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FED BATCH PHYTOREMEDIATION REGIME FOR ENHANCED NUTRIENT REMOVAL BY *SALVINIA MOLESTA* ON FISH FARM WASTEWATER

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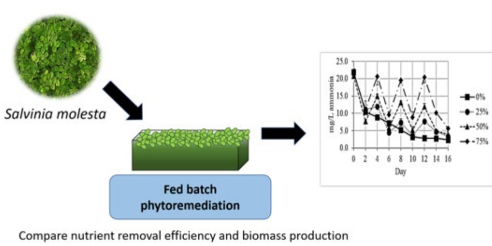
Graphical abstract



Abstract

Phytoremediation is a bioremediation process that uses various types of plants to remove, transfer, stabilize, and destroy contaminants in the soil and groundwater. In this study, the performance of fed batch (FB) phytoremediation by *S. molesta* on fish farm wastewater was investigated by varying percentage of fresh wastewater loading. This study was aimed to investigate the biomass production of *S. molesta* through different FB percentage cultivation along with nutrient removal aiming to treat wastewater. The water quality after phytoremediation was monitored throughout 16 days. The nutrient removal efficiency was high in higher percentage of FB but all the medium achieved standard discharge limit. Ammonia and phosphate removal showed very high (<95%) for 75% FB medium with total removal of 46.59 mg and 5.46 mg respectively. The removal of ammonia and phosphate by control set was 13.73 mg and 1.62 mg. Although the concentration of nitrate continued to increase throughout the study, FB cultivation minimized the increment where all percentage experienced decrease in nitrate value and 75% FB had lowest level of 2.40 mg/L compared to control which was 5.00 mg/L. However total nitrogen value in higher FB medium indicated highest value of 13.85 mg/L on day 16. VSS for FB medium was far below control. FB phytoremediation of *S. molesta* in fish farm water showed high efficiency in nutrient removal but no significant effect on its biomass. This study proved that FB phytoremediation of *S. molesta* has potential on nutrient removal from fish farm wastewater

Keywords: Phytoremediation, macrophytes, wastewater treatment, water quality, fed batch

1.0 INTRODUCTION

Salvinia molesta is an aquatic fern (macrophytes) and is classified in Salviniaceae family which belongs to eastern Brazil. Temperature of 20 -30 °C and pH range in between 4 and 9 are the most favourable growth conditions for *S. molesta* [1]. Under optimal condition it has the potential to double its biomass within 16 h to 2 days [2]. Macrophytes can grow in or near water and can be emergent, submerge or floating on the water surface. Macrophytes play an important role in a healthy ecosystem. It is one of the main sources of oxygen in the aquatic environment via photosynthesis. Its long roots serve as substrate for algae and shelter for fish and invertebrates.

In recent study, macrophytes play vital role in recycling nutrient along sediments and help in stabilizing stream and river [2]. *S. molesta* helps in stabilization of sediments by serving as filter to prevent suspended solids along with prevention against corrosion by reducing turbulence and flow velocities of water bodies [1, 2]. Some studies showed that macrophytes have potential in dye removal [1], detoxification of Cr(VI) [3], adaption to high NH_4^+ [4] and also nutrient removal in treated POME [2]. The success of these adaption is because of the ability of *S. molesta* to grow in high level of nitrogen, phosphorous, potassium, concentrates the minerals and accumulates protein concentration in plant [2].

The removal of contaminants from the environment by plant activities, or commonly known as phytoremediation, is a process whereby the plants remove, transfer, stabilize, or break down the contaminants in the soil and groundwater. Phytoremediation using microalgae is popular among researchers especially in wastewater treatment processes. However, the major cost in these operations is microalgae removal from wastewater treatment. Despite the high nutrient removal efficiency by microalgae, their presence in the effluent will contribute towards the BOD of the water if they are not removed from the water column. Their typical culture density, which is generally very low (below 1 g/L), further contribute to the difficulty in harvesting and escalating the cost [5-7]. Therefore, macrophytes such as *S. molesta* are considered as a potential substitute in treatment pond since macrophytes are easier to be removed since they float above the water surface. *S. molesta* can be removed through simple harvesting process and the study of feeding strategy is required to further enhance the treatment system. Even though fed batch strategy has been widely applied in phytoremediation with microalgae, there are lack of studies of application of fed batch mode on phytoremediation with macrophytes. Past studies of phytoremediation with macrophytes were mostly done in batch mode, where the removal efficiencies for ammonia, phosphate and nitrate ranging from 56- 95% [8, 9]. Fed-batch (FB) feeding mode is said to be the most economical and environmental-friendly treatment [9-12]. Therefore, this study aimed to investigate the different feeding strategies and evaluate the performance of *S. molesta* in nutrient removal from fish farm wastewater.

