PERFORMANCE OF *LEMNA* SP. AND *SPIRODELA* SP. ON NUTRIENT REMOVAL FROM FISH FARM WASTEWATER

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PERFORMANCE OF *LEMNA* SP. AND *SPIRODELA* SP. ON NUTRIENT REMOVAL FROM FISH FARM WASTEWATER

by

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LIST OF ABBREVIATIONS

BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
MLVSS	Mixed liquor volatile suspended solids
N	Nitrogen
NTU	Nephelometric Turbidity Units
P	Phosphorus
sp..	Species
TN	Total Phosphorus
TP	Total Nitrogen

PRESTASI *LEMNA* SP. DAN *SPIRODELA* SP. UNTUK MENGELUARKAN NUTRIEN DARIPADA AIR SISA LADANG IKAN

ABSTRAK

Fitopemulihan menggunakan tumbuh-tumbuhan untuk memulih tanah dan air sisa yang tercemar. Efluen daripada akuakultur mempunyai banyak pepejal, nitrogen dan fosforus di dalam ekosistem akuatik. Dengan menggunakan rawatan air sisa konvensional untuk merawat air kumbahan kolam ikan, ia melibatkan kos yang tinggi untuk menjalankan dan mengekalkan.Prestasi fitopemulihan oleh *Lemna* sp. dan *Spirodela* sp. dinilai untuk rawatan air sisa kolam ikan. Tumbuhan akna tumbuh atas air sisa dari koalm ikan. Tujuannya adalah untuk menentukan maklumat nutrien dan kesannya terhadap biomass. 4 kolam buatan manusia telah ditubuhkan untuk mengkaji. Antaranya ialah *Spirodela* sp., *Lemna* sp., campuran (*Lemna* sp. dan *Spirodela* sp.) dan kawalan (tanpa tumbuhan). Kualiti air dipantau juga. rawatan oleh fitopemulihan telah dilakukan di pelantar kolam untuk 2 minggu. Kecekapan penyingkiran fosfat ialah 89% dan 85% oleh *Lemna* sp. dan *Spirodela* sp. masing-masing. *Spirodela* sp. mengurangkan kepekatan nitrat kepada 3.6 mg/l, manakala *Lemna* sp. mengurangkan kepada 7.9 mg/l. *Spirodela* sp. mencapaikan 89.0% dalam kecekapan penyingkiran fosfat berbanding dengan *Lemna* sp. hanya 85.1%. *Lemna* sp. dan *Spirodela* sp. mengurangkan kekeruhan daripada 16.2 ± 4.0 NTU kepada 3.2 ± 2.4 NTU dalam masa 6 hari. Walau bagaimanapun, *Lemna* sp. and *Spirodela* sp. tidak berubah nilai pH. COD pepejal daripada air sisa kolam ikan. Secara keseluruhannya, *Lemna* sp. dan *Spirodela* sp. mempunyai kelebihan untuk menyingkirkan nutrien, meningkatkan kualiti air dan pertumbuhan biojisim

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ABSTRACT

Phytoremediation employs plants to remove contaminated soil and wastewater. The effluents from aquaculture has large amount of suspended solids and enriched with nitrogen and phosphorus in aquatic ecosystems. By using conventional wastewater treatment to treat fishpond wastewater, the cost is expensive to run and maintain. The performance of phytoremediation by using macrophytes, such as *Lemna* sp. and *Spirodela* sp., are evaluated on treatment of fishpond wastewater. The duckweeds were fed with wastewater from Catfish production facility. The objective was to determine the nutrient update and its effect towards biomass. 4 pond rigs were set up to study. They were *Spirodela* sp., *Lemna* sp., mix (*Lemna* sp. and *Spirodela* sp.) and control (without plant). The water quality was monitored as well. The treatment by phytoremediation was done in raceway pond rig for 2 weeks. The efficiency of phosphate removal was 89% and 85% by *Lemna* sp. and *Spirodela* sp. respectively. *Spirodela* sp. reduced nitrate concentration to 3.6 mg/L, while *Lemna* sp. managed to reduce to 7.9 mg/l. *Spirodela* sp. archived 89.0% in phosphate removal efficiency compared to that of *Lemna* sp. at only 85.1%. *Lemna* sp. and *Spirodela* sp. reduced turbidity from 16.2 ± 4.0 NTU to 3.2 ± 2.4 NTU in just 6 days. However, both *Lemna* sp. and *Spirodela* sp. did not change much in pH value. COD volatile solids of fishpond wastewater. In overall, *Lemna* sp. and *Spirodela* sp. were good for nutrient removal, improve water quality and growth of biomass.

