EFFECT OF PLANT BIOMASS HARVESTING ON WASTE NUTRIENT REMOVAL FROM FISH FARM WASTEWATER

DURRAH FAIQAH BINTI MOHD FARID

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by

DURRAH FAIQAH BINTI MOHD FARID

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DURRAH FAIQAH BINTI MOHD FARID

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LIST OF ABBREVIATIONS

COD	Chemical Oxygen Demands
DO	Dissolved oxygen
FW	Fresh weight
MLVSS	Mixed Liquor Volatile Suspended Solids
NTU	Turbidity Unit
POME	Palm oil mill effluent
sp.	Species
TN	Total nitrogen
TP	Total phosphorus
TSS	Total suspended solids
VS	Volatile solids

KESAN ORGANISMA TUMBUHAN TERHADAP PEMBUANGAN SISA NUTRIEN DI DALAM AIR SISA PENTERNAKAN IKAN

ABSTRAK

Tujuan utama kajian ini adalah untuk mengkaji kesan organisma tumbuhan terhadap potensi spesies *Lemna* dan *Spirodela* dalam pembuangan sisa nutrien yang terkandung dalam air sisa pertenakan ikan. Kesan organisma tumbuhan terhadap potensi spesies *Lemna* dan *Spirodela* dikaji melalui proses secara berkumpulan. Eksperimen dijalankan selama 14 hari dan sample air dikumpulkan setiap 2 hari. Jumlah berat kedua-dua spesies pada permulaan adalah 4.2 g bagi setiap bekas. Prestasi spesies *Lemna* dan *Spirodela* dinilai melalui enam jenis ujian iaitu ujian nitrat, fosfat, tahap kekeruhan air, ammonia, kapasiti air untuk menggunakan oksigen semasa proses penguraian bahan organik serta oksidasi bahan kimia bukan organik (COD) dan tahap kepekatan biomass dalam enapcemar diaktifkan (MLVSS). Jumlah nitrat yang terdapat dalam sampel sisa air ternakan ikan tanpa kehadiran spesies *Lemna* dan *Spirodela* ialah yang paling tinggi iaitu 1.7 mg/L NO_3^-N. Jumlah fosfat yang terdapat dalam sampel air dari spesies Spirodela yang dituai berkurang sehingga 0.04 mg/L PO_4^{3-} pada hari ke 14 iaitu hari terakhir eksperimen dijalankan. Corak perubahan menurun untuk tahap kekeruhan sampel air dapat dilihat untuk kesemua kumpulan eksperimen tetapi kadar penurunan tahap kekeringan lebih pantas dengan kehadiran spesies *Lemna* dan *Spirodela*. Jumlah ammonia mencapai bacaan 0 mg/L N pada hari ke 6 dengan spesies *Spirodela* manakala hari ke 10 untuk spesies *Lemna* dan hari ke 12 untuk kumpulan tanpa kedua-dua spesies. Spesies *Lemna* tanpa penuaian mencapai 0 mg/L O_2 paling terawal iaitu pada hari eksperiment yang ke 10. MLVSS meningkat banyak dengan kehadiran spesies *Lemna* dan *Spirodela*.

EFFECT OF PLANT BIOMASS HARVESTING ON WASTE NUTRIENT REMOVAL FROM FISH FARM WASTEWATER

ABSTRACT

The main purpose of this work is to study the effect of plant biomass harvesting on waste nutrient removal from fish farm wastewater using *Lemna* and *Spirodela* sp. The effect of biomass harvesting on the performance of *Lemna* and *Spirodela* sp. in the nutrient removal from fish farm wastewater was evaluated in feed batch processes. The experiment was conducted for 14 days and the water samples to be tested were collected for every 2 days. The initial weight of both sp. in every container is 4.2 g. The performance of *Lemna* and *Spirodela* were determined by six tests which were nitrate, phosphate, turbidity, ammonia, chemical oxygen demand (COD) and mixed liquor volatile suspended solids (MLVSS) tests. The nitrate concentration increases the highest at 1.7 mg/L NO_3^--N without the presence of *Lemna* and *Spirodela* sp. The concentration of phosphate in the water sample greatly reduced to 0.04 mg/L PO_4^{3-} on day 14 which the last day of the experiment in the harvested *Spirodela* batch. A decreasing trend is obtained in turbidity for all water samples but the decrease is faster with the presence of *Lemna* and *Spirodela*. The concentration of ammonia reached 0 mg/L N the fastest with *Spirodela* sp. which is on day 6 while day 10 and day 12 for *Lemna* and without duckweed respectively. The COD level reached 0 mg/L O_2 the fastest with *Lemna* sp. without harvesting. The MLVSS increased greatly with the presence of *Lemna* and *Spirodela* sp.

CHAPTER ONE

INTRODUCTION

1.1 Fish farming

Fish farming is raising fish in tanks or enclosures and it is usually for food. In general, fish farming is big, dirty and also dangerous just like the factory farming on land (Watch, 2017). Basically, there are two kinds of aquaculture in fish farming and they are extensive and intensive aquaculture. Extensive aquaculture is based on local photosynthetical production while intensive aquaculture is fishes are fed with external food supply. Nowadays, aquaculture provides a wide variety of fish which are freshwater and saltwater. Other than providing food sources to mankind, aquaculture is also seen as a way to provide a living for thousands of farmers and fishermen who always seen their usual crops lose value and their everyday catches disappear (Britannica, 2008).

Fish farming can be constructed not only along coastal areas but near inland rivers and lakes. To be precise, wherever water could be supplied then fish farm can be located. After fish farm is constructed, farmers themselves can choose the fish species they wish to raise and the fish growth can be controlled (Discussion, 2016). Without fish farming, they need to fish in wild waters where the fish are free for all and make them share in the common catch uncertain. Therefore, fish farming makes the fish produced as the owner's property. Effective land use is achieved through aquaculture. The land which is too poor and costly to drain for agriculture can be profitably devoted to aquaculture that is suitably prepared (Discussion, 2016).

1.2 Problem statement

Water from main sources like rivers and oceans are facing degradation of quality due to the release of untreated water from fish farming activity. The fishes that are

consumed in the world come from these types of facilities because producers want to get more profit thus producing fish as cheaply as possible. In order to do that, huge amounts of antibiotics, hormones and together with pesticides are used to keep disease at bay just to keep the fish alive in an overcrowded condition such as ponds and cages. The risk of contamination of this activity is very high to the surrounding water and within the enclosures themselves.

Fish farming has caused a big concern regarding its environmental impact. Uneaten food and faeces will become nutrients and organic matter in the water which will lead to the sediments enrichment. Disease and parasites outbreaks also can occur in the fish farm which later affects the water body it flows to. In order to conserve our water resources, fish farm water need to be treated before releasing to water bodies. Developing a value way to purify water in a simple, cheap and energy-efficient method is important (Zhao et al., 2014). Constructed wetland technology is one of the promising alternative treatment process for removing conventional and non-conventional pollutants from wastewater (Iatrou et al., 2015).

The main purpose of this work is to study the effect of plant biomass harvesting on waste nutrient removal from fish farm wastewater using *Lemna* and *Spirodela* sp.