2.0 METHODOLOGY

Cultivation of *S. molesta*

S. molesta used in this study was sub-cultured from original samples collected from Nibong Tebal, Penang [2]. Aseptic *S. molesta* was cultivated in closed glass bottles containing 70 mL of Hoagland medium [13] where the medium was sterilized at 121°C for 15 min. All the glass jars were placed in a closed room with fluorescent lamp at continuous light intensity of 1600 lux and the temperature was maintained at 25 ± 1 °C for 14 days for plants to achieve exponential growth. The resulting macrophytes were collected, filtered and washed several times with tap water to remove any medium adhering to the macrophyte. They were then dapped dry with absorbent paper before the biomass was used for subsequent experiments.

Fed Batch Cultivation Set-Up Run

The wastewater was collected from a fish farm located at Nibong Tebal, Penang. The quality of the wastewater tested was NH_3-N: 21.5 ± 0.64 mg/L, PO_4^{3-}: 2.39 ± 0.03 mg/L, NO_3^-: 0.13 ± 0.05 mg/L, TN: 16.5 ± 0.34 mg/L, COD: 164.5 ± 2.98 mg/L O_2, turbidity: 184.25 ± 2.25 NTU, pH: 7.64 ± 0.19. The collected wastewater was stored in containers at 4 °C till prior use. The healthy floating macrophyte was chosen and weighed (8.4 g) before they were placed evenly in the container (16.5 cm long x 11.5 cm wide x 7.1 cm deep) with fish farm wastewater to stimulate a small-scale pond with a total liquid volume of 700 ml. The experiment was conducted for 16 days and three replicates were obtained. The experiment duration (16 days) was based on the amount of time required by the macrophyte to reduce the nutrient to the lowest possible concentration. The 4-day interval for medium replenishment were chosen as the data shows that most major nutrient were significantly consumed by that duration. Four sets of culture were prepared with different fed batch mode percentage which were no FB (control), 25% FB, 50% FB and 75% FB which were labelled as 0%, 25%, 50% and 75% respectively. The medium was added every 4 day of cycle with total duration of 16 days. At each cycle fed batch mode, the mentioned percentage of medium was removed and topped up with fresh medium. About 12 ml of treated wastewater sample was collected in 2 days intervals. The water level in the bottle was ensured to be constant. Tap water was added to the container to replace the evaporated volume. Water samples were collected after refilling with the indicated percentage of feed batch volume to be tested for nitrate, phosphate and ammonia to study nutrient uptake by S. molesta. COD, turbidity and VSS test were conducted on the last day of the experiment to investigate the water quality after phytoremediation.

Analytical Analysis

Determination of nitrate and phosphate concentration for water sample

The supernatant of the samples was obtained by centrifugation at 10000g for 15 min. The supernatant was tested for nitrate and phosphate content. The nitrate was determined by Cadmium Reduction Method (HACH strategy 8039) utilizing 5 Nitrate Reagent Powder Pillows. The phosphate was determined by Ascorbic Corrosive Method (HACH technique 8048) utilizing 3 Phosphate Reagent Powder Pillows. The phosphate determination was in accordance with USEPA technique 365.2 and Standard Method 4500-P-E for wastewater.

Determination of ammonia concentration, total nitrogen and COD for wastewater with Vario Tube Test

For ammonia, the detectable range was 1 – 50 mg/L NH_3-N with usage of 0.1 mL sample supernatant. The method used was VARIO Am tube test reagent, Set HR, F5 (ammonia salicylate along with ammonia cyanurate). Meanwhile COD test was conducted by dichromate/H_2SO_4 method by LOVIBOND method 131 with 2 mL of sample supernatant and detectable range of 0 – 1500 mg/L O_2. The Total Nitrogen (TN) used hydroxide LR digestion vials along with persulfate and TN reagent powder pack. The detectable range was 0.5 – 25 mg/L N.