CHAPTER ONE

INTRODUCTION

1.1 Research Background

In Malaysia, aquaculture field are developed to provide protein sources for human beings. One of the popular aquaculture are catfishes. They are cultured in open ponds, which the effluents are discharged to the environment with enhanced nutrient and solid concentrations (Endut et al., 2009).

Fertilizers and feeds are applied to ponds to promote fish production, normally no more than 25% to 30% of the nitrogen and phosphorus applied to ponds in fertilizers and feeds is recovered in fish at harvest (Schwartz and Boyd, 1994b). The rest remain at the ponds fish excrement and leftover food particles.

As a results, ponds often have higher concentrations of nutrients, plankton, suspended solids, and oxygen demand than the water bodies into which they discharge (Schwartz and Boyd, 1994b). The organic loading from fish farm wastewater reduces the dissolved oxygen levels, while high nutrient loading reduces water quality by stimulating excessive phytoplankton production. Hence, the effluent is harmful to environment if discharge without any treatment.

Wastewater treatment is crucial to ensure the water comply with standard before discharging. The goal is to reduce or remove organic matter, solids, nutrients, disease causing organisms and other pollutants from wastewater (Cao and Wang, 2010). Conventional method, such as physical, chemical, and biological methods, have been applied in aquaculture systems. The purpose physical method is to remove solid, such as sedimentation and mechanical filtration. Chemical methods, include microbes, O_3 water and ClO_2, are used in the sterilization of effluent. Biological processes are submerged biofilters, trickling filters, rotating biological (Cao and Wang, 2010).

However, after treatment, nearly all the nutrients will be lost. Conventional wastewater treatment is relatively costly and beyond the technological and financial capabilities of many regions in the developing countries (Cao and Wang, 2010).

Alternative method to treat wastewater is phytoremediation. It is a plant based green technology and has received increasing attention. The plant has ability to remove pollutants from the environment or to make them harmless or less dangerous by accumulate, translocate, and concentrate high amount of certain toxic elements in their above-ground/harvestable parts (Rahman and Hasegawa, 2011). At the same time, the plants can be used as biomass after harvesting. The biomass is used as feed stock for animals.

1.2 Problem Statement

The most important constraint to wastewater treatment has most often been the culturists’ concern of losing nutrient. Conventional treatment processes suffer from some disadvantages. The efficiency are depending upon the nutrient to be removed and the chemical processes often lead to pollution (Noüe et al., 1992).

Conventional wastewater treatment is relatively costly and beyond the technological and financial capabilities of many regions in the developing countries (Cao and Wang, 2010). The waste treatment systems comprises of activated sludge units, aerated lagoons, and trickling filters. They are capital intensive to build because of machinery and construction materials and are also expensive to run and maintain because of the cost of electricity and skilled employee. There is no return on the investment since they only treat the waste. Such systems are likely to become considerably more expensive to build and maintain in the long run (Edwards, 1980).

Traditional treatment methods such as activated sludge were not designed to pathogen. Organisms that can survive wastewater without treatment include bacteria, protozoa, helminthes, and viruses. Wastewater disinfection will eliminate them, but its costs are high. Most of these pathogens affect the human body by contacting the waste contaminated water and food (Edwards, 1980).

Therefore, the project emphases on the treatment process of fish farm wastewater by phytoremediation. Duckweeds, such as *Lemna* sp. and *Spirodela* sp., are chosen as free floating plants to treat the fish farm wastewater. At the same time, *Lemna* sp. and *Spirodela* sp. grow into large amount of biomass with high protein content. They are valuable as feedstock of animals.

1.3 Research Objectives

The main objectives of this study are:

i. To evaluate the performance of both *Lemna* sp. and *Spirodela* sp. on nutrient removal from fish farm wastewater.

ii. To evaluate the growth of both *Lemna* sp. and *Spirodela* sp. on fish farm wastewater.

iii. To study the feasibility of *Lemna* sp. and *Spirodela* sp. phytoremediation on fish farm wastewater.

1.4 Scope of Study

The scope of the study is focuses on wastewater treatment, which is emphasized on how to treat fish farm wastewater by using phytoremediation. Fish farm wastewater was taken from Kampung Sungai Kota, Perak. *Lemna* sp. and *Spirodela* sp. were used to treat the wastewater. The quality of water was tested after treatment process. The parameter of water quality are studied, for instance, total nitrogen, nitrate, ammonia, total phosphorus, phosphate, turbidity, pH and MLVSS.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fish Farming

The primary aims of fish farming are to maximize survival and growth of fishes at minimal cost (Knights, 1985). It is a form of aquaculture. Aquaculture is the technique of raising useful aquatic species under some control of the organism and environment. It is expected to supplement fishing by adding significantly to supplies of protein food (Idyll, 1973).

