PHYTOREMEDIATION OF FISH FARM WASTEWATER INCORPORATING PHYSICAL TREATMENT

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UNIVERSITI SAINS MALAYSIA

2018

PHYTOREMEDIATION OF FISH FARM WASTEWATER INCORPORATING PHYSICAL TREATMENT

by

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Thesis submitted in partial fulfilment of the requirement

for the degree of Bachelor of Chemical Engineering

June 2018

ACKNOWLEDGEMENT

First and foremost, I would like to convey my sincere gratitude to my supervisor, Associate Professor Derek Chan Juinn Chieh for his precious encouragement, guidance and generous support throughout this work.

I would also extend my gratitude towards all my colleagues for their kindness cooperation and helping hands in guiding me carrying out the lab experiment. They are willing to sacrifice their time in guiding and helping me throughout the experiment besides sharing their valuable knowledge.

Apart from that, I would also like to thank all SCE staffs for their kindness cooperation and helping hands. Indeed, their willingness in sharing ideas, knowledge and skills are deeply appreciated.

Once again, I would like to thank all the people, including those whom I might have missed out and my friends who have helped me directly or indirectly. Their contributions are very much appreciated. Thank you very much.

Navin Kumar

June 2018

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LIST OF ABBREVIATIONS

APHA	American Public Health Association
ADFDMW	Anaerobically digested flushed dairy manure wastewater
COD	Chemical oxygen demand
HRT	Hydraulic retention time
ISO	International Organization for Standardization
MLVSS	Mixed liquor volatile suspended solids
TSS	Total suspended solids
UASB	Upflow anaerobic sludge blanket
USEPA	United States Environmental Protection Agency

FITOPEMULIHAN AIR SISA KOLAM IKAN DIGABUNG RAWATAN FIZIKAL

ABSTRAK

Fitopemulihan mempunyai potensi untuk rawatan dan penggunaan semula air sisa dalam sistem akuakultur intensif dan teknik ini mengurangkan pencemaran yang disebabkan oleh ternakan ikan dan akan memelihara kualiti air. Dalam kajian ini, prestasi fitopemulihan oleh macrophytes *Spirodela Polyrhiza* digabungkan dengan rawatan fizikal pada air sisa ladang ikan akan disiasat. Objektif kajian ini adalah untuk menilai prestasi rawatan fizikal digabungkan dengan fitopemulihan. Pengurangan nutrien oleh *S.polyrhiza* dari air sisa kolam ikan dan kualiti air selepas fitopemulihan telah dipantau. Fitopemulihan air sisa oleh *S. polyrhiza* telah dijalankan dalam tanah lembap berskala kecil untuk 14 hari. Keputusan menunjukkan bahawa penapis Fendi dengan saiz liang 20 μm lebih berkesan dari Sterlitech (3.0 μm) dan Sartorius (0.2 μm) dari segi kadar turasan (L/m^2) dan segi kos berdasarkan kuantiti 2 L yang diperlukan untuk eksperimen ini. Fendi menunjukkan kadar turasan tertinggi iaitu 25000 L/m^2 dan kos yang paling rendah untuk menapis 2 L air sisa dengan RM 37.40. Untuk pengurangan nutrien, tapisan lebih berjaya berbanding kawalan kerana ia menunjukkan nilai akhir yang lebih rendah pada hari 14. Nitrat, fosfat dan ammonia menunjukkan nilai terakhir 9.4 mg/l, 0.27 mg/l dan 1.4 mg/l masing-masing. Kesemua tiga nutrien dapat memenuhi Piawai A (untuk melepaskan ke dalam mana-mana perairan pedalaman dalam kawasan tadahan yang merupakan had yang paling ketat), had pelepasan untuk badan air tertutup. Bagi kualiti air, tapisan dapat memenuhi Piawai A untuk tahap COD dan jumlah had pepejal terampai pelepasan mencapai nilai akhir 109 mg/l dan 20 mg/l. Nilai pH pada hari 14 iaitu 7.45 adalah dalam lingkungan Piawai A dan kekeruhan

menurun dari 110 NTU kepada 25 NTU dalam tempoh 14 hari iaitu di bawah kelas IIA (bekalan air II) yang melepasi untuk rawatan konvensional untuk menghasilkan air minuman yang bersih. *S. polyrhiza* menunjukkan kenaikan biojisim untuk ditapis dan kawalan. Kajian ini menunjukkan bahawa gabungan rawatan fizikal dengan fitopemulihan menunjukkan potensi yang lebih besar dalam merawat air sisa kolam ikan.