1.3 Research objectives

The main objectives of this study are:

i. To evaluate the effect of biomass harvesting on the performance of *Lemna* and *Spirodela* sp. in the nutrient removal from fish farm wastewater.
ii. To study the feasibility of the system.

1.4 Organization of thesis

This thesis consists of five main chapters and each chapter contributes to the sequence of this study. The following are the contents for each chapter:

Chapter 1 introduces the effect of fish farming, problem statement, research objectives and organization of thesis.

Chapter 2 discusses the literature review of this study. An insight into *Lemna* and *Spirodela* sp. and waste nutrient removal.

Chapter 3 covers the experiment materials and the details of methodology. It discusses on the description of equipment and materials used, plant cultivation, experimental design and analytical methods.

Chapter 4 covers the results obtained throughout this study together with the discussions about the trend and values obtained.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fish farm wastewater

Fish farming is raising fish commercially in tanks or enclosures and it is usually for food (Farms.com, 2017). Basically, there are two kinds of aquaculture in fish farming and they are extensive and intensive aquaculture. Extensive aquaculture is based on local photosynthetic production while intensive aquaculture is fishes are fed with external food supply. Fish farming is important to the aquaculture industry to supply feed and feeders, filtration systems, hatchery supplies, heating and cooling systems, predator control and so many more (Farms.com, 2017). However, fish farm wastewater leaves impacts to the environment.

Fish farm wastewater contains nutrients and organic matter in the form of uneaten food and faeces which may cause organic enrichment of the sediments beneath the cages, thereby affecting the benthos. Disease and parasites outbreaks also can occur in the fish farm wastewater which later affects the water body it flows to. When untreated fish farm wastewater flows into water body, it can result in oxygen deficiency thus resulting in a decline of water quality. Other than that, it will also lead to eutrophication and turbidity in the receiving body it flows to (Commission, 2010).

The fish farm wastewater also causes conflicts with the main users of the water bodies that the wastewater flows into. Since the wastewater from fish farm brings waterborne disease along, it may affect the people's health who use the water sources directly. The contaminated water can cause many types of diarrheal diseases, including Cholera and also other serious illnesses such as Guinea worm disease, Typhoid and Dysentery (Vestergaard, 2014).

2.2 Conventional treatment

There are several conventional treatments that have been employed in order to treat fish farm effluents such as sedimentation, mechanical filtration and many more. Sedimentation is a process where particles in suspension in water is allowed to settle out of the suspension under the effect of gravity (Publishing, 2017). The differences in density between the particles and water that leads the particles which is suspended solids such as fish faeces to travel downward in a slowly moving liquid. However, the specific gravity of fish faeces and water do not differ too much thus the sedimentation rate is low (Mousavi, 2015). The minerals such as sand will settle faster since they have higher specific gravity. Sedimentation requires a large surface area and the arrangements must always be made for later removal of the suspended solids. Sedimentation has several treatments such as channels, cones, lamellar settlement tanks and hydro clones but all of them are not that suitable to treat fish farm effluents because of some limitations (Mousavi, 2015). Therefore, this method is not efficient to treat fish farm wastewater.

Next is the mechanical filtration system that use barriers to prevent solids from passing through. This filters will trap settlings solid together with those that will not settle due to their small size or low density and this is achieved by using a packed medium or mesh (Mousavi, 2015). Before using filtration, few important things need to be considered like the type of solids to be filtered, concentration of solids within the effluent, flow capacity and energy requirement to operate filter. There are several type of filters such as pressure, gravity, drum filters and the most efficient and widely used in aquaculture is drum filter. The other treatment is treatment with coagulant and flocculants (Mousavi, 2015). This treatment should precede passage through a second filter or a settling tank and usually the concentration of suspended solids achieved is around 15-20% (150 – 200 g/L).

2.3 Phytoremediation

The direct use of living green plants for the removal of contaminants in solids, sludges, sediments, surface water and also groundwater is called phytoremediation. Phytoremediation has emerged as a green, passive, solar energy driven and cost effective approach for environmental cleanup when compared to physico-chemical and even other biological methods (Khandare and Govindwar, 2015). Phytoremediation based on the synergistic actions of plants and their associated microorganisms has been recognized as a powerful in situ approach to soil remediation. Suitable combinations of plants and their associated endophytes can improve plant growth and enhance the biodegradation of organic contaminants in the rhizosphere and/or endosphere, dramatically expediting the removal of organic pollutants from soils (Feng et al., 2017). In the 1990s, remediation methods that use plants to investigate and extract contaminations were developed. According to their proponents, these technologies have considerable potential for greening remediation and to develop a more sustainable trajectory for revitalization (Bleicher, 2016).

In order to remove pollutants from soil, sediment or water, plants can break down or degrade organic pollutants and stabilise metal contaminants by acting a filters or traps. The principal mechanisms for preventing contaminant toxicity are found through the root system where the uptake of contaminants in plants occurs primarily. The root system has a huge surface area that absorbs and accumulates the water and nutrients essential for growth. Researchers found that by using trees rather than smaller plants is more effective in treating deeper contamination because the tree roots can go deeper into the ground. Other than that, plant roots cause changes at the soil-root interface since they release both organic and inorganic compounds in the rhizosphere. Phytoremediation is an in situ remediation technology that utilises the inherent abilities of living plants, ecologically

friendly, solar-energy driven clean-up technology based on the concept of using nature to cleanse nature. This makes phytoremediation at an advantage (UNEP).

Duckweed is a free-floating aquatic plant that proliferates through vegetative budding of new fronds and accumulates biomass at rates greater than most of other plants including field crops. There are 37 species belonging to 4 genera (*Lemna*, *Spirodela*, *Wolffia*, *Wolfiella*). Duckweed species primarily reproduce asexually, grow fast and increase biomass rapidly. Many species of duckweed can double their biomass every 2 or 3 days (Zhao et al., 2014). Duckweed also has a longer growing period compared to most other plants. In some areas with warm climates, duckweed can grow in all seasons. Duckweed has been used for tertiary treatment of municipal and industrial for more than a decade. Effluent quality was at secondary level and met criteria for reuse of agricultural irrigation (Cheng et al., 2001).

Duckweed is used because it has rapid growth rates and can achieve high levels of nutrient removal. Besides that, duckweed has low fibre and high protein contents make it a valuable fodder (Priya et al., 2011). It can easily be harvested and cold tolerant which suppresses the growth of algal. Duckweed can assimilate nutrient in wastewater, thus integrate of wastewater purification and biomass production. In addition, duckweed is also known to tolerate high ammonia nitrogen and has an excellent ability to uptake nitrogen with a preference for ammonium, the dominant nitrogen form in wastewater (Zhao et al., 2014). Duckweed is also considered as a potential bioenergy source for bioethanol production due to its excellent growth and starch accumulation capability (Zhao et al., 2014). There are many types of plants that have been used in phytoremediation. Young mangrove species *Rhizophora apiculate* was used for the removal of chromium (Richter et al., 2016).