The fish production is growing continuously, at an average annual rate of 3.2% in the world. The per capita fish consumption increases from 9.9 kg in 1960 to 19.2 kg in 2012 (Villamil et al., 2017). Fish is an important source of animal protein. Some countries such as Bangladesh, Indonesia and the Solomon Islands depend on fish for above half of their animal protein consumption (Khan et al., 2016)

2.2 Quality of Fish Pond

In aquaculture, fish pond has high levels of nitrogenous waste due to over feeding and waste excretion. Only about 25 % (range 11–36 %) of the total fixed nitrogen ingested by fish is then converted to fish biomass (Nakphet et al., 2016). If 25% of input nitrogen compound is retained by fish, then 75% of input of nitrogen compound is excreted.

Nitrogen excretion can be partitioned into dissolved (62%) and particulate (13%) fractions (Folke and Kautsky, 1989). Taking an example of a feeding rate at 100 kg $ha^{-1}d^{-1}$(consist of 32% protein feed), the ammonia excretion would be 317 mg N m^{-2} d^{-1} (Hargreaves, 1998). Some of the ammonia is released as unionized ammonia

NH_3 and ionized ammonium NH_4^- , nitrite (NO_2^-), and nitrate (NO_3^-) into the environment (Nakphet et al., 2016).

During protein catabolism process, fish digest the protein in their feed and excrete ammonia through their feces, which settle to the sediment along with phytoplankton and other particulate organic matter. When mass approach 13% nitrogen as particulate solids, 67 mg N m^{-2} d^{-1} are excreted as faecal solids at a feeding rate of 100 kg ha^{-1} d^{-1} (Hargreaves, 1998). Alternately, faecal solids can weight for up to 50% by mass of dry weight feed applied to the pond (Colt and Orwicz, 1991). Most of this organic matter faeces is hydrolyzed and mineralized (Hargreaves, 1998).

The nature of the major nitrogenous end-product of a species, ammonia, is dependent on the availability of water (Colt and Tchobanoglous, 1978). For freshwater pond, it has abundant of water. Fresh water fish, which have access to large amounts of water, will eliminate ammonia by exchange with the environment. The ammonia is eliminated by converting it to a non-toxic end-product such as urea or uric acid (Colt and Tchobanoglous, 1978).

Ammonia will become toxic if it is accumulated in pond. Once human beings consumed water from the pond, which contained high toxicity of ammonia, human beings will suffer from the symptoms such as, hyperactivity, convulsions, loss of equilibrium, lethargy and coma will be happened (Hargreaves, 1998).

At the same time, nitrate is exist in the fish pond. If nitrate increased over a specific limit, it will be toxic to fish eaters and cause nitrate pollution. Nitrite is another toxic nitrogenous compound. Nitrite is released as an intermediate product during nitrification and denitrification (Hargreaves, 1998). People, who consumed the fish, suffer from methemoglobinemia disease. It caused the hemoglobin to have less capacity to carry oxygen and will not be able to carry oxygen to the rest of human organs (Khater et al., 2015).

2.3 Conventional Treatment

Aquaculture systems, which incorporate waste treatment are developed to minimize wastewater. Aquaculture waste treatment systems can be classified into three categories, namely physical treatment, chemical and biological methods (Cao and Wang, 2010).

Sedimentation is type of physical treatment method. Sedimentation is the processes which is suspended solids, that have a higher density gravity than water, can settle down (Cao and Wang, 2010). Suspended solids, from fish pond, are originated from feces and uneaten fish food. The suspended solids will sink to the bottom of pond. According to natural deposition principle, most of the suspended solid wastes can be removed.

For chemical treatment method, ultra-violet filter and ozone water are used. Any pathogens, parasites and diseases in fish farm wastewater are destroyed after passing through ultra-violet filter or treated with ozone (Cao and Wang, 2010). The benefits of using ozone are it does not have pollution such as organic chloride and oxygen consumption is small with fast reaction rate. (Liltved and Cripps, 1999). Chemical oxidation by ozonation can be applied to reduce the organic load in conventional waste water treatment (Metcalf and Eddy, 1991).In a recirculating system for fish culture, chemical oxidation by ozonation is effective in degradation of organic matter and sterilization (Cao and Wang, 2010)

Another way to treat aquaculture wastewater is by using microbes. For example, they are photosynthetic bacteria, lactic acid bacteria, *sporangium bacillus*, nitration bacteria and etc. The bacteria decomposes and absorbs the sedimentary organic nitrogenous, the ammonia nitrogen, the nitrite nitrogen or convert them to beneficial substances (Cao and Wang, 2010). Alternative name for this method is called suspended film system. It contains suspending microorganisms in aquaculture wastewater (Midlen and Redding, 1998).