PHYTOREMEDIATION OF FISH FARM WASTEWATER INCORPORATING PHYSICAL TREATMENT

ABSTRACT

Phytoremediation has the potential for treatment and reuse of wastewater in intensive aquaculture systems. This technique serves the purpose of reducing the pollution caused by fish farming which will preserve the surface and ground water quality. In this study, the performance of phytoremediation incorporated with physical treatment by *Spirodela Polyrhiza* macrophytes on fish farm wastewater is investigated. The objectives of the study are to evaluate the performance of physical treatment incorporated with phytoremediation. The nutrient uptake by *S.polyrhiza* from the fish farm wastewater and water quality after phytoremediation was monitored. The wastewater phytoremediation by *S. polyrhiza* was conducted in small-scale constructed wetlands for 14 days. The results showed that filter Fendi with pore size 20 µm out-performed Sterlitech (3.0 µm) and Sartorius (0.2 µm) in terms of filtrate rate (L/m^2) and costing based on the quantity required of 2 L for this experiment. Fendi showed the highest filtrate rate of 25000 L/m^2 and the lowest cost for filtering 2 L of wastewater with RM 37.40. For nutrients uptake, filtered was more successful compared to control as it resulted in a lower final value at the end of day 14. The final nitrate, phosphate and ammonia level were 9.4 mg/l, 0.27 mg/l and 1.4 mg/l respectively. All three nutrient level were able to meet the standard A (applicable to discharge into any inland waters within catchment area which is the most stringent limit), discharge limit for enclosed water body. As for water quality, filtered was able to meet the Standard A for COD level and total suspended solids discharge limit achieving final value of 109 mg/l and 20 mg/l. The pH level day 14 of 7.45 was in the range of Standard A and as for turbidity it decreased from 110 NTU to 25 NTU in 14 days which was below the class IIA (water supply II) which passes for conventional treatment to produce clean drinking water. *S. polyrhiza* showed biomass increment for both filtered and control. This study indicates that phytoremediation incorporated with physical treatment shows a greater potential in the treating fish farm wastewater.

CHAPTER ONE

INTRODUCTION

1.1 Fish farm wastewater

In fish farming, the wastewater exhibits environmental impacts when effluent from fish farm is released to receiving waters. The main contaminants of this wastewater effluent are suspended solids, ammonium, organic nitrogen and phosphorus and removal of this are essential for aquaculture wastewater treatment to protect receiving waters and for potential reuse of the treated water. In a research done by Ghaly (2005), they found that the production of 1 ton (1000 kg) of live channel catfish releases 1190 kg of dry matter, 60 kg of nitrogen and 12 kg of phosphorus to the culture water as metabolic wastes. Nitrogen and phosphorus are capable of stimulating the growth of aquatic plants in the receiving waters.

Furthermore, fish farm wastewaters effluent exhibits environmental impact when discharged to receiving waters as organic matter loading reduces dissolved oxygen levels and causes to the buildup of bottom sediments and high nutrient loading impairs water quality by stimulating excessive phytoplankton production. Apart from that a report by EC-European Communities (2006) stated that it poses a great danger to public health as pollutants can enter food through agricultural products or leach into drinking water. It is also reported that the production of fish has been affected with high concentrations of toxins and/or heavy metals and infestation of non-desirable phytoplankton and/or zooplankton species. Moreover, eutrophication is the enrichment of a water body with nutrient and is a serious environmental problem since it results in deterioration of water quality. It is well documented that from the total nitrogen supplemented to the cultured organisms only 20 to 50% is retained as biomass by the farmed organisms and meanwhile the rest is eventually discharged in the effluents toward the receiving ecosystems which causes diverse impacts such as production of red tides which is decolourization of water, burring (the disperse of pollutants by aquatic cultures), death of benthic organisms and also undesirable odors as well as presence of pathogens in the discharge sites (Martinez-Porchas and Martinez-Cordova, 2012).