Determination Of Turbidity and pH of Water Sample

Turbidity was measured using HANNA HI 93703 microprocessor turbidity meter by 890 nm along with 0-1000 NTU. Samples were ensured to be well mixed before pouring it gently into a clean cuvette to avoid air bubble on the cuvette wall. The collected water samples were tested for pH value using pH meter.

Determination of VSS

50 mL sample was filtered using 47 mm glass microfiber filters with mini air pump and the residue obtained was dried in an oven at 105 °C for 1h to obtain constant weight. Increment in filter weight indicated total suspended solid (TSS). The residue filter obtained were then ignited in furnace at 550 °C for 20 min until constant weight was obtained where the solid left indicates fixed solid and loss in weight is volatile solid (VSS).

Determination Of Biomass (Fresh Weight)

The macrophytes was harvested, washed with distilled water and dapped dried with absorbent paper before being weighed [13, 14].

3.0 RESULTS AND DISCUSSION

Effect on Nutrient Uptake by *S.molesta* in fish farm wastewater through FB phytoremediation

Ammonia removal by S.molesta through FB phytoremediation

The amount of ammonia removal is shown in Figure 1. The total removal of ammonia for FB percentage of 0%, 25%, 50%, and 75% in 16 days were 13.73 ± 0.30 mg, 26.36 ± 0.50 mg, 34.06 ± 0.60 mg, and 46.59 ± 0.80 mg respectively. The trend obtained corresponds to the studies conducted by [9, 15, 16]. The FB mode of 25%, 50%, and 75% showed higher ammonia uptake which were 2.3 times, 3.0 times, and 4.2 times larger than control set (cultivated without FB), respectively. Ammonia concentration at the end of this study for all percentage of FB were below standard discharge limit which is 5 mg/L [8].

The main ideas behind the fed batch cultivation mode employed in this study were to replenish the depleted nutrients concentration in the wastewater as to sustain the growth of the *S. molesta* and also to ensure consistent availability of the nutrients and enhance the total removal of the particular nutrient. Ammonia reduction was not only caused by absorption but also due to volatilization of a small portion to the atmosphere. This phenomenon could be caused by pH of the medium, initial ammonia concentration and due to aeration [11]. The rate of absorption increases as the FB percentage increases in which 0.86 mg/L/Day, 1.65 mg/L/Day, 2.13 mg/L/Day, and 2.91 mg/L/Day for 0%, 25%, 50%, and 75%, respectively. Free-floating macrophytes uptake ammonia via their fronds and roots [17]. Nutrients can be uptake passively where ammonia concentration being sufficiently high in wastewater, diffusion occurs through the root membranes. Plants can also use the active uptake system where it depends on specific transporter protein on the cell membrane. For the uptake of ammonia from the wastewater, the roots rely on AMT gene [18]. The continuous decrement of ammonia concentration was also partially contributed by aerobic digestion of Ammonia Oxidizing Bacteria (AOB) and Nitrate Oxidizing Bacteria (NOB) through nitrification. Excessive ammonia concentration in the medium tends to show a slower growth rate of *S. molesta* in wastewater. *S. natans* grew well at ammonia concentrations up to 90 mg/L. However, at ammonia concentration of 255 mg/L, old parts of plants died but new leaves were produced, so plants still showed positive yet reduced growth rates [19]. Based on the study done by [4], the plant exhibited damage leaves or rotted roots when the ammonia concentration in medium exceeded concentration of 17 mg/L during the 40 days of study. For 75% FB cultivation, medium recorded higher than the tolerance toxicity level, between the range of 23.10-18.80 mg/L. However, the macrophyte did not suffer any adverse effect due to the FB cultivation mode eliminates excessive build-up of ammonia from the intermittent feeding strategy. Similar studies by other researcher also have shown that the removal of ammonia can occurs even when the concentration exceeds toxicity level due to the feeding regime used [10, 12].