The microorganisms grow in size and number after absorbing organic matter and nutrients from the wastewater. After the microorganisms have been suspended for several hours, they are settled out as sludge. Some of the sludge is pumped back into the incoming wastewater, while the remaining is collected and sent to a sludge treatment process. The purposes of treatment of sludge are to stabilize the sludge, reduce odors, remove some of the water, decompose some of the organic matter, kill disease causing organisms and disinfect the sludge (Cao and Wang, 2010).

One of the drawback of microbe treatment, such as activated sludge, is usually sophisticated and expensive. Some advanced technologies are beyond the financial capabilities of many developing countries. Microbe treatment do not allow for reuse of nutrient resources contained in wastewater because they convert wastewater nutrients to gas (e.g. N_2) and sludge (Zhao et al., 2014).

2.4 Phytoremediation

The term “Phytoremediation” consists of the Greek prefix “phyto”, meaning plant, attached to the Latin root “remedium”, meaning to correct or remove an evil (Ghosh and Singh, 2005). It is a type of treatment by using plant based on green technology. This technology can be applied to organic and inorganic pollutants, which are present in soil (solid substrate), water (liquid substrate) or the air in environmental friendly way (Salt et al., 1998).

Green plants, for instance, grasses, forbs, and woody species, are utilized to remove, contain or render environmental contaminants. They can be heavy metals, trace elements, organic compounds, and radioactive compounds in soil or water. Phytoremediation has few advantages of its selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, contaminant storage and degradation abilities (Hinchman et al., 1996).

Phytoremediation is easy to operate and low in cost. The conventional methods of remediation may cost from $10 to 1000 per cubic meter of macrophytes. Phytoremediation costs are estimated to be as low as $ 0.05 per cubic meter (Kruger et al., 1997).

In fish farm, phytoremediation can efficiently absorb dissolved compounds in wastewater as nutrients for plant growth. The nutrients, which are excreted directly by the fish or generated by the microbial breakdown of organic wastes, are absorbed by aquatic plants (Endut et al., 2009). At the same time, it can utilized the nutrient from wastewater to grow into valuable biomass.

2.4.1 Types of Phytoremediation

Phytoremediation includes several types namely, phytoextraction, phytodegradation, rhizofiltration, phytostabilization and phytovolatilization (Rahman and Hasegawa, 2011), which is shown in Table 2.1.

Rhizofiltration process is usually performed by aquatic plants. The hyperaccumulating aquatic plants adsorb and absorb pollutants from aquatic environments (water and wastewater). The technology has been tested with uranium contaminated water at concentrations of 21-874 µg/L; the treated U concentration was less than 20 µg/L before discharge into the environment (Dushenkov et al., 1997).

Phytostabilization does not remove pollutants from contaminated sites but reduces mobility and excludes metals from plant uptake. The plants reduce mobility and phytoavailibility of contaminants in the environment (Rahman and Hasegawa, 2011)..

Table 2.1 Processes and mechanisms of contaminant removal by Phytoremediation adapted from Ghosh and Singh, 2005

Types	Mechanism	Contaminant
Rhizofiltration	Rhizosphere accumulation	Organics/Inorganics
Phytostabilization	Complexation	Inorganics
Phytoextraction	Hyper-accumulation	Inorganics
Phytovolatilization	Volatilisation by leaves	Organics/Inorganics
Phytotransformation	Degradation in plant	Organics

Phytoextraction removes pollutants form the contaminated sites. This process is observed in hyperaccumulating plants resistant to the pollutants. The plants uptake pollutants from soil and water and translocate to and store in the harvestable biomass of the plants environment (Rahman and Hasegawa, 2011).

Phytovolatilization utilizes plants to take up contaminants from the soil, transforming them into volatile form and transpiring them into the atmosphere. Phytovolatilization has been used for the removal of mercury, the mercuric ion is transformed into less toxic elemental mercury (Ghosh and Singh, 2005).

Phytodegradation breaks down organics taken up by the plant to simpler molecules, which can be incorporated into the plant tissues (Chaudhry et al., 1998). Plants contain enzymes that can breakdown and convert chlorinated solvents such as trichloroethylene and other herbicides. The enzymes are dehalogenases, oxygenases and reductases (Cunningham and Jones, 1995).

2.5 Duckweed in Phytoremediation

Floating macrophytes are used for the removal of nitrogenous and phosphorous compounds in the water (Steward, 1970). Duckweed is a floating aquatic macrophyte, which belong to belong to family *Lemnaceae* and taxonomically belong to monocotyledons and have four genera: *Lemna*, *Spirodela*, *Wollfia* and *Wolffiella* (Gupta and Prakash, 2013).

