 0 to 75 mg/L at day 14. Meanwhile, COD level for *S. polyrhiza* dropped gradually from 77 mg/L at day 0 to 30 mg/L towards the end of the run. Overall COD reduction for *S. molesta* after 14 days of phytoremediation was about 60% whereas *S. polyrhiza* was able to remove about 61 % COD in the wastewater. Both plants achieved highest COD removal on day 12, whereby 68% for *S. polyrhiza* and 63% for *S. molesta.* The COD decreased significantly during the treatment because the plants were fully developed causing the filtration capacity of the roots towards suspended solids and the absorption of dissolved nutrients increased [19].

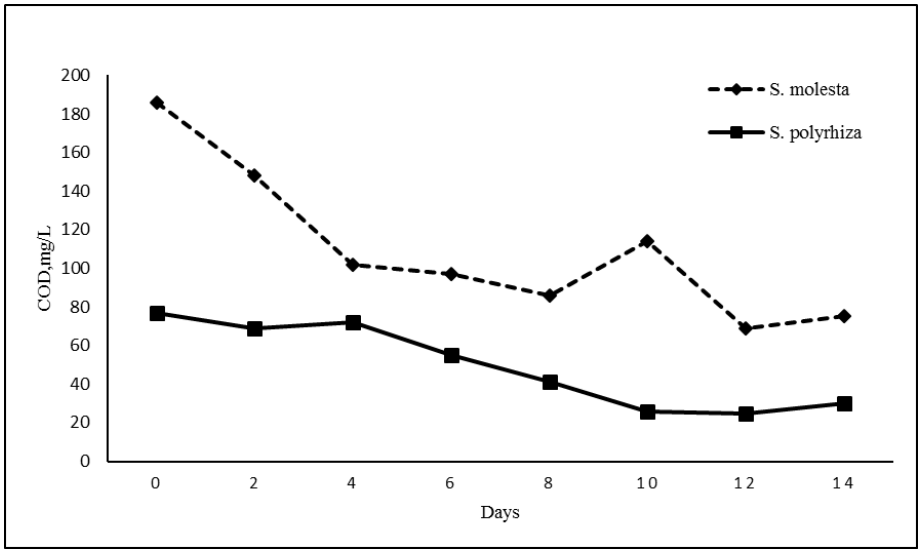

Figure 4. Concentration profile of COD in fish farm wastewater grown with *S. molesta* and *S. polyrhiza* species.

For pH analysis, Figure 5 showed that pH value did not fluctuate much for *S. molesta* macropyhtes with 7.69 at day 0 and 7.67 towards the end of the experiment whereas pH reading for *S. polyrhiza* at day 0 and day 14 is 7.71. Both plants had similar trend in pH value and pH fluctuations were minimum for both plants, with a range between 7.62 to 7.77. Akinbile and Yusoff [23] stated the pH level reduction is due to microbial activities and increase in CO_2 level in photosynthesis. Nitrification occurs over a wide pH range in soil while the optimal pH was estimated to be between pH 6.6 to 8.0 [17]. This may show that nitrification activity in our phytoremediation system is in optimal condition where its pH lain within 6.6 to 8.0, causing increasing nitrate level in the system.