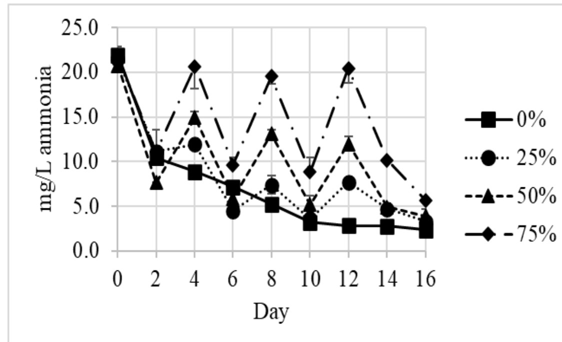


Figure 1 Effect of phytoremediation on ammonia concentration throughout the experiment

Phosphate removal by S. molesta through FB phytoremediation

The plant, *S. molesta* showed healthy growth in all mediums which indicated good phosphate removal. The phosphate profile in the medium is shown in Figure 2 throughout 16 days of experiment. The initial concentration of phosphate in the medium was lower that allowable limit which in around 2.40 mg/L. The total phosphate removal achieved by 0%, 25%, 50%, and 75% were 1.62 mg, 3.14 mg, 3.68 mg, and 5.46 mg. As the FB feeding percentage increases, the percentage of removal decreases. The percentage of removal were determined to be at 97 %, 82 %, 78 %, and 87 % for 0 %, 25 %, 50 %, and 75 % FB mode respectively. However, the largest quantity of phosphate removed was in 75 % FB mode which was shown to be 3.4 times greater than non-FB medium. Although in terms of removal efficiency, 75% FB was lower compared to the control, but the strategy of FB feeding proved to be capable of achieving higher phosphate removal rate and greater capacity of treating wastewater. All the phosphate level for FB and non-FB system showed huge decrement on the last day of experiment indicating that *S. molesta* has great potential in storing phosphate via its widely spread root system [20-22]. Vegetation roots in the water tend to absorb most of phosphate from the water column and transfer it to the entire plantlets to support growth [20]. An energy-driven phosphate transporter that relies on Pht1 genes move phosphate through the plant membrane into the plant root cells [23]. Based on the mass balance in the non-axenic study, it was shown that the uptake of phosphorus by the macrophytes was the dominant mechanism of removal of phosphate [23].

Phosphate is a basic supplement for plant development but at high level it turns into an ecological contaminant since it triggers eutrophication to take place. Phosphate is vital macronutrient for ADP and ATP component in plant where these components been used for storage and translocation in photosynthesis and respiration. Phosphate is also required in formation of biochemical structures such as nucleic acid, nucleotide and sugar phosphate during their exponential growth stage for multiplication of plant. Phosphate component especially orthophosphate in medium will be used up by *S. molesta* for biomass production. Hence the decrement of phosphate is to be observed drastically in all medium.

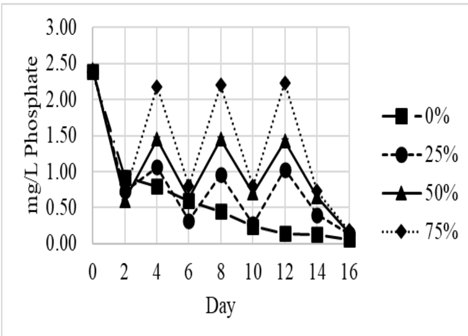


Figure 2 Effect of phytoremediation on phosphate concentration throughout the experiment

Nitrate removal by S. molesta through FB phytoremediation

FB feeding strategy indicated reduction in nitrate level as shown in Figure 3. The final nitrate concentration for 0%, 25%, 50% and 75% were 5.00 ± 0.20 mg/L, 3.1 ± 0.05 mg/L, 2.90 ± 0.05 mg/L and 2.40 ± 0.10 mg/L respectively. The nitrate concentration profile by *S. molesta* through FB phytoremediation on 0% and 25 % experienced increment about 4.82 mg/L and 0.15 mg/L, whereas 50% and 75% showed decrement in nitrate concentration about 0.40 mg/L and 4.15 mg/L respectively. FB with 75% of loading gave maximum nitrate removal rate of 0.26 mg/L/Day along with lowest nitrate concentration 2.40 mg/L/Day on day 16. On FB cultivation, the nitrate concentration fluctuated until day 12 and then started to decrease. Throughout the study, nitrate concentration was below the discharge limit value which is 10 mg/L.