Duckweed grows in both still and running freshwater, such as lakes, rivers, and streams. Any plant of the genus *Lemna* sp. can be considered duckweed. The plants usually have small vestigial roots and grow in the form of thick green carpets of rounded free-floating thalloids, flattened structures which resemble leaves (Gupta and Prakash, 2013).

Duckweed in wastewater treatment was found to be very effective in the removal of nutrients, soluble salts, organic matter, heavy metals, eliminating suspended solids, algal abundance and total and fecal coliform densities (El-Kheir et al., 2007). Duckweed has a high mineral absorption capacity and can tolerate high organic loading as well as high concentrations of micronutrients (Velichkova and Sirakov, 2013).

Duckweed treatment and farming systems have relatively high land requirements. 2 to 3 m^2 per inhabitant are necessary for duckweed-based wastewater treatment systems. Duckweed based pisciculture requires a duckweed/fish pond area ratio of at least 1:1 to provide enough duckweed to sustain fish production (Iqbal, 1999).

2.5.1 Chemical Composition in Duckweed

Duckweed have high protein content and low fiber content. According to Culley Jr and Epps (1973), the protein content of duckweed harvested from natural water bodies is 140-260 g (kg duckweed)$^{-1}$, while 290-410 g (kg duckweed)$^{-1}$ of protein of duckweed harvested from wastewater ponds. Based on natural freshwater site and wastewater ponds, the nitrogen content of 20-40 g nitrogen (kg duckweed)$^{-1}$ and 40-60 g nitrogen (kg duckweed)$^{-1}$ respectively, phosphorus content of 2-10 g phosphorus (kg duckweed)$^{-1}$ and 13-29 g phosphorus (kg duckweed)$^{-1}$ respectively, and fiber content of 90-120 g (kg duckweed)$^{-1}$ and 60-90 g (kg duckweed)$^{-1}$ respectively. Typical water content of duckweed is 94-95% in mass (Bonomo et al., 1997).

The nutrient content in wastewater is higher compared to natural waters. It will promote to the vigorous growth of duckweed kept young through a frequent harvesting (Bonomo et al., 1997).

2.6 Removal Mechanism

2.6.1 Total Suspended Solid Removal

Total suspended solid (TSS) is contributed by sedimentation and biodegradation of organic particle. A minor fraction is absorbed by the roots of the duckweed, where organic particles undergo aerobic biodegradation by microorganisms. Part of the degraded products is assimilated by the plants (Iqbal, 1999).

A large amount of algae contribute to TSS concentrations. Although algae takes up considerable amounts of nitrogen, phosphorus and other nutrients, the nutrients are released again by biodegradation when algae die off (Iqbal, 1999).

Consequently, concentration of TSS increases. A complete mat of duckweed on the surface of pond inhibits penetration of light and growth of algae at the same time.

2.6.2 Nitrogen Removal

The nitrogen balance in a duckweed treatment system is determined by plant uptake, denitrification, volatilization of ammonia, microbial uptake, and sedimentation. Approximately 50 % (± 20 %) of the total nitrogen load is assimilated by duckweed. The remaining nitrogen is removed by loss to the atmosphere by denitrification and volatilization of ammonia (Iqbal, 1999).

At alkaline pH above 8, the ammonium-ammonia (NH_3) balance shifts towards the unionised form which results in a loss of nitrogen through volatilization of ammonia (Iqbal, 1999).

Ponds with aerobic and anaerobic environments favour microbial nitrification and denitrification. For nitrification process, ammonium is first oxidized to nitrite and nitrate. In denitrification process, the nitrite and nitrate are reduced to nitrogen, which is released to the surrounding.

2.6.3 Phosphorous Removal

Phosphorous is removed by plant uptake, adsorption to clay particles and organic matter, chemical precipitation with Ca^{2+}, Fe^{3+}, Al^{3+}, and microbial uptake. Except for plant uptake, the latter three mechanisms cause a storage of phosphorous in the system. Phosphorous removal is only possible by plant harvesting. The plants uptake capacity depends on the growth rate, harvesting frequency and available ortho-PO_4^{3-}, which is the favoured form of phosphorous for duckweed growth. When the growth rate is highest, phosphorous removal rate is also highest (Iqbal, 1999).

Adsorption and precipitation are also mechanisms for phosphorous removal in a duckweed treatment system. These particle/sediment-water phase interactions are very complex and depend on the redox potential, pH and concentrations of reactants. Aerobic conditions contribute to the precipitation of phosphorous through oxidized forms of Fe and Al. However, phosphorous is again released under anaerobic conditions prevailing in the sediments (Iqbal, 1999).