Conventional fish farm wastewater treatment consists of a combination of physical, chemical, and biological processes and operations to remove solids, organic matter and nutrients from wastewater. Preliminary, primary, secondary, and tertiary wastewater treatments are the terms used to describe different degrees of treatment for wastewater treatment (Pescod, 2017). In fish farm wastewater preliminary treatment is needed first as it focuses on the removal of large materials and the most common preliminary treatment is by running it through filters or coarse screening. Meanwhile, the objective of primary treatment is the removal of settleable organic and inorganic solids by sedimentation, and floating materials are removed by skimming. Then, it is followed by secondary treatment which involves the removal of biodegradable dissolved and colloidal organic matter using biological processes such as submerged biofilters, trickling filters and activated sludge process which is employed for the oxidation of organic matter, nitrification or denitrification. Tertiary treatment wastewater is employed when specific wastewater constituents which cannot be removed by secondary treatment must be removed. This treatment processes are usually combined with primary or secondary treatment such as chemical to remove phosphorus. However, these conventional methods pose certain drawbacks as they are costly in terms of needing constant high electrical energy requirements and maintenance requirements. In addition, the general cost of construction will be high as it requires highly skilled worker and the issue of ecological disposal of the sludge waste from the secondary treatment is also a major concern (Turcios and Papenbrock, 2014).

1.2 Problem Statement

Duckweed is a small, free-floating aquatic plant which can be easily recognized as the plant from the Lemnaceae family. Growth of duckweed spans the globe and 40 species belonging to four genera (*Lemna*, *Spirodela*, *Wolffia*, and *Wolffiella*) have been determined thus far (McCutcheon and Schnoor, 2004). The study of wastewater treatment using *Spirodela polyrhiza* has been done before and has been identified as promising duckweed by Xu and Shen (2011). It has shown significant reduction in nitrogen, phosphorus, chemical oxygen demand (COD) and turbidity. However the turbidity reduction can still be increased by the removal of total suspended solids in the early stages. In this study this can be done by incorporating physical treatment to remove total suspended solids before proceeding with rhizofiltration. *S. polyrhiza* or giant duckweed which is characterized by its widespread availability and its potential to grow at wide temperature range is most commonly accepted wetland plant for the phytoremediation of wastewater.

Phytoremediation involves the use of vascular plants, algae and fungi either to remove and control waste or to spur waste breakdown by microorganism. On top of that, it is evolving into an effective way of waste managing especially excess organic matter and nutrients. This is proving to be an effective solution due to its low costs, in situ nature of the treatment, applicability to extensive areas of limited contamination such as urban brown-fields and attractiveness as a green technology.

Rhizofiltration is the use of plant roots to remove excess nutrients in contaminated groundwater. The contaminated water from the site is used to acclimatize the plants to the environment and these plants are grown in greenhouses in water instead of soil. These plants are then planted on the site of contaminated groundwater where the roots take up the water and contaminants. Once the roots are saturated with the contaminant, the plants are harvested including the roots (Dushenkov, 1995).

1.3 Research Objective

The aims of the research are as follows;

1) To evaluate the performance of physical treatment incorporated with phytoremediation.
2) To evaluate the uptake of nutrients of test plants in fish farm wastewater and the water quality after phytoremediation.

1.4 Scope of study

In this research, the efficiency of *S. polyrhiza's* nutrient removal capability and improvement of waste quality was studied in fish farm wastewater. The effect of physical treatment during pre-treatment to the fish farm wastewater was studied as well to observe how it affects the *S. polyrhiza's* nutrient removal capability and improvement of water quality.