At the start of the phytoremediation study, nitrate concentration of all medium increased gradually. The nitrate concentration in the wastewater was mainly contributed by nitrification, denitrification, plant and microbial uptake. Nitrification is a process where ammonia is biologically oxidised to nitrite and further oxidation into nitrate, thus nitrification rises nitrate concentration in medium. The other mentioned processes decrease in nitrate concentration. The reason is probably due to the combination of *S. molesta* and filamentous algae present in the system when the wastewater was exposed to sunlight (fluorescent light in this study) during the experiment. With sufficient light, the *S. molesta* and algae will produce oxygen through photosynthesis and the oxygen will dissolve in the water column. This dissolved or available oxygen supported the nitrification process which has high oxygen demand and thereby increases its presence in the wastewater. Oxygen level in the medium tends to increase due to photosynthesis process by photosynthetic plants which leads to nitrification favour condition, thus the nitrate level increases. The rate of nitrification was higher in non-FB medium, caused rise in nitrate level throughout the studies. The presence of highly oxygenated water suppressed the denitrification process caused the nitrate concentration to increase. Assimilation of ammonia also requires less energy as compared to nitrate [19]. Moreover, macrophytes tend to absorb ammonia compared to nitrate as nitrogen source due to the higher affinity towards ammonia ions which then resulted in rises in nitrate concentration [24-26]. The fluctuation in nitrate level proves that the medium exhibit good nitrogen cycle. This analysis illustrate that FB has positive impact in lowering nitrate concentration in medium which again stated by [10, 12, 27].

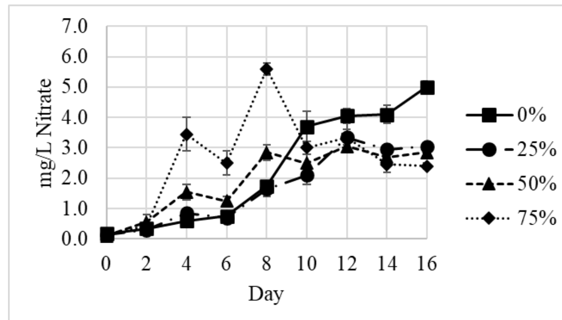


Figure 3 Effect of phytoremediation on nitrate concentration throughout the experiment

Nitrogen removal by S. molesta through FB phytoremediation

Nitrogen source in this study was mainly coming from the combination of organic and inorganic nitrogen in the medium. Figure 4 showed total nitrogen level throughout 16 days of experiment where FB achieved lower nitrogen level in medium compared to control. The initial total nitrogen amount in the medium was 16.6 mg/L, where it exceeded the standard nitrogen level 15.0 mg/L [19]. Phytoremediation with and without FB, the nitrogen level on day 16 was 19.50 ± 0.70 mg/L, 12.70 ± 0.20 mg/L, 10.95 ± 0.65 mg/L, and 13.85 ± 0.25 mg/L for 0%, 25%, 50% and 75% respectively. Hence, this proved that phytoremediation with FB the respective final effluent successfully achieved the standard discharge limit. The total removal of nitrogen by *S. molesta* 25%, 50%, and 75% were 11.65 mg/L, 12.8 mg/L and 19.75 mg/L, respectively whereas control group showed increment of 2.90 mg/L. This indicated that medium without FB had poor removal in nitrogen source as well as production of nitrogenous compound. *S. molesta* in 75% of FB absorbed 6.8 times higher than in control medium. The highest FB percentage (75%) showed highest nitrogen uptake but at 50% FB gave lower nitrogen concentration on day 16 which 10.95 mg/L compared to other 25% and 75% FB. In ecological wastewater treatment systems, a combination of nitrogen transformation mechanisms are responsible for N removal, including: mineralization of organic nitrogen to ammonium; decay of plant biomass to organic nitrogen; volatilization of ammonia; nitrification of ammonium to nitrate; denitrification of nitrate to nitrogen gas and plant uptake of ammonium and nitrate [28]. Possibly, a combination of these processes was contributing to nitrogen removal in the medium, making it difficult to attribute nitrogen removal to any one mechanism.

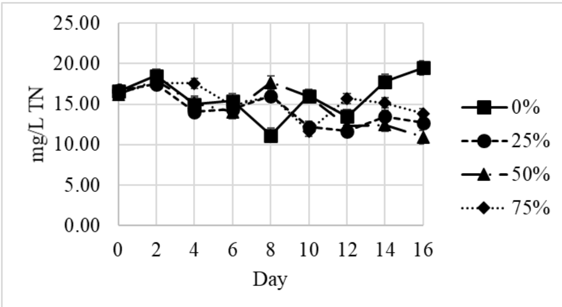


Figure 4 Effect of phytoremediation on total nitrogen concentration throughout the experiment

Wastewater Quality After FB Phytoremediation

COD test is a measure of oxygen level in water to the organic substance in wastewater which able to decompose chemically and oxidation of inorganic compounds such as ammonia and nitrite by utilizing dichromate in acid solution. This method ensures that nearly all organic as well as inorganic substances can be oxidized chemically where dichromate able to react with component such as chloride, nitrite, ferrous ion and sulphate [2]. Figure 5 showed COD changes throughout FB phytoremediation.