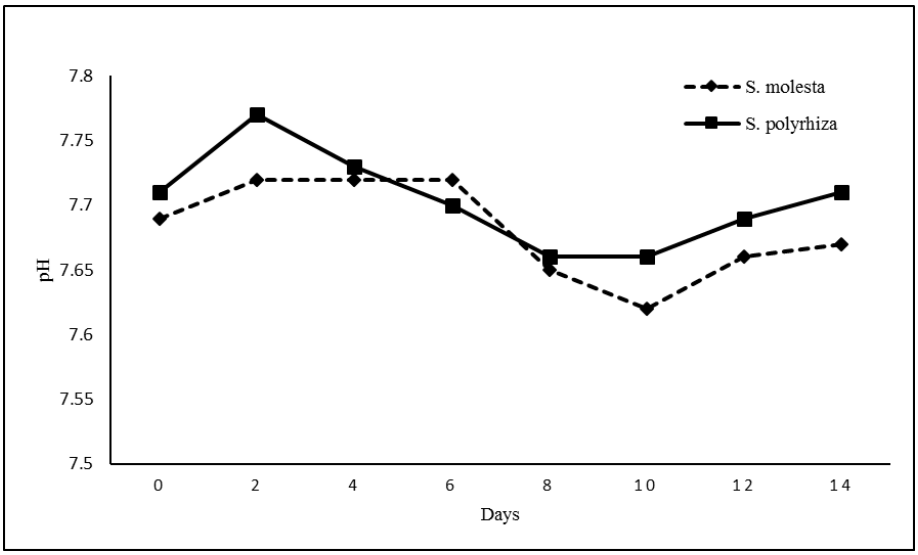

Figure 5. Concentration profile of pH in fish farm wastewater grown with *S. molesta* and *S. polyrhiza* species.

The results showed that significant improvement had taken place in increasing clarity of fish farm wastewater after 14 days of phytoremediation. Obvious change can be observed in the clarity of the fish farm wastewater when the level of turbidity drastically reduced during the first 6 days of

experiment. As the results shown in Figure 6, the turbidity reading for *S. molesta* decreased gradually from 298 NTU at day 0 to 54 NTU at the last day of experiment. Same goes to *S. polyrhiza,* the turbidity decreased from 137 NTU to 5 NTU at day 14. 82% and 96% reduction in turbidity were achieved for *S. molesta* and *S. polyrhiza* respectively. It is believed that the microbes and biofilms that grew, suspended in the wastewater column and resided in long hairy extensive roots eg. *S. molesta* as shown in Figure 7 consumed the organic suspended solids for their growth or specifically respiration. The organic suspended solids were metabolized or used up by the cells and being converted to energy, carbon dioxide and water. This would explain the reduction of COD and turbidity level in the fish farm effluent.

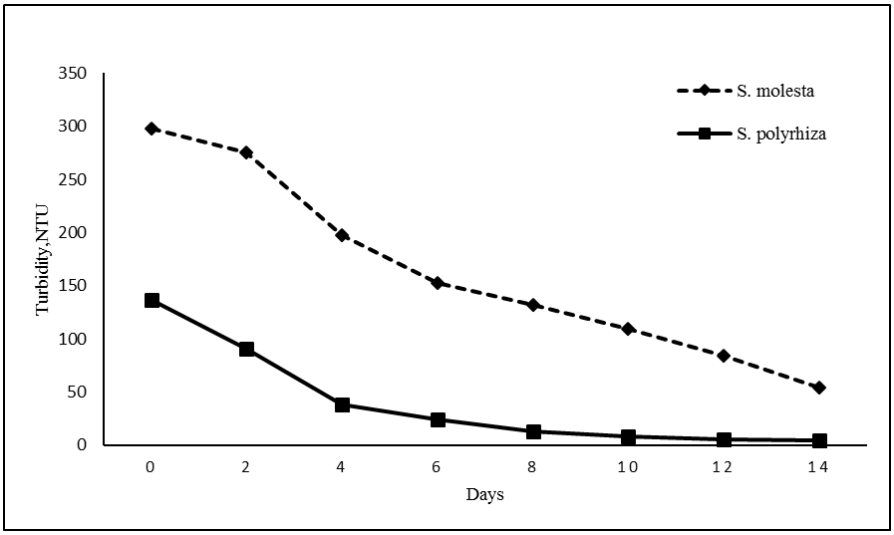

Figure 6. Turbidity profile of fish farm wastewater grown with *S. molesta* and *S. polyrhiza* species.