1.5 Organization of thesis

The following are the contents for each chapter in this study:

Chapter 1 introduces fish farm wastewater, problem statement, research objectives, scope of study and organization of thesis.

Chapter 2 discusses the literature review of this study which includes aquaculture, phytoremediation and the effect of *S. polyrhiza* on the nutrients and solids along with the efficiency of pre-treatment.

Chapter 3 covers the materials and details of methodology. It discusses on the description of equipment and materials used and experimental procedure.

Chapter 4 refers to the experimental results and discussions of the data obtained with further elaboration on the cause and affects.

Chapter 5 concludes all the findings obtained in this study. Recommendations are included as well.

CHAPTER TWO

LITERATURE REVIEW

2.1 Aquaculture

Aquaculture were only limited to areas where the water resources were almost unlimited but now with techniques involving the recirculation of the wastewater after biological treatment and/or filtration can make aquaculture almost independent of water availability. Nevertheless, the content of nutrients in the waste stream from these systems has to be below the allowable limit (Ghaly, 2005).

Aquaculture systems produce large quantities of organic matter and nutrients that bring adverse environmental impacts when the effluent from these systems is discharged to receiving waters as organic matter loading reduces dissolved oxygen levels and contributes to the buildup of bottom sediments and high nutrient loading impairs water quality by stimulating excessive phytoplankton production (Joyner, 1992).

2.1.1 Fish Farm Wastewater

Organization for Economic Cooperation and Development (OECD) stated that "eutrophication is an enrichment of water by nutrient salts that causes structural changes to the ecosystem such as increased production of algae and aquatic plants, depletion of fish species, general deterioration of water quality and other effects that reduce and preclude use". The effluent of fish farm wastewater contributes to eutrophication and this is emerging as a serious environmental issue as deterioration of water quality is one of the major hindrance to achieving the quality objectives established by the Environmental Quality (Sewage) Regulations 2009 (Environment, 2010).

Eutrophication is a significant increase of algae due to the greater availability of one or more growth factors necessary for photosynthesis which are sunlight, carbon dioxide and nutrients such as nitrogen and phosphorus (Environment, 2010). When algae grows in an uncontrolled manner, an increasingly large biomass is formed which will degrade. In deep water, a large amount of organic substance accumulates is represented by the algae having reached the end of their life cycle.

Then, to degrade all this dead algae, an excessive consumption of oxygen is required and in some cases almost total by microorganisms. Therefore an anoxic condition in which an oxygen free environment is created on the bottom of the water bodies with the growth of organisms capable of living in the absence of oxygen (anaerobic) will degrade the biomass. The microorganisms will decompose the organic substance in the absence of oxygen and releasing compounds that are toxic such as ammonia and hydrogen sulphide. The absence of oxygen reduces biodiversity which causes even the death of animal and plant species. All this happens when the rate of degradation of the algae by microorganisms is greater than that of oxygen regeneration (Environment, 2010).

The main effects of eutrophication are abundance of particulate substances (phytoplankton, zooplankton, bacteria, fungi and debris) that determine the turbidity and colouration of the water, abundance of inorganic chemicals such ammonia, nitrites, hydrogen sulphide and phosphorus which promotes the formation of harmful substances such as nitrosamines suspected of mutagenicity, abundance of organic substances that give the water disagreeable odours or tastes and reduction of oxygen concentration especially in the deeper layers of the water bodies (McCutcheon and Schnoor, 2004).

The conventional way of treating this have been tested in the past as reported by Chislock (2013) which includes the alteration of excess nutrients, physical mixing of the water, application of powerful herbicides and algaecides but all this have proven to be ineffective, expensive and impractical for large ecosystems. Nowadays, the main focus is on prevention techniques, namely removal of the nutrients that are introduced into water bodies from the water. It would be sufficient to reduce the concentrations of one of the two main nutrients (nitrogen and phosphorus), in particular phosphorus which is considered to be the limiting factor for the growth of algae.