2.4 Types of nutrient able to be treated by phytoremediation

There are many types of nutrients that are able to be treated by phytoremediation. *Salvinia molesta* achieved 95% phosphate removal efficiency from the wastewater, and lowering the concentration to 0.17 mg/L. At the end of experiment, nitrate concentration was determined to be at 0.50 mg/L. Ammonia concentration showed a dynamic fluctuating trend with average value of 2.62 mg/L. The turbidity decreased from 7.56 NTU to 0.94 NTU in just 2 days' time. COD removal efficiency was determined at 39% (Ng and Chan, 2017).

The duckweed, *Spirodela oligorrhiza* was able to remove 83.7% total nitrogen (TN) and 89.4% of total phosphorus (TP) (Xu and Shen, 2011). *Lemna gibba L*. caused a decrease in pH which is 9–13% and the yield rate of protein and carbohydrates ranged from 1.19-1.95 g/m^2 (dry weight) and 22.72-35.58 g/m^2 day (dry weight) respectively in duckweed systems (Verma and Suthar, 2014). Nitrate removal was greatly dependent upon the presence of other nutrients, namely sulphate and phosphate which caused lower nitrate uptake by water hyacinth (Ng and Chan, 2017).

2.5 Parameters that affect the performance of phytoremediation

There are several parameters that can affect the performance of phytoremediation. The concentration of medium where the duckweeds grow can affect the performance. At the concentration of 12% and 10% of swine lagoon water, the duckweed uptake for nitrogen and phosphorus were both impaired while the ammonia volatilization was increased. This is because that the detrimentally high nutrient level made it impossible for the duckweed to fully cover the water surface. The improved light penetration caused the thriving of phototrophic algae in the water which consequently resulted in rise of water pH due to exhaustion of CO_2 by algae growth. Since alkaline pH favours a high equilibrium yield of ammonia, ammonia volatilization played a much more important role

in nitrogen removal which could probably explain the lower ratios at which TN and TP were removed (Xu and Shen, 2011). Therefore, it can be said that pH also affects the performance of phytoremediation.

A higher duckweed density is positively related to absolute biomass production due to the fact that more fronds produce more biomass while too high density will inevitably inhibit duckweed growth (Skillicorn et al., 1993). Harvesting duckweed more frequently generally resulted in better nutrient removals (Xu and Shen, 2011) thus affecting the performance of phytoremediation. The amount of nitrogen also plays a role in the performance of phytoremediation. The reduction rated of both TN and TP were pretty high at the initial stage of the experiment and this probably because that NH_4-N, the preferred form of nitrogen for duckweed was abundant at the initial stage (Xu and Shen, 2011).

The choice of plant itself affect the performance of phytoremediation. By using *Salvinia molesta,* I was able to increase clarity of POME characteristic wastewater in short time and maintain the low turbidity value in long time due to system itself. As *S. molesta* plants have long hairy extensive roots; it was observed that at day 2, large amounts of dark brownish particulates were attached to the plants' roots and the particulates prone to reattach back to the roots after being disturbed (Ng and Chan, 2017).

2.6 Usage of biomass

The production of valuable biomass through nutrient recovery was found to have huge differences among various duckweed species and geographical isolates. Therefore, selecting the best duckweed strain from a collection of local strains is a prerequisite for the establishment of an effective duckweed cropping system. However, only single species such as *Landoltia punctata, Lemna minor* and *Wolffia arhiza* was reported at a

time as high potential candidates for domestic wastewater treatment and biofuel production (Zhao et al., 2014). Duckweed biomass can be used as a high value supplement for animal feed due to its high protein content ranging from 15% to 45% (Landolt and Kandeler, 1987). Cheng and Stomp (2009) concluded in their review paper that duckweed could supply a large proportion, if not all, of the protein required by the animals with no adverse effects and animals supplied with a plant-based diet supplemented with duckweed normally had a higher growth rates.

The conversion of wastewater nutrients into rich plant biomass has drawn increasing attention because it is not only addresses the problems of nutrient pollution but also provides a value-added by product that can be used as source for energy and feed generation (Ge et al., 2012, Mohedano et al., 2012). The total biomass harvested was 5.30 times that of the starting amount (Xu and Shen, 2011).

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental activities

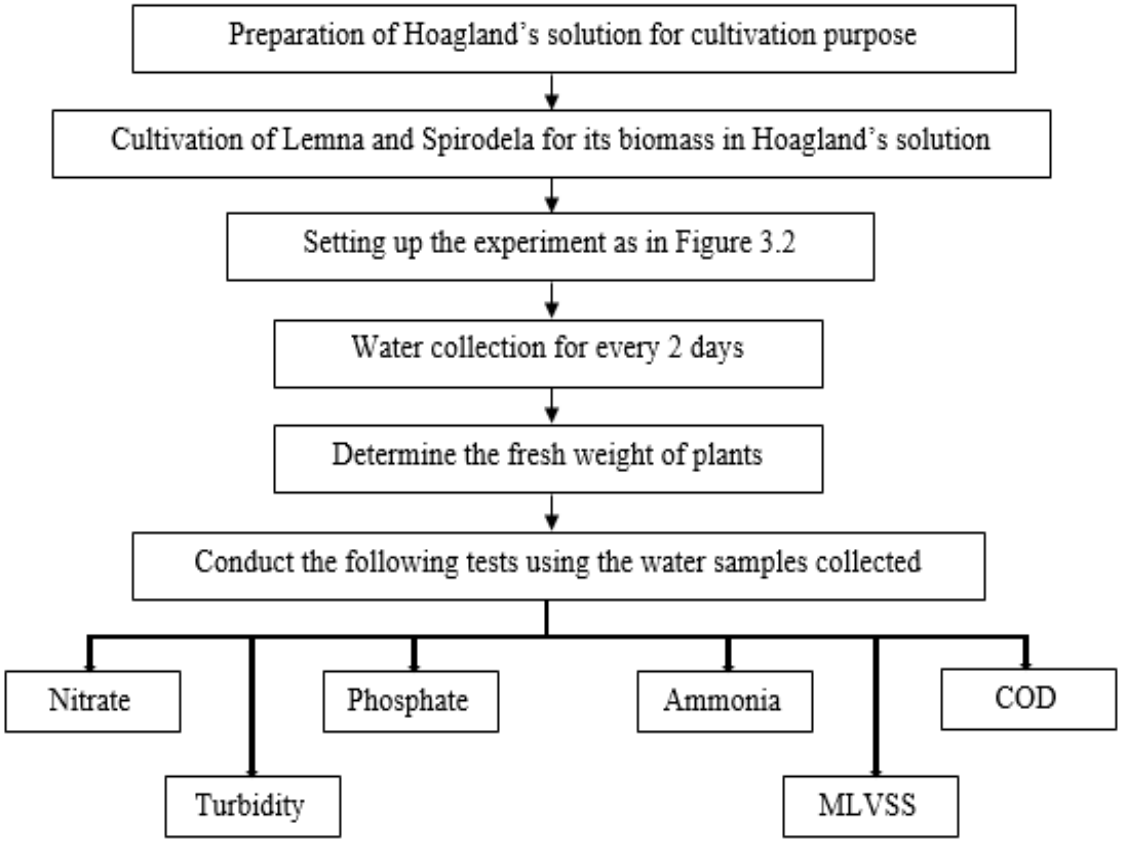

Figure 3.1: The flow of experimental activities

3.1.1 Materials

In this study, fish farm water near Universiti Sains Malaysia and two species of duckweeds; *Lemna* and *Spirodela* are used.