For non-FB phytoremediation, trend of decrease in COD at day 2; 117.50 mg/L O2, then increment of 22.50 mg/L O2 on day 4, then gradual decrement until day 16 where the final COD value was 79.00 mg/L O2. The total COD removal attained by 0% medium was 86.50 mg/L with 52.27% of removal efficiency at rate of 5.41 mg/L/Day O2. FB medium showed same trend for all percentage where decrease in COD level at day 2; 118.00 mg/L. After loading of fresh wastewater (FB) on day 4, increment in COD level was experienced with FB 25%, 50%, and 75% were 194.00 mg/L O2, 251.00 mg/L O2, and 295.00 mg/L O2 respectively due to FB feeding. On day 16, COD level in the medium decreased for FB 25%, 50%, and 75% were 160.00 mg/L O2, 247.00 mg/L O2, and 304.00 mg/L O2 respectively. The total reduction of COD in 25% FB was highest which 124.50 mg/L O2 at rate of 7.78 mg/L/Day O2. For 50% and 75%, total COD reduction were 94.00 mg/L O2 and 100.50 mg/L O2 with rate of 5.88 mg/L/Day O2 and 6.28 mg/L/Day O2, respectively. All FB medium showed higher reduction in total COD level compared to non-FB medium in which FB 25%, 50%, and 75% had reduction of 1.44 times, 1.08 times, and 1.16 times higher than non-FB medium. This showed that the efficiency of COD reduction decreases as FB percentage increases where 25%, 50% and 75% showed 17.2 %, 10.5 %, and 9.7 % reduction efficiency. Even though FB phytoremediation offered greater amount of total reduction for COD, on day 16 only the non-FB was below limit level which is 120 mg/L O2 [17]. In phytoremediation, increased in COD value is due to degradation of organic carbon in fish farm wastewater into carbon dioxide and water while simultaneously COD level reduced due to absorption of organic carbon of wastewater suspended solid attached to *S. molesta* widespread root system [2]. COD level is also affected when degradation by microorganism in plant root leads to release of organic carbon into the medium [29]. For the non-FB set, organic decomposition by bacteria took place and without fresh medium added in throughout 16 days, no increment in COD was observed. Hence, with the loading of medium in FB sets, increment of COD was observed as organic matter was continuously introduced to the system. Figure 6 and 7 showed significant increase in turbidity and TSS for the FB sets due to the biodegradation of the accumulated organic matter in the system. In FB mode, the lower the percentage of FB, the lower COD level was achieved.

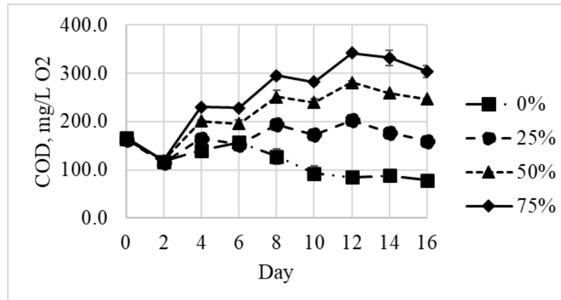


Figure 5 Effect of FB phytoremediation on COD value throughout the experiment

Turbidity is an optical assurance of wastewater clearness. Measurement of turbidity is important since it can alter water chemistry, photosynthesis efficiency and promote contamination. Figure 6 showed that FB phytoremediation proved the capability of improving the clarity of fish farm wastewater in short period of time. For the FB medium, peak was observed after fresh loading. The clarity of wastewater on day 16 by *S. molesta* in different FB of 0%, 25%, 50% and 75% were 27.00 NTU, 45.00 NTU, 58.50 NTU and 61.50 NTU respectively. This showed that non-FB medium had the best clarity compared to FB medium. However, higher percentage of FB indicated higher total clarity removal where 75% FB showed total turbidity reduction of 553.50 NTU. Whereas 0%, 25%, and 50% had reduction of 156.00, 198.50, and 358.50 NTU. This indicated FB performed well for water clarity. At high FB percentage feeding, the clearer the clarity as more particulate of wastewater been attached to its root.