2.6.4 BOD Removal

BOD is removed by both aerobic and anaerobic microorganisms associated with the plants' surfaces, suspended in the water column and present in the sediment. Aerobic BOD removal depends on oxygen supply and surface area available for attached bacterial growth. *Lemnaceae,* however, possess a relatively small surface area for attached growth of mineralising bacteria compared to other aquatic macrophytes with larger submerged root and leaf surfaces (Iqbal, 1999).

2.7 Performance of Duckweed to Remove Nutrient

In India, Ayyasamy et al. (2009) used water hyacinth to treat nitrate-contaminated groundwater from Kar, Pali and Marwar. The study showed water hyacinth reduced the nitrate level to 64% in a synthetic medium containing 100 mg/l of nitrate. The efficiency of nitrate removal was further increased to 83% with initial nitrate concentrations of 300 mg/l, respectively. This was caused by osmotic pressure at higher concentrations not supporting the uptake of nitrate. Water lettuce and salvinia showed lower nitrate removal efficiencies. (Ayyasamy et al., 2009).

A study conducted by Lin et al. (2002) to evaluate system performance in removing inorganic nitrogen and phosphate from aquaculture wastewater. Water spinach (Ipomoea aquatica) and native weed (Paspalum vaginatum) were used to treat aquaculture wastewater. They found out nitrogen removals were excellent with efficiencies of 86% to 98% for ammonium nitrogen (NH_4 –N) and 95% to 98% for total inorganic nitrogen (TIN). Phosphate removal of 32% to 71% occurred. (Lin et al., 2002). Removal of ammonium and nitrite (effluent concentrations < 0.3 mg NH_4 –N /l and 0.01 mg NO_2 –N /l) were sufficient for recycle in the aquaculture system without danger of harming the fish (Lin et al., 2002).

Spirodela oligorrhiza was used s for nutrient recovery from swine wastewater and biomass production. *Spirodela oligorrhiza* were capable of removing 83.7% and 89.4% of total nitrogen (TN) and total phosphorus (TP) in eight weeks at a harvest frequency of twice a week. The total biomass harvested was 5.30 times that of the starting amount (Xu and Shen, 2011).

(Lu et al., 2010) studied on performance of water lettuce (P. stratiotes) on stormwater detention ponds (East and West Ponds) in 2005–2007. Water turbidity was decreased by more than 60%. Inorganic N (NH^{4+} and NO^{3-}) concentrations in treatment plots were more than 50% lower than those in control plots (without plant). Reductions in both PO_4^{3-} and total P were approximately 14–31%, as compared to the control plots. Water lettuce contained average N and P concentrations of 17 and 3.0 g kg^{-1}, respectively, and removed 190–329 kg N ha^{-1} and 25– 34 kg P ha^{-1} annually (Lu et al., 2010)

Generally, nitrogen removal by macrophyte systems was in the order of water hyacinth followed by water lettuce, pennywort, *Lemna* sp., *Salvtnia* sp., *Spirodela* sp. and Egeria during the summer season, while pennywort ranked first during the winter followed by water hyacinth, *Lemna* sp., water lettuce, *Spirodela* sp., *Salvinia* sp. and egeria. Phosphorus removal in summer was highest by water hyacinth and egeria systems, while pennywort and *Lemna* sp. showed high P removal rates during the winter compared to other plants. Nitrogen and P removal were generally higher in summer than winter (Reddy and De Busk, 1985).

Most of the research emphasize on the nitrogen and phosphorus removal. However, contaminated plant biomass, which caused by pathogen or disease, may cause a concern.

CHAPTER THREE

METHODOLOGY

3.1 Approach Research

The study was to analyse the water quality of phytoremediation based on *Lemna* sp. *and Spirodela* sp. The performance of *Lemna* sp. and *Spirodela* sp. were evaluated based on nutrient removal from fish farm wastewater. In order to run the phytoremediation, rigs were built to place the fish farm wastewater.

3.2 Experimental Flow Chart

The flow of experiment is shown at Figure 3.1.

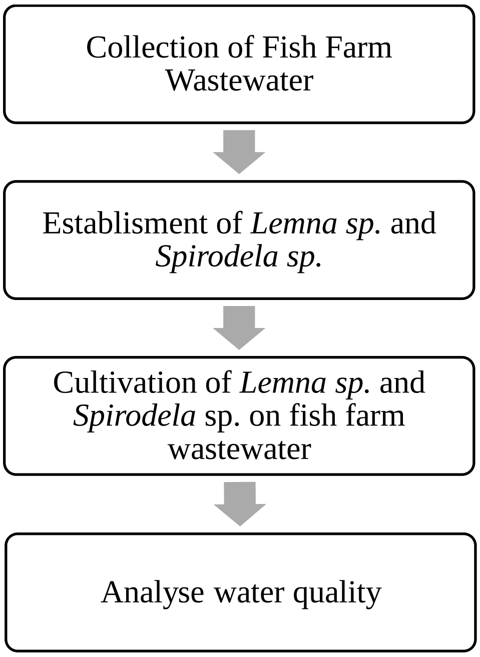

Figure 3.1 Experiment flow chart

3.3 Material and Chemicals

In this study, *Lemna* sp. and *Spirodela* sp. was used. The plant stock was obtained from USM School of Chemical Engineering Research Lab. The fish farm wastewater was take from Kampung Sungai Kota, Perak. 4 different fish ponds were randomly selected as sample of wastewater for experiment.