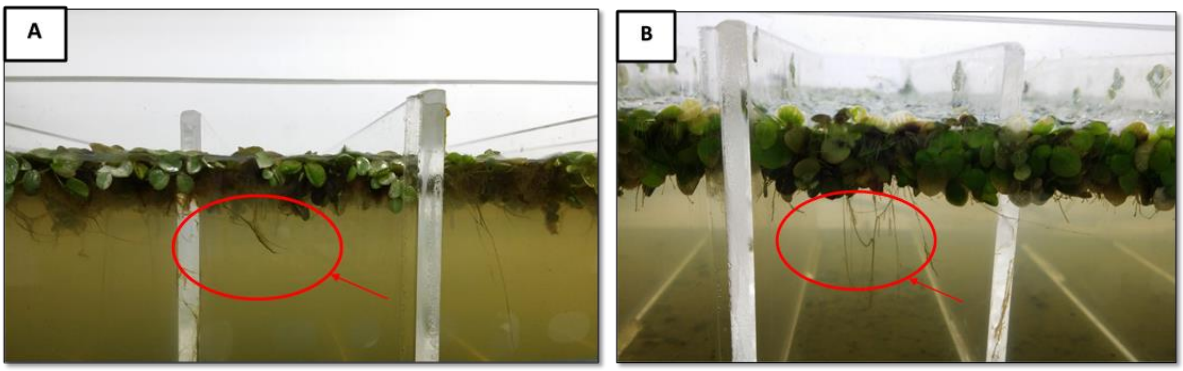

Figure 7. Biofilms or filamentous algae formed and attached to the roots of macropytes (A) *S. molesta* and (B) *S. polyrhiza*.

The red arrow in Figure 7 indicates the location where biofilms or filamentous algae attach to the roots in both macropyhtes system. They can be seen after 4 days. The roots of the plants become the place for bacteria to grow and the hairy roots makes nutrients easy to being absorbed into their roots.

Figure 8 showed the MLVSS result for both macrophytes. It showed that the concentration of total suspended solid (TSS) for both *S. molesta* and *S. polyrhiza* macropyhtes gradually decreased to 40 mg/L and 23 mg/L respectively in the end of the experiment. *S. molesta* reduced 79% of TSS while *S. polyrhiza* decreased 86% of TSS. Meanwhile, *S. molesta* achieved 67% reduction whereas *S. polyrhiza* managed to achieve 84% reduction in term of volatile solids. The concentration of fixed solids also

depicted decrement in which 93% of reduction was observed for *S. polyrhiza* and 96% reduction for *S. molesta* in phytoremediation system. The suspended solids concentrations declined during the experimental period due to several reasons. First, the plants were fully developed and the filtration capacity of the roots increased with the growth of the plants. Biofilms or filamentous algae were able to grow on them. This allowed those microbes to multiply and consumed the organic suspended solids causing the TSS to decrease throughout the experiment. Brix [24] stated that the reduction in suspended solids caused by the reduction of water current, which enhance the sedimentation of suspended solids and the filtration of solids by the plant tissue. TSS was thus further reduced in the wastewater. There was a study conducted by Lin et al. [25] in which more than 90% reduction in suspended solids of aquaculture wastewater using subsurface flow wetland planted with reed named *Paspalum vaginatum.*

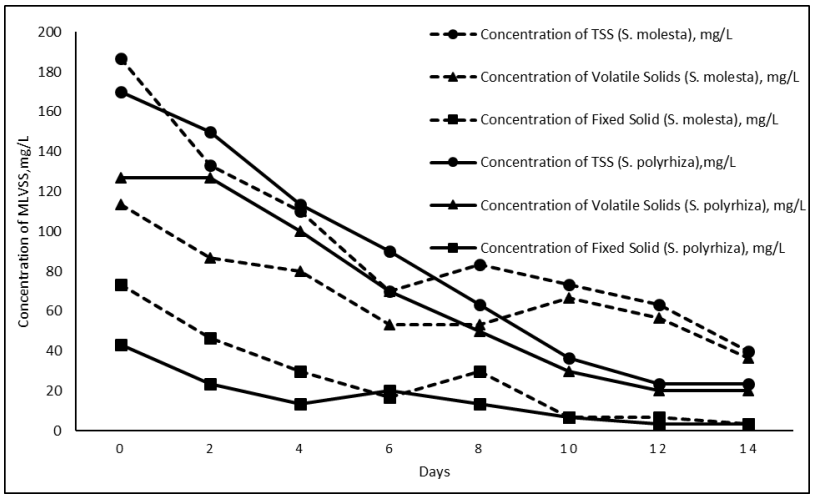

Figure 8. Concentration profile of MLVSS in fish farm wastewater grown with *S. molesta* and *S. polyrhiza* species.