3.2 Plant cultivation

Duckweeds (*Lemna* and *Spirodela* sp.) are cultured in sterile Hoagland's solution in a culture room at 25 ± 2 ℃ with a 24-h photoperiod for 14 days using sterile jars. The cultivation process is carried out in the laminar flow cupboard and everything must be sterile with 75% alcohol several times including the jars, knife, gloves, lighter and also

the surface of the cupboard itself where the process will take place. The amount of *Lemna* and *Spirodela* needed for each jar is around 5-6 plantlets.

3.3 Experimental design

In this study, two replicates are used in order to increase the accuracy of the results obtained. The average readings will be analysed. This experiment is carried out using containers with measurement of 10cm x 15.5cm x 5.8cm and are covered with black sugar paper to prevent lights from penetrating from the sides of the containers. The amount of wastewater being filled up in each container is 700mL and 4.2g of *Lemna* and *Spirodela* needed for every 700mL water sample. For every 2 days, the water samples will be collected and tested for the determination of nitrate, phosphate, COD, ammonia, nitrite, total nitrogen, total phosphorus, MLVSS, turbidity and pH level.

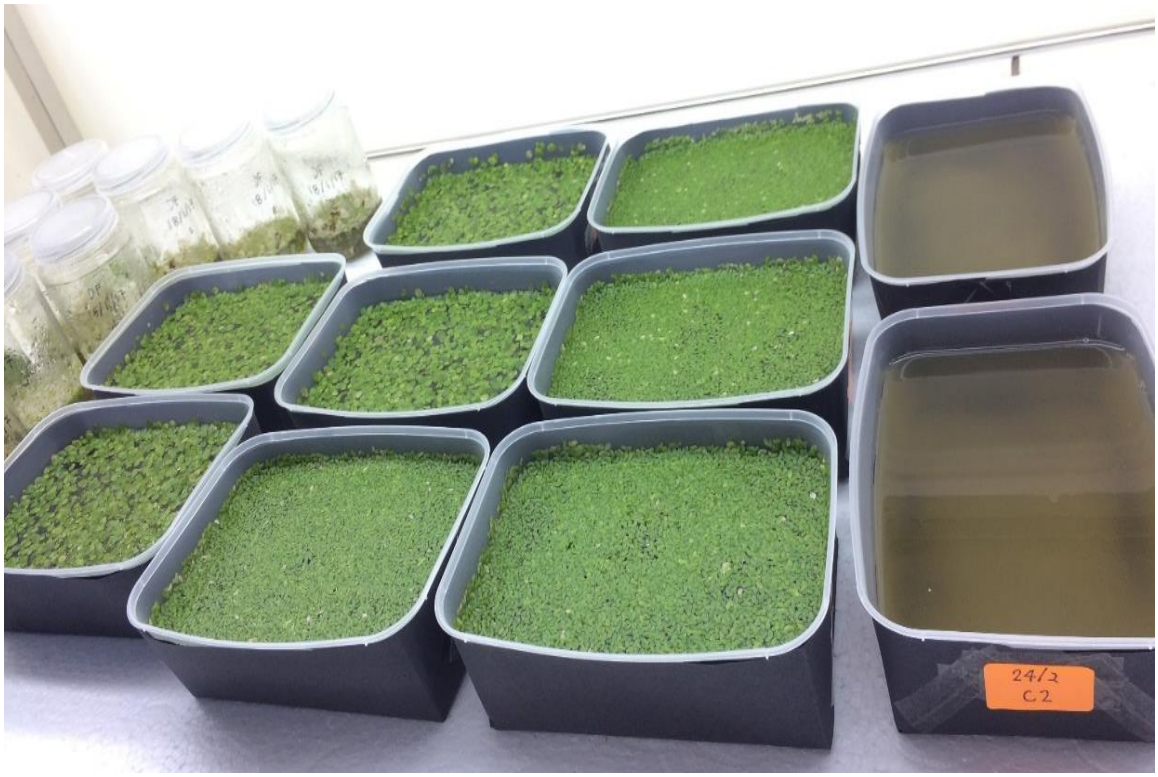

Figure 3.2: Set up of the experiment

Each replicate has five batches of wastewater together with the assigned duckweeds weighing 4.2 g for each container except for the control which consists of fish farm wastewater only. The batches are as follows; control, *Lemna*, *Spirodela,* harvested *Lemna* and harvested *Spirodela.* All the batches are left in a clean room under the lamp light for 24h and until they reached day 14. The set-up of the experiment is shown in Figure 3.2. At day 7, the two batches which are harvested *Lemna* and harvested *Spirodela* will be harvested 50% from the total surface area of the containers used. The harvested duckweeds are dried and weighed in order to know their actual weight at the end of the experiment. The harvested *Lemna* and *Spirodela* batches are shown in the Figure 3.3 and Figure 3.4. At the end of cultivation process which is day 14, duckweed was harvested to obtain their fresh weight (FW).

Figure 3.3: Harvested Lemna

Figure 3.4: Harvested Spirodela

3.4 Water samples collection

Water samples are collected on day 0, 2, 4, 6, 8,10, 12 and 14. Every time before each collection, the volume is to be corrected back to 700 mL by distilled water and mixed well. The amount of water collected is 12 mL and is kept in a 20 mL tube with a cap. All the collected water samples need to be stored in the fridge to prevent degradation. The water samples collected is shown in Figure 3.5.

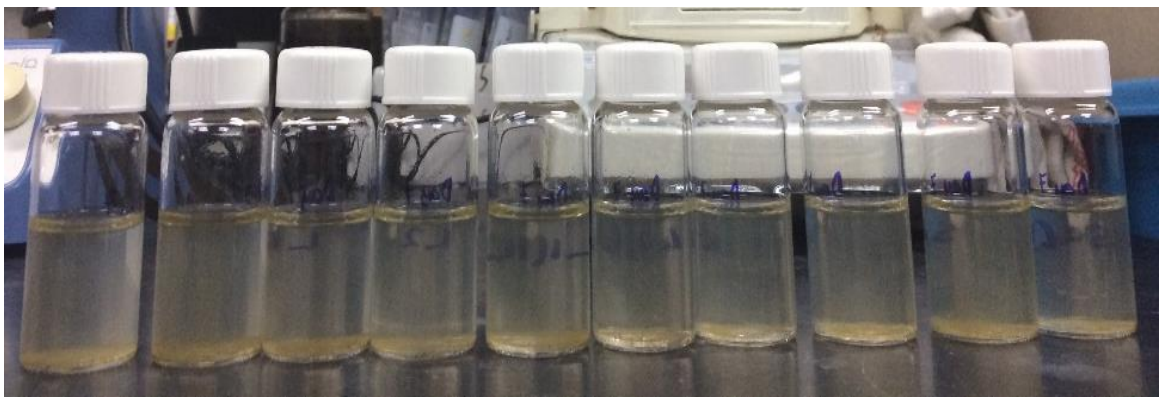

Figure 3.5: Water samples collected

3.5 Equipment and instrumentation

3.5.1 DR 2800 spectrophotometer

The concentration of nitrate and phosphate are determined using the DR 2800 spectrophotometer. For nitrate and phosphate concentration tests, the blank must be prepared first in order to zero the reading of the spectrophotometer. The method used for both nitrate and phosphate tests is powder pillows. NitraVer 5 nitrate reagent powder pillow is poured inside the cuvette for 1 minute and to be left for 5 minutes' period for the reaction. An amber colour will develop if nitrate is present. Meanwhile for the phosphate concentration test, PhosVer 3 phosphate reagent powder pillows are poured into the cuvette and shake it for 30 seconds after stopper is already put on. The reaction time is 2 minutes and after that it can be tested to obtain the reading. The measurement unit for nitrate and phosphate are mg/L NO_3^--N and mg/L PO_4^{3-}.