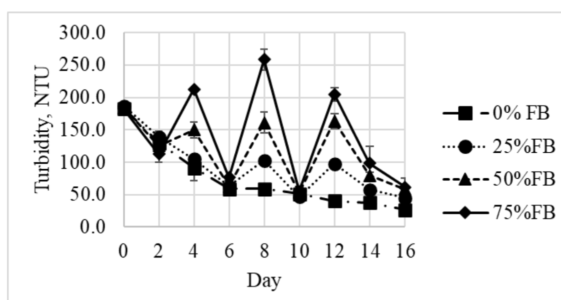


Figure 6 Effect of FB phytoremediation on turbidity value throughout the experiment

Both total suspended solid (TSS) and volatile suspended solid (VSS) tests were conduct and the tabulated result was shown in Figure 7. Based on Figure 7 (a), TSS during phytoremediation for FB and non-FB medium started with range of 0.22 g/L TSS to 0.23

g/L TSS. For 0%, 25% and 50% medium showed decrement in TSS present in fish farm wastewater in which were 0.07, 0.10 and 0.16 g/L but 75% FB medium showed increase in TSS by 0.01 g/L TSS on day 16. This was because 75% FB medium experienced higher loading of suspended solid compound. FB proved to increase in TSS amount because fresh medium introduced more suspended solids. This also proved that FB caused the higher turbidity level as TSS component was greater in presence compared to non-FB. The higher the FB percentage, the higher turbidity level of the medium. The filtrate of 75% FB showed highest amount of TSS present in fish farm wastewater and control was the least where the control showed same trend as turbidity which was discussed in earlier part.

For VSS present in fish farm wastewater initially within the range of 0.16 to 0.21 g/L VSS based on Figure 7 (b). While the VSS amount at end of FB phytoremediation at percentage of 0%, 25%, 50% and 75% FB were 0.07 g/L VSS, 0.10 g/L VSS, 0.15 g/L VSS, and 0.20 g/L VSS with removal amount throughout the study 0.10 g/L VSS, 0.09 g/L VSS, 0.05 g/L VSS, while 75% FB increased by 0.01 g/L VSS. The VSS displayed the same trend obtained from TSS indicating that the total suspended solids present were volatile solids in phytoremediation which probably could be organic substance likely from microorganism and the plant itself.

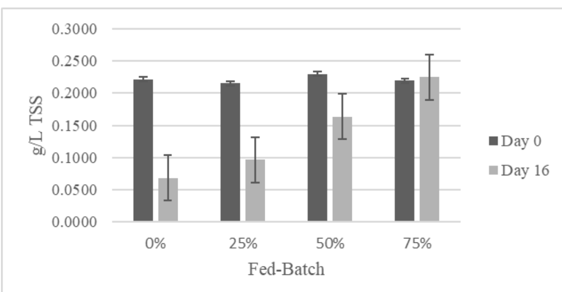


Figure 7(a) Effect of FB phytoremediation on TSS value throughout the experiment

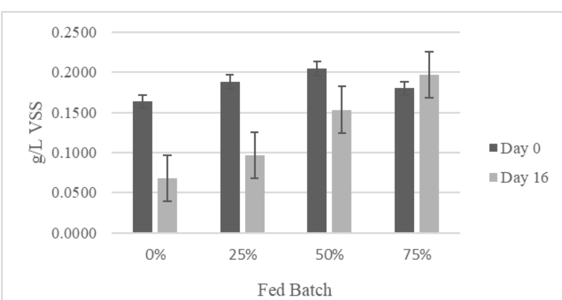


Figure 7(b) Effect of FB phytoremediation on VSS value throughout the experiment

Effect Of Fed Batch Towards Biomass

During cultivation, 6.0 g/L of *S. molesta* were used for each replicate. On day 16 the plant was harvested and cleaned with tap water then dapped dried with tissue towel before weighing it. Figure 8 showed the average fresh weight of S. molesta on day 0 and day 16 for each set of FB phytoremediation.