In order to achieve this, the improvement of the purifying performance of waste water treatment plants is needed by installing tertiary treatment systems to reduce nutrient concentrations. Tertiary treatment is the final treatment stage to further improve water quality before it is discharged to the receiving water bodies. Examples of tertiary treatment include ultra violet treatment, membrane and sand filters. However these methods pose certain drawbacks as they are costly in terms of needing constant high electrical energy requirements and maintenance requirements. In addition, the general cost of construction will be high as it requires highly skilled worker (Martinez-Porchas and Martinez-Cordova, 2012).

2.2 Phytoremediation

Phytoremediation comes in several forms. Phytoextraction removes metals or organics from soils by accumulating them in the biomass of plants. Phytodegradation or phytotransformation which is the use of plants to uptake, store and degrade organic pollutants meanwhile rhizofiltration involves the removal of pollutants from aqueous sources by plant roots. Phytostabilization reduces the bioavailability of pollutants by immobilizing or binding them to the soil matrix and phytovolatilization uses plants to take pollutants from the growth matrix, transform them and release them into the atmosphere (Peuke, 2005).

Phytoremediation have the potential for treatment and reuse of wastewater in intensive aquaculture systems. Combining aquaculture with phytoremediation technique serves the purpose of reducing the pollution caused by fish farming which will preserve the surface and ground water quality (Snow, 2008). A number of technologies have been developed for the nutrient removal from aquaculture wastewaters but among all the technologies, the conversion of nutrients into valuable plant biomass has drawn increasing attention because it does not only addresses nutrient pollution but also provides a profitable way to recycle the nutrients by producing a variety of value-added products such as commercial fertilizers from postharvest biomass (Xu and Shen, 2011).

On top of that, phytoremediation is proving to be an effective solution due to its low costs, *in situ* nature of the treatment, applicability to extensive areas of limited contamination such as urban brown-fields and attractiveness as a green technology (Dushenkov, 1995). Aquaculture systems integrated with plant culture involve the use of nutrient-rich effluent from aquaculture production tanks to provide water and nutrients to plants. The method of hydroponics is where the plant is cultivated in nutrient rich enriched water with or without the support of a medium such as sand or gravel (Bukaveckas, 2007).

A study by Ghaly (2005) have studied the possibility of using five plants (barley, white clover, fall rye, oat and alfalfa) in reducing the pollution potential of aquaculture wastewater by measuring the initial and final total solid (TS), chemical oxygen demand (COD) , nitrogen compounds, phosphorus (P) and potassium (K) and also the nutritional content and suitability of the plants as fish feed. The study was conducted for 21 days and of the five plants used three (oat, barley and rye) have the ability to reduce the pollution potential of aquaculture wastewater and have the potential use as fish feed. Clover and alfalfa were infected with fungus shortly after germination, and their roots were completely destroyed by day 14. Oat, barley and rye had the fastest growth and showed much better resistance to fungal disease compared with alfalfa and clover.

Another study by Xu (2015) have focused on duckweed which is a small floating aquatic plant within the family *Lemnaceae*. The study carried out on five local duckweed strains of the Yangtz River Delta (*L. minor, Lemna perpusilla, S. polyrrhiza, Spirodela oligorrhiza and Wolfflia arrhiza*) by using synthetic nutrient-rich wastewater and it showed that *S. oligorrhiza* had superior tolerance to high nutrient levels and low temperatures, and produced the highest protein among it.

Other than that, Cheng (2002) reported using *Spirodela punctate 7776* to recover nitrogen and phosphorus from synthetic artificial medium (SAM) which was formulated to resemble typical swine lagoon water it showed that the duckweed grew well at high nitrogen and phosphorus levels (240 mg NH_4-N/L and 31.0 mg PO_4-P/L).