3.5.2 MD 600 photometer

The level of ammonia and chemical oxygen demand (COD) are determined by MD 600 photometer. For determination of ammonia level, the vial that mixed with 0.1 mL of deionised water is the blank and another vial is mixed with 0.1 mL of water sample to be tested. One vario ammonia salicylate F5 powder pack and one vario ammonia cyanurate F5 powder pack are added into each vial and swirled after the vials are closed tightly. The reaction period is 20 minutes and the blank can be put into the sample chamber for zeroing purpose and then the sample can be tested for the reading. The results are in mg/L N.

In order to test the COD level, blank sample is prepared by mixing the content in the vario test tube with 2 mL of deionised water while the test sample is prepared by mixing it with 2 mL of water sample. The vials need to be inverted gently a few times to

mix the liquids. At here, precaution step need to be taken because the vial will become hot. The vials need to be heated for 120 minutes at 150℃. Let the vials cool down until they reached around 60℃ and be sure to invert the vials several times while they are still hot. The blank sample can be used to zero the reading and test all the water samples in the sample chamber of MD 600. The results are in mg/L COD.

3.5.3 Mixed liquor volatile suspended solids (MLVSS)

MLVSS test is performed in order to know the concentration of biomass in the water samples. Firstly, filter paper is ignited at 550℃ for 30 minutes. The weight of blank filter paper is labelled as weight (A) as shown in Equation (3.1). The filter paper (A) is placed on the glass and began suction by vacuum pump. Make sure the wastewater is homogeneous and only then pour the wastewater onto the filter paper. After that, the filter paper together with the residue is dried in an oven at 105℃ for 1 hour or until constant weight is gained. After drying, the filter paper together with the residue is labelled as weight (B) as shown in Equation (3.2).

In order to obtain the amount of total suspended solids (TSS) in the water samples, weight (A) needs to be deducted by weight (B) as shown in Equation (3.3).

$$Weight\ (A) = Weight\ of\ filter\ paper \tag{3.1}$$

$$Weight\ (B) = Filter\ paper + Total\ Suspended\ Solid\ (TSS) \tag{3.2}$$

$$TSS = Weight\ (B) - Weight\ (A) \tag{3.3}$$

The filter paper with the TSS is ignited again in maffle furnace at 550℃ for 20 minutes until constant weight is achieved. After this second ignition in maffle furnace, the weight (C) are obtained from Equation (3.4). Volatile solids are determined by using Equation (3.5). Therefore, the MLVSS for each water samples are obtained through Equation (3.6) which is by summing up Equation (3.3) and (3.5).

$$Weight\ (C) = Filter\ paper + Fixed\ solids \quad (3.4)$$

$$Volatile\ solids\ (VS) = Weight\ (B) - Weight\ (C) \quad (3.5)$$

$$MLVSS = TSS + VS \quad (3.6)$$

3.5.4 Determination of turbidity

The turbidity of the water samples is determined using turbidimeter. The 400 NTU solution is prepared. The turbidity meter is calibrated using the 400 NTU solution by adjusting the calibration knob. Then calibrate the turbidity meter to 0 NTU using distilled water and by adjusting the calibration knob. The turbidity meter is read by inserting the sample.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

This chapter presents the experimental results together with discussions which consist of two main sections which are the effects of the performance of *Lemna* and Spirodela on the nutrients removal and the feasibility of the system.

4.1 Effects of the performance of duckweeds on nutrients removal

4.1.1 Removal of nitrate

The 14-days of experiment was conducted to find out the effect of *Lemna* and *Spirodela* on the removal of nitrate in the fish farm wastewater. From Figure 4.1, the concentration of nitrate is affected by the presence of both duckweeds. The mean concentration of nitrate in control, *Lemna*, harvested *Lemna*, *Spirodela* and harvested *Spirodela* at day 14 are 1.7 mg/L NO_3^--N, 0.25 mg/L NO_3^--N, 0.4 mg/L NO_3^--N,0.3 mg/L NO_3^--N and 0.4 mg/L NO_3^--N. A great increase in the nitrate concentration can be seen from Figure 5 when there is no presence of both duckweeds which is the control batch. However, the difference in value is very small for *Lemna* with harvesting *Lemna* and *Spirodela* with harvesting *Spirodela* which are 0.15 mg/L NO_3^--N and 0.1 mg/L NO_3^-N. The plants are surrounded by nitrogen in the atmosphere but unlike their capability to take up carbon dioxide, they cannot directly take up nitrogen. This need is met by bacteria living in the roots. The energy needed by bacteria to perform nitrogen uptake is provided by plants, however, in the form of carbon compounds released in the root zone (Landmeyer, 2011). From this test, it can be concluded that nitrate is produced during this process.