The initial biomass for each set (0%, 25%, 50%, and 75%) were same which was 6.00 g/L. The trend showed that as the fed batch percentage increases, the biomass of plant decreases slightly. Therefore, the biomass of *S. molesta* with and without FB were approximately same. The final fresh weight of *S. molesta* during FB phytoremediation of 0%, 25%, 50%, and 75% were 32.00 ± 0.75 g/L, 31.00 ± 1.55 g/L, 30.00 ± 1.92 g/L, and 29.00 ± 1.04 g/L respectively. Therefore, it was concluded that average biomass on day 16 for all set was 30.58 ± 0.81 g/L. However, phytoremediation of *S. molesta* in fish farm wastewater posed positive growth with rate of production 1.63 g/LD. Based on [30], pH would affect the biomass production of macrophyte and further analysis on pH of the medium was done on this study. Typically, pH of wastewater would be around 6.50-7.80 [31] and 6.20-7.80 [32] while standard effluent discharge of pH is between range of 5.00-9.00 [17]. Figure 9 showed pH value of medium throughout the study. Most of the macrophyte demonstrated ideal development on biomass production at pH of 7.5 [29].

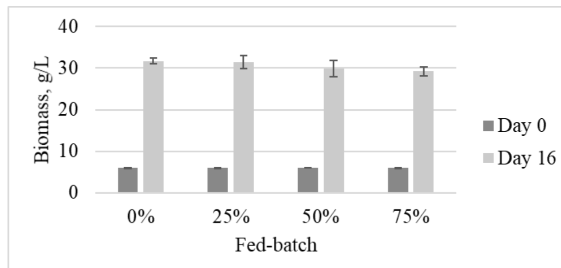


Figure 8 The biomass (fresh weight) of *S. molesta* for 16 days of FB phytoremediation

The pH increased as the FB percentage increased as shown in Figure 9. FB medium with 75% showed highest pH among other which was 8.52 at day 12 while 0%, 25% and 50% gave maximum pH of 7.31; day 8, 8.08; day 10, and 8.24; day 12. Besides pH, ammonia concentration also contributed to *S. molesta* biomass production. By referring to Figure 9, the pH of FB medium was in the range of 8-9, resulted in the medium to be less favourable for nitrification since nitrification rate is high at pH of 6-8 [2]. Variations of pH was related to ammonia and nitrate uptake. When roots of plants take up charged molecule such as ammonium or nitrate from the medium, they tend to release identically charged molecule back into the medium in order to maintain balanced pH. Uptake of ammonium will release H+ ion to the surrounding medium causing the pH declines and vice versa for nitrate uptake[33].

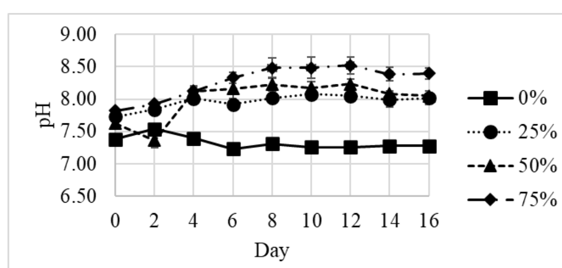


Figure 9 Effect of FB phytoremediation on pH value throughout the experiment

4.0 CONCLUSION

The present study suggests that FB phytoremediation of *S. molesta* in fish farm wastewater is a successful mode of treatment for their nutrient removal. All FB phytoremediation successfully removed the nutrients of wastewater to the standard discharge level, 5mg/L for ammonia and phosphate, 10 mg/L for nitrate and 15 mg/L for total nitrogen. Ammonia and phosphate removal achieved by FB is almost 3.40 times higher than non-FB medium, where 75% FB show highest removal with 46.59 and 5.46 mg/L of ammonia and phosphate, respectively. Final nitrate level in non-FB was 2.90 mg/L higher than day 0 while 75% FB showed reduction by 4.15 mg/L. Total nitrogen in the medium on day 16 for 75% FB was lower than non-FB medium in which experience rose about 4.48 mg/L. *S. molesta* in FB medium tend to clear the wastewater about 553.50 NTU removal for 75% of FB medium. FB mode also show highest TSS and COD removal which 6.97 and 100.50 g/L respectively for 75% of FB. The nutrient removal by *S. molesta* in fish farm wastewater increases as FB loading percentage increases. However, FB phytoremediation showed negative effect on *S. molesta* biomass production where non-FB had higher biomass compared to all percentage of FB. Therefore, phytoremediation using FB cultivation of S. molesta showed good improvement in nutrient removal but no significant change in biomass production.

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