Moreover, further studied by (Ng, 2017) have been carried out to precisely evaluate nutrient removal efficiency of NO_3^--N, PO_4^{3-}, NH_3-N, COD and pH in the water sample through phytoremediation by *S. polyrhiza*, *Salvinia molesta* and *Lemna* sp. axenically in synthetic wastewater under controlled condition. All the macrophytes showed biomass increment and *S. polyrhiza* outperformed other macrophytes in nutrient removal despite lower biomass production.

2.2.1 *Spirodela polyrhiza*

Duckweed is a small floating aquatic plant within the family Lemnaceae. It proliferates through vegetative budding of new fronds and produces biomass faster than most other plants. The geographic ranges of duckweed span the entire globe and 40 species belonging to four genera (Lemna, Spirodela, Wolffia, and Wolffiella) have been identified so far (Landolt, 1986). Duckweed of various species has been studied for the nutrient recovery from synthetic or real swine wastewaters due to its rapid proliferation, tolerance to high nutrient levels and excellent nutrient uptake ability (Bergmann, 2000). Compared with most plants, duckweed fronds have little fiber as little as 5 percent in cultured plants because they do not require structural tissue to support leaves or stems. Therefore virtually all tissue is metabolically active and useful as a feed or food product (Skillicorn, 1993).

Furthermore, duckweed species can adapt to a wide variety of geographic and climatic zones and can be found in all but waterless deserts and permanently frozen polar region. They can survive extremes temperature but grow fastest under warm, sunny conditions and are spread by floods and aquatic birds (Skillicorn, 1993). Great duckweed (*S. polyrhiza*) is known for its fast growth, wide distribution, short life span and stability to environmental changes. Moreover, *S. polyrhiza* have the largest fronds, measuring as much as 20 mm across (Rahman, 2007). Duckweed species like *S. polyrhiza* has an inherent capability to exploit favorable ecological conditions by growing extremely rapidly. The most favorable circumstance is water with decaying organic material to provide duckweed with steady supply of growth nutrients and trace elements.

In order to cultivate duckweed a farmer that mimic the natural environmental like of duckweed is needed to be organized and maintained for example a pond-like culture plot, sheltered and a constant supply of water and nutrients from organic or mineral fertilizers condition. Furthermore, wastewater effluent rich in organic material is a particularly valuable asset for cultivating duckweed because it provides a steady supply of essential nutrients and water (Skillicorn, 1993). Duckweed reproduction is primarily vegetative. Daughter fronds bud from reproductive pockets on the side of a mature frond and an individual frond may produce as many as 10 generations of progeny over a period of 10 days to several weeks before dying (Landolt, 1986). Duckweed plants can double their mass in less than two days under ideal conditions of nutrient availability, sunlight, and temperature and this is faster than almost any other higher plant.

On top of that, cultured duckweed also has high concentrations of trace minerals and pigments, particularly beta carotene and xanthophyll that make duckweed meal an especially valuable supplement for poultry and other animal feeds. The total content of carotenoids in duckweed meal is 10 times higher than that in terrestrial plants which grows on or in or from land (Skillicorn, 1993).

2.3 Nutrient and solids removal efficiency

Duckweed plants are efficient at removing elements which are used as growth nutrients by them. These include some organic compounds as well as ions of elements such as nitrogen, phosphorus, potassium, magnesium, calcium, sodium, chlorine, boron, and iron others as well. On top of that, 99 percent by bioaccumulation of the nutrients and dissolved solids contained in wastewater can be removed by duckweed wastewater treatment system (Skillicorn, 1993).

2.3.1 Nitrate

Nitrates cause the most serious problems when dispersed in water. Nitrate is oxidized forms of nitrogen and if it is not removed, discharge of the incompletely treated wastewater will cause the depletion of aquifers and the eutrophication of rivers. On top of that, excess algae growth in rivers and streams and these excess algae depletes oxygen which results in the death of fish and other important organisms as well as odor problems.