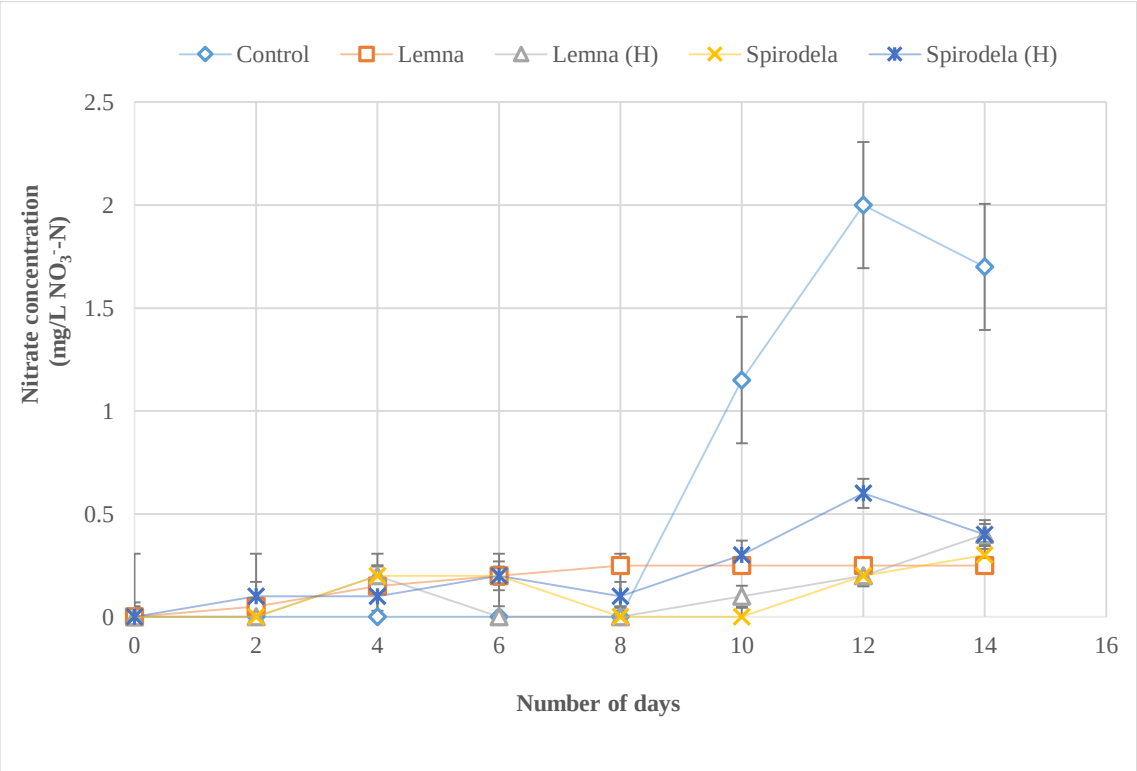

Figure 4.1: The concentration of nitrate versus the day which the samples were taken

4.1.2 Removal of phosphate

From Figure 4.2, it shows a decreasing trend for the concentration of phosphate in the fish farm wastewater. At day 0, the concentration of phosphate in control, *Lemna*, harvested *Lemna*, *Spirodela* and harvested *Spirodela* are 1.34 mg/L PO_4^{3-}, 0.31 mg/L PO_4^{3-}, 0.215 mg/L PO_4^{3-}, 0.245 mg/L PO_4^{3-} and 0.24 mg/L PO_4^{3-} respectively. For control batch, at day 6 there is a sudden increase from 0.395 mg/L PO_4^{3-} to 0.76 mg/L PO_4^{3-}. As for day 14, all the batches decreasing from its original amount on day 0. Harvested *Spirodela* gave the lowest concentration of phosphate among those four batches which is 0.04 mg/L PO_4^{3-}. As for the control, it stays the highest concentration of phosphate on day 14 at 0.085 mg/L PO_4^{3-}. From the removal efficiency calculation, it is true that harvested *Spirodela* gave the highest removal efficiency at 83.33%. Phosphate is taken up by plants in order for their growth. Phosphorus is a component of the complex nucleic acid structure of plants which regulates protein synthesis. Thus, it is important for cell

division and development of new tissue. Other than that, it is also associated with complex energy transformations in the plant (eLibrary, 2017). This concluded that *Lemna* and *Spirodela* affected the rate of removal of phosphate in the fish farm wastewater and the best method is by harvesting the Spirodela.

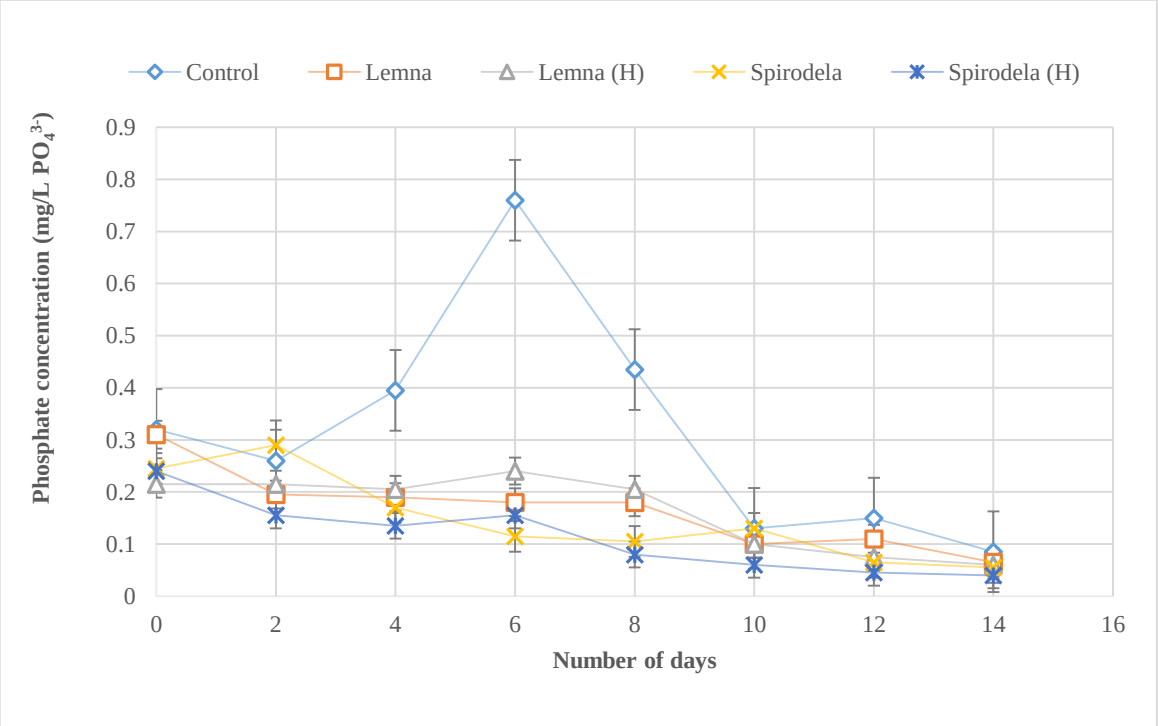

Figure 4.2: The concentration of phosphate versus the day which the samples were taken

4.1.3 Removal of ammonia

When plants take up ammonium (NH4+), it releases a proton (H+) to the water. By increasing the protons concentration, it decreases the pH around the plants' roots. Hence, the pH level of the wastewater decreases. The uptake rate of ammonia is affected by the presence of *Lemna* and *Spirodela* sp. As shown in Figure 4.3, the trends for all batches is the same which is decreasing in the concentration of ammonia in the fish farm wastewater and the difference is the day which all the ammonia is removed. The control batch took the longest which is day 12. Meanwhile, the *Spirodela* sp. removed ammonia

completely the fastest on day 6 for both *Spirodela* batch and harvested *Spirodela* batch. *Lemna* and harvested *Lemna* batches completely removed ammonia in the water samples on day 10.