3.4. Effect of phytoremediation towards biomass

After the phytoremediation, the macrophytes were checked for changes of their fresh weight, so that effect of phytoremediation towards biomass can be known.

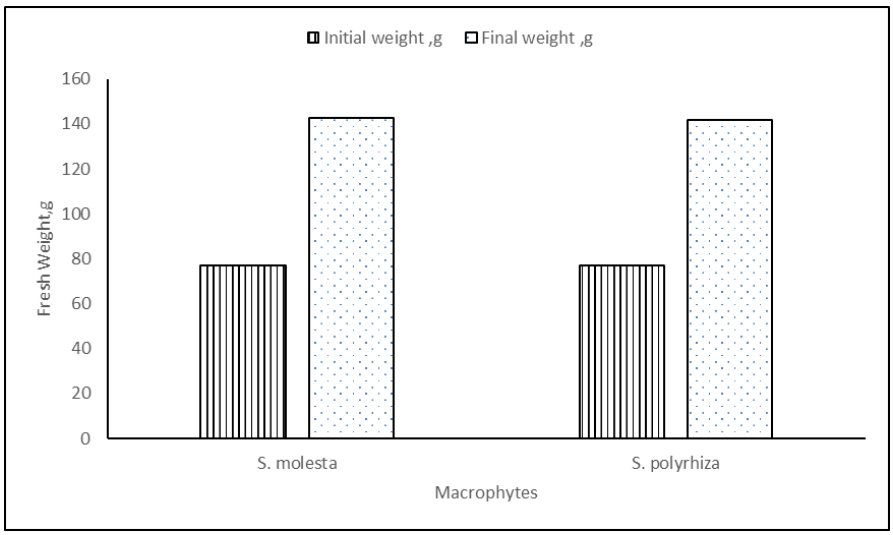

Figure 9. Changes of biomass (fresh weight) of different macropyhtes in phytoremediation system

As shown in Figure 9, fresh weight of *S. molesta* macropyhtes increased to 142.43g in which 85 % increase in weight was achieved. On the other hand, fresh weight of *S. polyrhiza* macropyhtes rose up to 141.62g where 84 % increase in weight was obtained. Both showed similar increment in biomass. The positive increment of biomass was attributed to the sufficient nutrients available in the fish farm wastewater to support the growth of macrophytes. The nutrients came from inorganic dissolved nutrients as well as mineralization of the organic suspended solids which were originated from uneaten feed and fish excretion. It showed suitability of the effluent in cultivating the plants, which was also shown in [26]. The huge increment of biomass for both species also allowed aquaculture farmers to produce valuable by-products such as fertilisers [12], biofuel [11, 13] and fish feed [10, 14] which can ultimately generate side income. Figure 10 and Figure 11 showed the appearance of both macrophytes on raceway pond at day 0 and day 14.