3.4 Experimental Procedure

3.4.1 Establishment of *Lemna* sp. and *Spirodela* sp.

After the nutrient solution had cooled down, *Lemna* sp. and *Spirodela* sp. are subcultured in Hoagland liquid medium, which is shown in Appendix A. *Lemna* sp. and *Spirodela* sp. were then placed at culture rack under fluorescent lamp.

3.4.2 Cultivation of *Lemna* sp. and *Spirodela* sp. in fish farm wastewater

The fish farm wastewater was collected from Kampung Sungai Kota, Perak. *Lemna* sp. and *Spirodela* sp. were cultivated in raceway pond rig with circulation system using motor pump. The amount of duckweeds were added which able to fill up the surface of the raceway pond rig. 72 g of duckweeds were used in each pond rigs except control set. The duckweeds were then distributed evenly on the surface of wastewater. *Lemna* sp. and *Spirodela* sp. were cultivated in raceway pond rig with circulation system. 4 pond rig were set up to accommodate the duckweeds. The schematic diagram of pond rig were shown in Figure 3.2 and Figure 3.3. The duckweeds in each pond rigs were listed as follow:

i. Pond Rig 1: *Spirodela* sp.
ii. Pond Rig 2: *Lemna* sp.
iii. Pond Rig 3: mixture culture of *Lemna* sp. and *Spirodela* sp.
iv. Pond Rig 4: control set

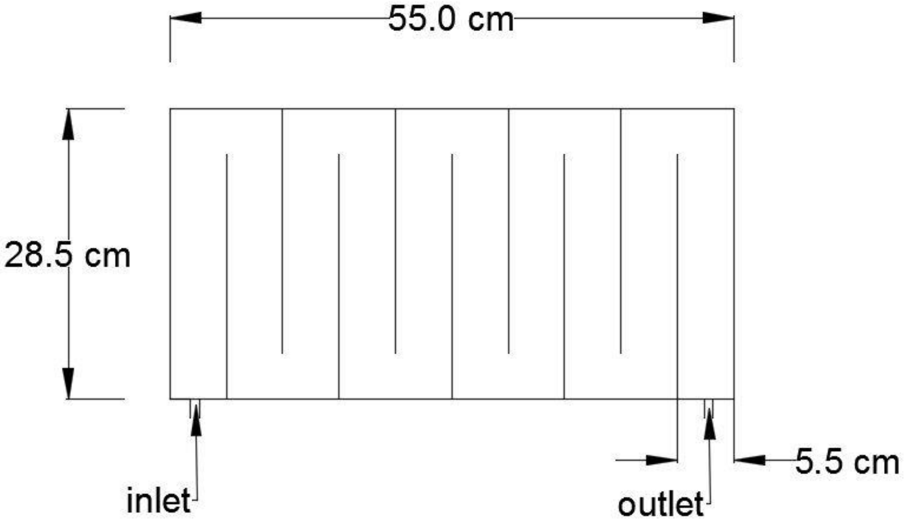

Figure 3.2 Top view of pond rig

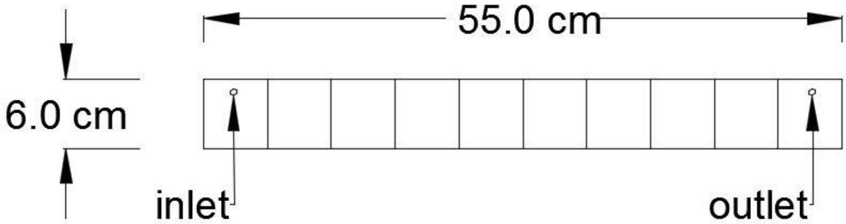

Figure 3.3 Front view of pond rig

The pond rigs were placed indoor with under florescent light. Mesh was installed at the end or outlet of raceway pond rig to avoid the duckweeds from being washed out from the pond. The experiment was run for 14 days. Two replicates batch was done in order to obtain average result.

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3.4.3 Nutrient Uptake by *Lemna* sp. and *Spirodela* sp.

150 ml of treated wastewater sample was collected from outlet of pind rigs every 2 days starting from day 0 using the centrifuge tubes throughout 14 days of experiment. The water level in the storage tank was ensured to be maintained at the initial marked level before collection as the water level will drop due to evaporation. Distilled water was added to the storage tank to replace the lost water.