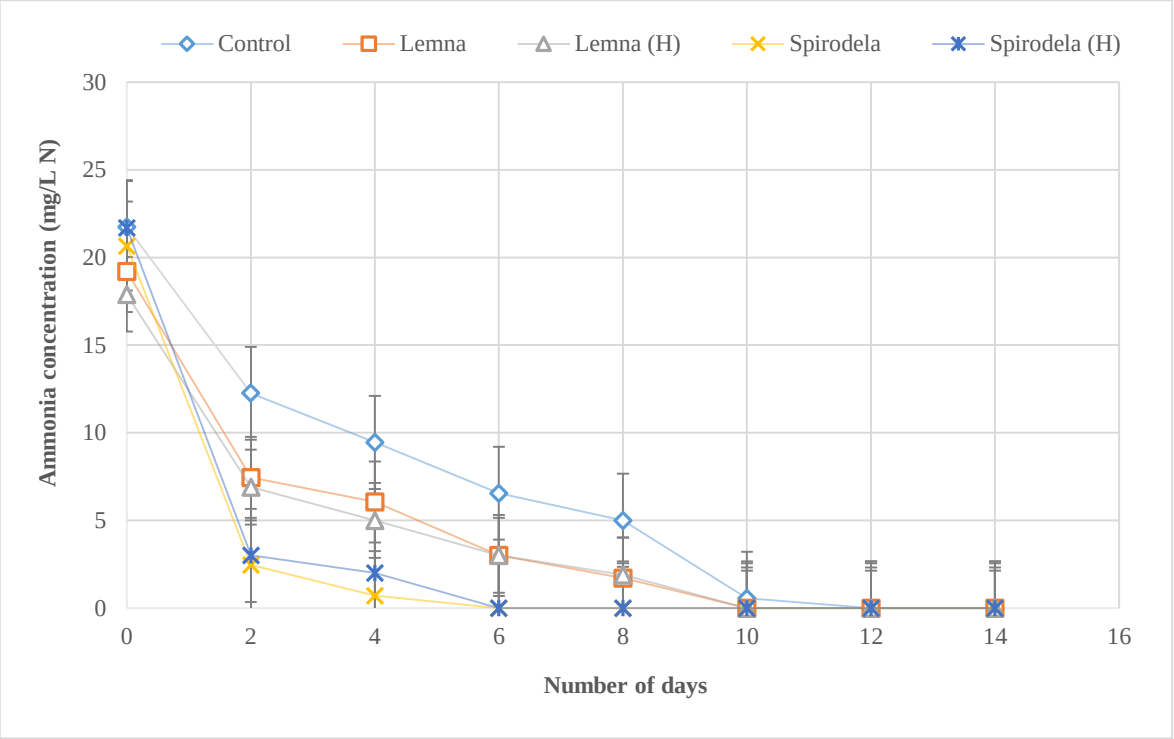

Figure 4.3: The concentration of ammonia versus the day which the samples were taken

4.1.4 COD

From Figure 4.4, the COD level on day 0 for all batches are ranging from 114 mg/L O_2 to 214 mg/L O_2. This may due to the degradation of microorganism in the water samples that caused by the distance of the containers with the lights. The COD level with *Lemna* decreased the fastest compared to the others and on day 10, the COD level with *Lemna* is already 0 mg/L O_2. For the control batch, the COD level reached 0 mg/L O_2 on day 14 and for the others which are harvested *Lemna*, *Spirodela* and harvested *Spirodela* did not reach 0 mg/L O_2 until day 14. Generally, they are decreasing in trend. This concluded that *Lemna* sp. without harvesting can decrease the COD level the fastest and this is good because high level of COD will reduce dissolved oxygen (DO) levels in water

thus lead to anaerobic conditions. The reduction in level of COD influenced by total suspended solids in the wastewater (Jingsheng et al., 2006). When the total suspended solids settle on the surface of the container is higher, the COD level will decrease.

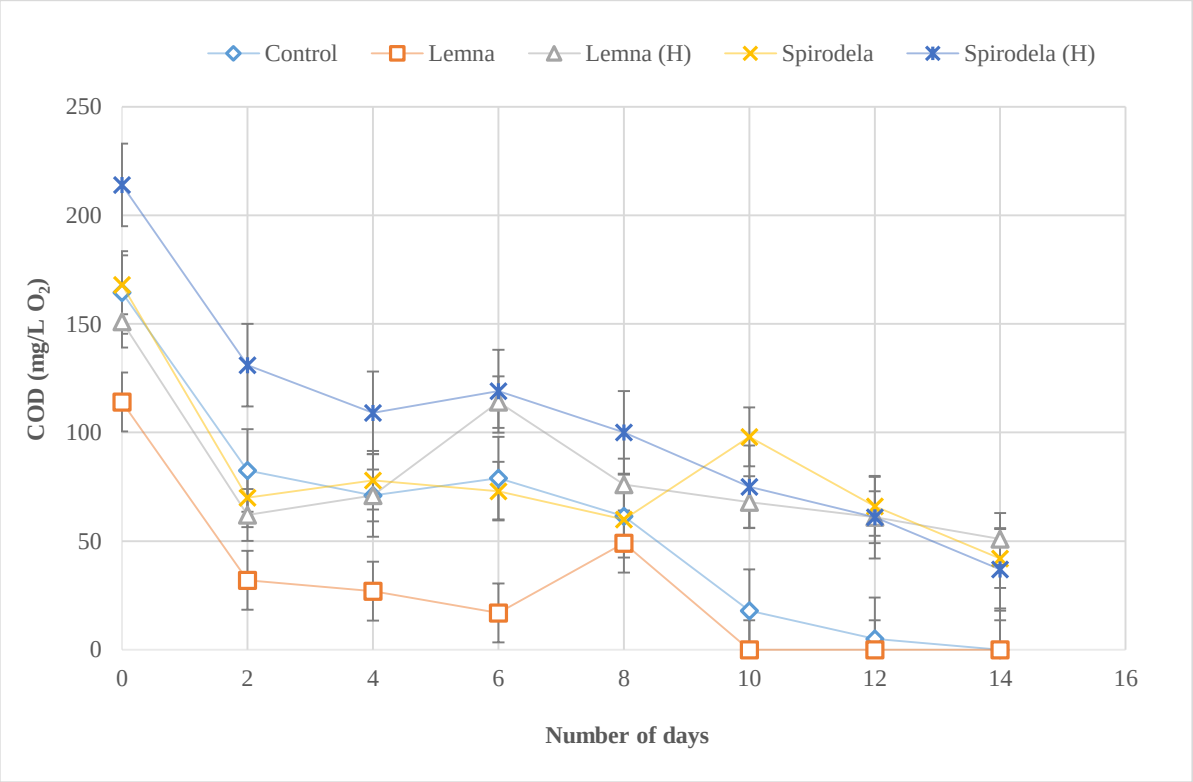

Figure 4.4: The concentration of ammonia versus the day which the samples were taken

4.1.5 MLVSS

The MLVSS values for all the batches on day 0 are the same because they are from the same source of fish farm wastewater. The MLVSS on day 0 is 3.05 g/L and an increasing trend can be seen from Figure 4.5 for the MLVSS in the water samples except for harvested *Spirodela*. The highest value of MLVSS is given by *Lemna* batch at 6.89 g/L followed by harvested *Lemna*, control, *Spirodela* and harvested *Spirodela* and the readings are 5.67 g/L, 5.11 g/L, 3.5 g/L and 2.89 g/L respectively. From MLVSS test, it can be concluded that MLVSS in harvested *Spirodela* batch decreases.