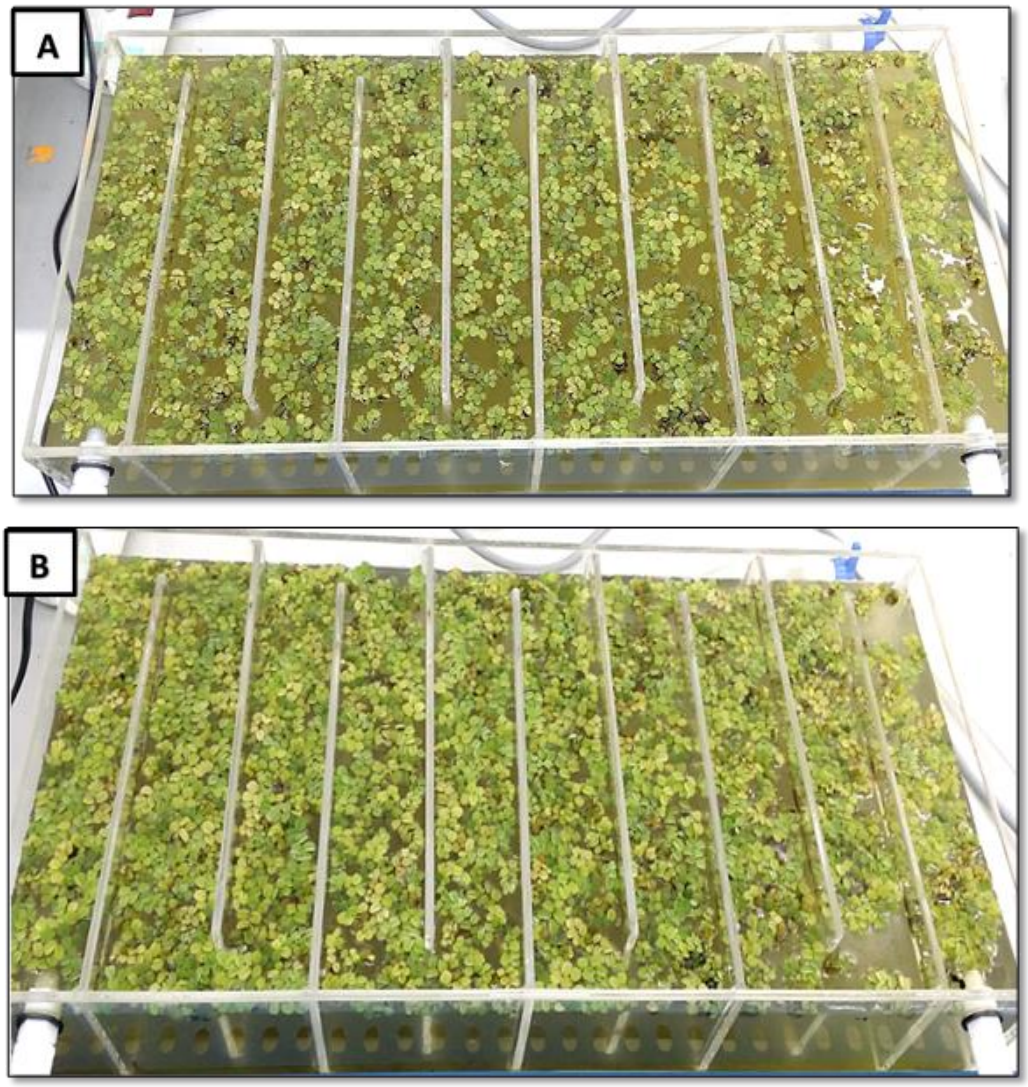

Figure 10. The appearance of *S. molesta* on the raceway pond at (A) day 0 and (B) day 14 of phytoremediation period