The water samples were tested for its concentration of nitrite, nitrate, phosphate, total nitrogen, total phosphorus and ammonia to determine respective nutrients uptake by *Lemna* sp. and *Spirodela* sp., COD, turbidity, pH and MLVSS test were also conducted on the samples to evaluate the water quality.

3.5 Analytical Analysis

3.5.1 Determination of nitrate and phosphate concentration

The supernatant of water samples was obtained by centrifugation at 10000g for 15 min. The supernatant was used to determine the concentration of nitrate and phosphate. The equipment used is DR 2800 Spectrophotometer.

The nitrate was determined by Cadmium Reduction Method. NitraVer®5 Nitrate Reagent Powder Pillows were used at 500 nm with allowable detection of 0.3 to30.0 mg/l NO_3^--N.

The phosphate was determined by Ascorbic Acid Method (HACH method 8048) using PhosVer®3 Phosphate Reagent Powder Pillows at 880 nm with detection range of (0.02 – 2.50 mg/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 10 ml of collected wastewater samples.

3.5.2 Determination ammonia and COD for water samples

The equipment used was MD 600 Photometer. Initially, the supernatant of water samples was obtained by centrifugation at 10000g for 15 min. 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) at 660 nm with detectable range of 0 to 50 mg/l NH_3-N. This method consumes 0.1 ml water sample.

The COD was determined by Dichromate/H_2SO_4 Method (Lovibond method 131) using COD VARIO 0 – 1500 mg/l tube test Reagent at 610 nm with detectable range of 0 to 1500 mg/l COD/CSB.

3.5.3 Determination of Turbidity of water samples

The equipment used was HANNA HI 93703 microprocessor turbidity meter peaking at 890 nm with range of 0 – 1000 NTU. The measurement was tested based on ISO 7027 International Standard. To ensure the water samples was well mixed, the centrifugal tube was shaken well before filling cuvette with water sample until the indicated level mark. It was inserted into measurement cell to measure its turbidity value in NTU.

3.5.4 Determination of MLVSS

A blank glass microfiber filters was ignite at temperature of 550 °C for 20 minutes in muffle furnace. The blank will represent filter paper weight. It was then placed at vacuum chamber which run by vacuum pump.

The centrifuge tube were shaking vigorously to ensure water samples were well mixed. 30 ml water sample was filtered. The residues were dried in an oven to a constant weight at 105 °C for 1 h. The weight represents the suspended solid weight.

The filters with the residues were ignited in furnace until constant weight was gained. It was done at 550 °C for 20 min. The solids left was the fixed solids while weight lost on ignition was the volatile solids.

3.5.5 Determination of pH

The pH is determined based on ISO standard 10523:2008 (Water quality – Determination of pH). The standard can be applied for a pH range from 2 to 12 between 0 °C and 50 °C for an ionic force lower than 0.3 mol/kg of solvent (equivalent to conductivity lower than 2000 mS/m at 25 °C).

The equipment used was edge® Multiparameter pH Meter - HI2020. The probe was rinsed with deionized water before 50 mL of the sample was measured. The sample was stirred for 15 seconds and then the pH value was recorded without stirring. The probe was rinsed between samples.

3.5.6 Determination of Total Nitrogen (TN)

Persulfate digestion method was used to determine the total nitrogen content in water samples. Water sample of 1 ml was well mixed with 1 ml of deionized water in a TN Hydroxide LR vial. The vials, which contained the TN Persulfate Reagent Powder, was heated at 100 °C for 30 minutes. It was left to cool down to room temperature. TN Reagent A and Reagent B Powder Pack were added before transferring to Acid LR/HR vial by 2ml. TN was determined by using Maxidirect MD600 photometer at wavelength of 430 nm with detection range of 0.5 to 25 mg/l N.

3.5.7 Determination of Total Phosphorus (TP)

Acid Persulfate Digestion method was used to determine the total phosphorus content in water samples. Water sample of 1 ml was well mixed with 4 ml of deionized water in a digestion tube. The vials, which contained the Potassium Persulfate F10 Powder, was heated at 100 °C for 30 minutes. It was left to cool down to room temperature. 2 ml 1.54 N Sodium hydroxide solution and Phosphate Reagent F10 Powder was added. TP was determined by using Maxidirect MD600 photometer at wavelength of 660 nm with detachable range of 0.02 to 1.1 mg/l P.

3.5.8 Determination of Biomass

The fresh *Lemna* sp. and *Spirodela* sp. biomass were washed with tap water twice. They were then washed with distilled water. After washing, they were casually dried by blotting them with a clean cloth before weighting.