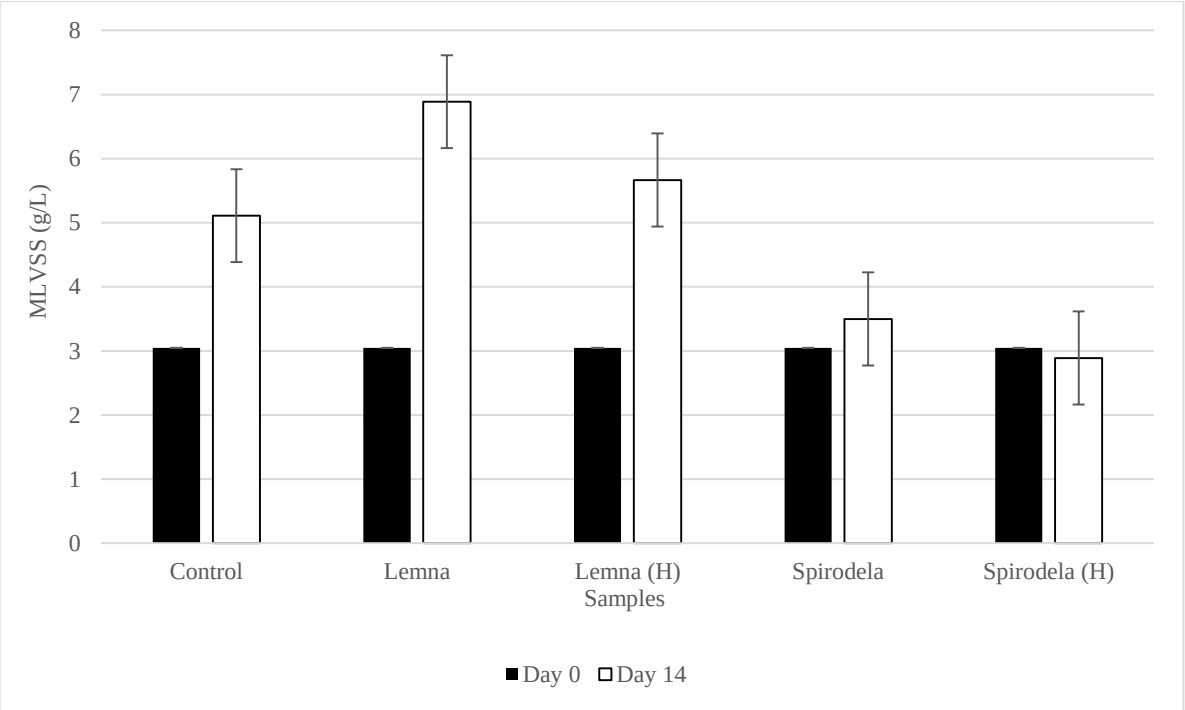

Figure 4.5: MLVSS in water samples on day 0 and day 14

4.1.6 Turbidity

A decreasing trend in turbidity can be observed from Figure 4.6 for all batches of experiment. A decreasing trend is a good thing because it means less cloudiness or haziness of the water samples that caused by large number of individual particles. The Lemna sp. managed to reduce from 202.5 NTU to 98 NTU in just 2 days' time. The control batch has the slowest reduction in turbidity level but has the highest reducing efficiency which is 93.6%. However, the difference in reducing efficiency is small; 92.6%, 86.9%, 88.4% and 92.7% for *Lemna*, harvested *Lemna*, *Spirodela* and harvested *Spirodela* respectively. For both harvested *Lemna* and *Spirodela*, there is slightly sudden increase on day 6 and this may due to microorganism activities. From this turbidity test, it can be concluded that the presence of *Lemna* and *Spirodela* sp. still reduce the turbidity but the reducing efficiency is slightly lower than without the presence of any duckweeds in the fish farm wastewater.

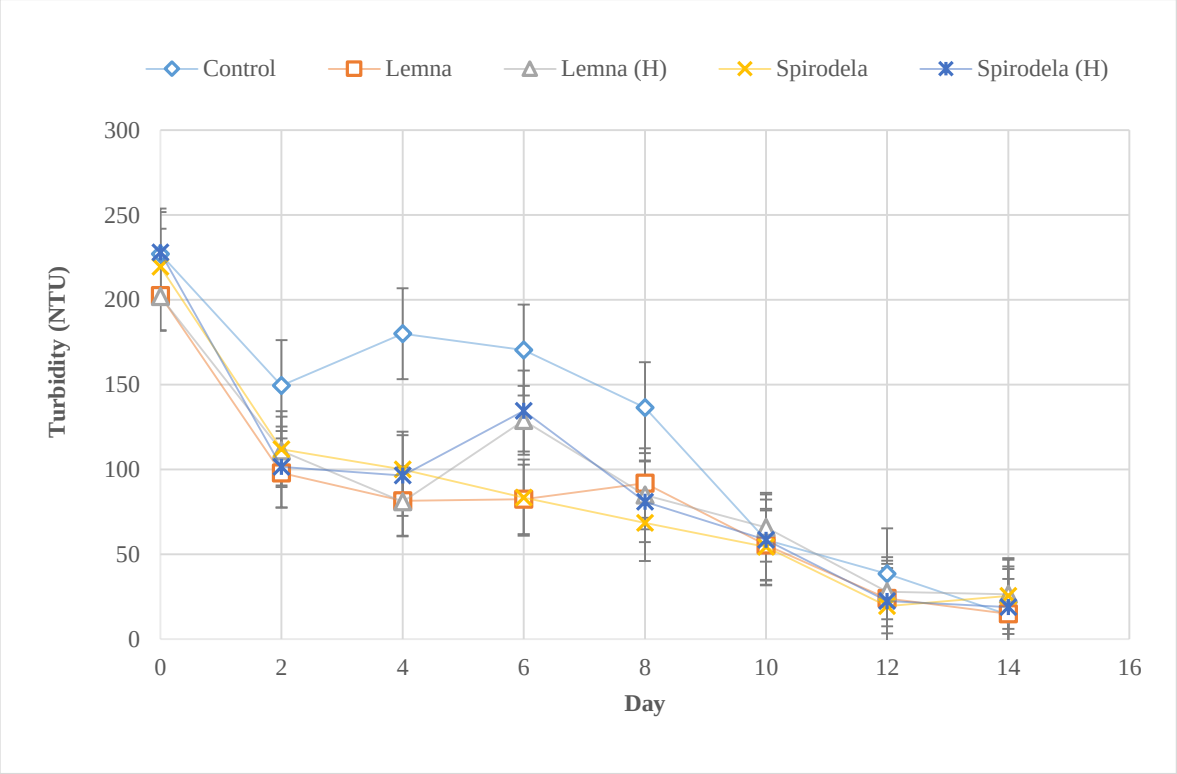

Figure 4.6: The turbidity of the water samples versus the day which the samples were taken

4.2 Feasibility of the system

The fresh weight (FW) of the *Lemna* and *Spirodela* sp. at the beginning of the experiment for all batches except the control batch is 4.2 g. At the end of the experiment, all the FW increased greatly compared to day 0 as shown in Figure 4.7. The FW on day 14 for *Lemna*, harvested *Lemna*, *Spirodela* and harvested *Spirodela* are 31.03 g, 31.88 g, 22.93 g and 24.03 g respectively. As can be observed, the FW if harvesting is done can increase slightly more than no harvesting. From this result, it can be concluded that if there is increase in duckweeds thus more nutrients can be removed from the water. As for the feasibility of the system, this method can be easily done since only small weight is needed but the plants can grow a lot and a lot more with harvesting done. From Table 4.1 and 4.2, it can be seen the rate of nutrient uptake at the beginning of the experiment and the last day of experiment for *Lemna* and *Spirodela* sp. respectively.