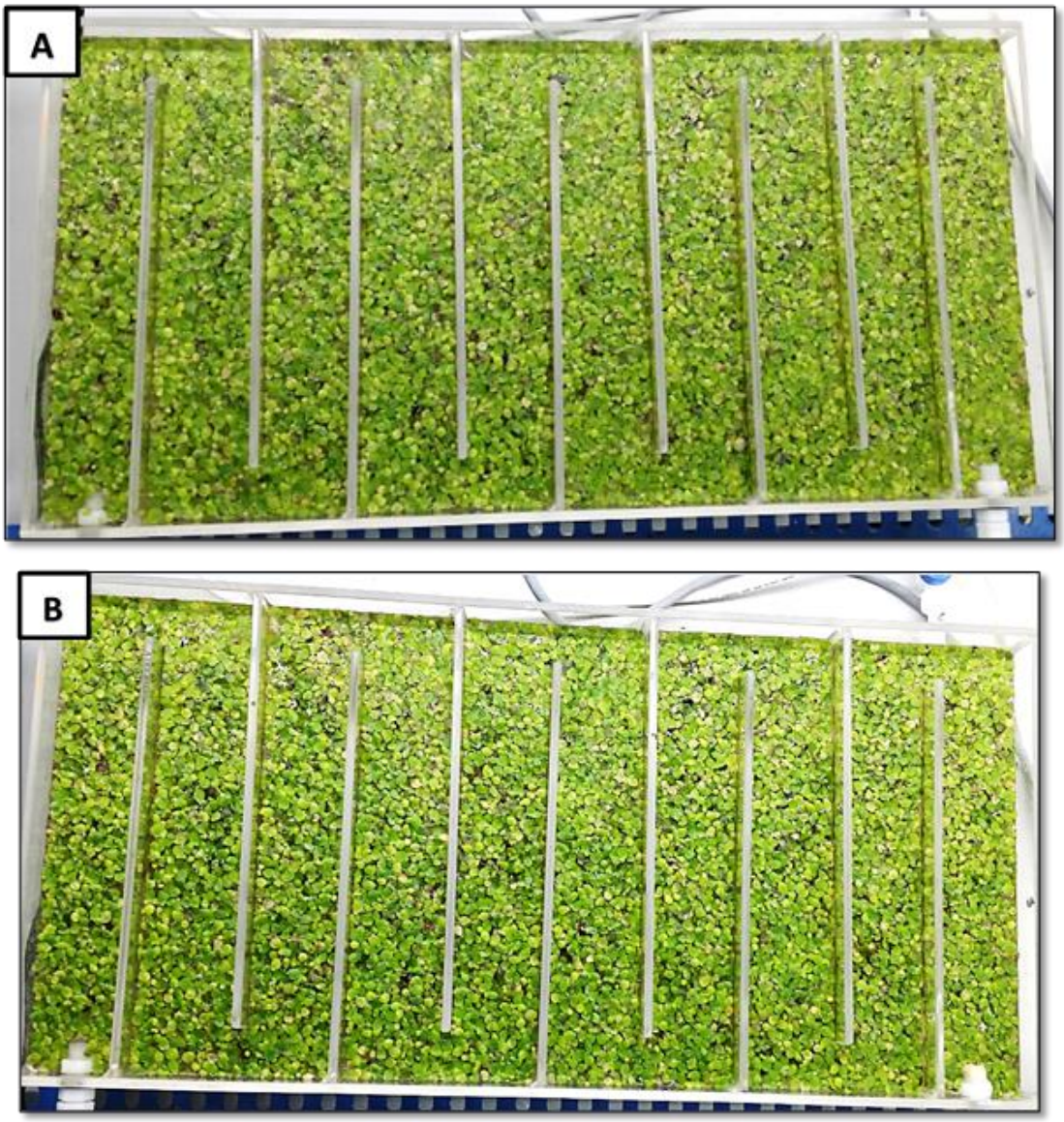

Figure 11. The appearance of *S. polyrhiza* on the raceway pond at (A) day 0 and (B) day 14 of phytoremediation period

4. Conclusions

The macropytes namely *S. molesta* and *S. polyrhiza* showed promising result in treating the fish farm wastewater. It was demonstrated that the phytoremediation system was capable of depurating the dissolved nutrients such as phosphate and ammonia and decreasing the suspended solids resulted from fish culture. Turbidity and COD was also lowered significantly. *S. polyrhiza* was more efficient and better in phosphate uptake as it removed phosphate faster and up to 95% in the end of the experiment whereas *S. molesta* removed only 72% at day 10. Both macrophytes had similar ammonia removal rate and managed to almost all ammonia within 8 to 10 days. This will surely help in minimizing the occurrence of eutrophication in receiving water since the effluent discharged were low in phosphate and ammonia. *S. polyrhiza* and *S. molesta* achieved highest COD removal on day 12 with 68% and 63% respectively. The total suspended solids in the wastewater was reduced drastically after phytoremediation as observed for both *S. polyrhiza* and *S. molesta.* It aids in avoiding the depletion of

oxygen in the water course that leads to suffocation and stress of aquatic living things. Turbidity of the wastewater was also significantly reduced in which 96% and 82% decrement shown for *S. polyrhiza* and *S. molesta* respectively. The clarity of fish farm effluent was improved tremendously eventually which allowed light to penetrate the water to ensure healthy living of the organisms beneath and prevent water-borne diseases.

Both macrophytes also exhibited high productivity in biomass in the fish farm wastewater besides their ability in treating the effluent. Biomass increment in fresh weight for *S. polyrhiza* was 84% while 85% was shown for *S. molesta.* The huge biomass harvested can be utilized by the aquaculture farmers to become fish feed, fertilizer or biofuel that in turn gain extra benefit.

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