A three-year investigation by Dalu (2003) into the potential use of duckweed (*L. minor*) based wastewater stabilizations ponds for wastewater treatment was carried out at two small urban areas in Zimbabwe. The duckweed based waste stabilization ponds have performed well in relation to nitrogen uptake as the effluent levels are below the stipulated normal band limit of 10 mg/l N. When the nitrate influent and effluent levels are compared reductions up to 70% are observed.

2.3.2 Phosphate

Phosphorus (P) is a nutrient that is vital to a plants growths, it occurs mostly as phosphates (PO_4^{3-}) and are classified as orthophosphates (reactive phosphates), condensed phosphates (pyro, meta, and polyphosphates) and organic phosphates. Orthophosphates found in natural water provide a good estimation of the amount of phosphorus available for algae and plant growth and this can be removed by phytoremediation.

From a study by Sooknah (2004), the potential of water hyacinth (*Eichhornia crassipes*) to improve the water quality of anaerobically digested flushed dairy manure wastewater (ADFDMW) was evaluated and in terms of phosphate the removal efficiency is by 96.5% in a 31-day batch growth.

Furthermore, a three-year investigation by Dalu (2003) into the potential use of duckweed (*L. minor*) based wastewater stabilizations ponds for wastewater treatment was carried out at two small urban areas in Zimbabwe. Despite the high phosphate levels in the effluent, there were reductions of up to 80% when influent and effluent levels were compared when using this system.

2.3.3 Ammonia

Ammonium is the preferred form of nitrogen for duckweed species. The main source of ammonium for wild colonies of duckweed is from biodegradation of organic material by anaerobic bacteria. Duckweed plants reportedly utilize all available ammonium before beginning to assimilate nitrate and appear to grow more quickly in the presence of ammonium than with nitrate.

From a study by Sooknah (2004), the potential of water hyacinth (*E. crassipes*) to improve the water quality of ADFDMW was evaluated and in terms of ammonium the removal efficiency is by 99.6% in a 31-day batch growth.

Moreover, another study by El-Shafai (2007) used a pilot-scale wastewater treatment system comprised a 40 L UASB reactor (6-h HRT) followed by three duckweed (*L. gibba*) ponds in series (total HRT 15 days reported the treatment system achieved a residual values of ammonia which was 0.41 mg N/l with a removal efficiencies of 98%.

2.3.4 COD

The chemical oxygen demand (COD), is the amount of oxygen available for chemical reactions (oxidation processes) within the system. The organic carbon represented by COD affects the efficiency of duckweed. The removal of COD is done through the root systems in the duckweed cultures. The less polluted the wastewater is the less oxygen is needed to chemically oxidise the organics present. COD is a very important pollution indicator in wastewater quality assessment as it gives an idea about the totality of effluent pollution indices. Discharge of effluent of high COD load into the environment or water courses could lead to the reduction in dissolved oxygen to aquatic flora and fauna which will the affect fish stock as some types of fish cannot survive in very low dissolved oxygen environment (Skillicorn, 1993).

A study by Adhikari (2015) has reported that duckweed-based wetlands used to treat high-strength manure-containing wastewaters for primary treatment and secondary treatment has the capability of removing COD. COD removal rate in primary duckweed wetlands was significantly higher than COD removal in secondary wetlands with primary wetlands removing approximately 43% of the COD meanwhile secondary wetlands removed 12 %.

Furthermore, another study by El-Shafai (2007) using a pilot-scale wastewater treatment system comprised a 40 L UASB reactor (6-h HRT) followed by three duckweed (*L. gibba*) ponds in series (total HRT 15 days) reported the treatment system achieved a removal value of 93%.

2.3.5 Turbidity and MLVSS

Turbidity is a measure of the clarity of water. Under normal circumstances total duckweed cover would suppress the growth of algae thereby reducing debris due to algal death, producing a clear wastewater. Meanwhile, the removal of total suspended solids (TSS) in wastewater stabilization ponds in mainly affected by biodegradation of organic particles, settling of particles to sediment and production of algae within the pond system. When phytoremediation is applied, the duckweed plants exhibit widespread roots where the suspended solids are mainly attached to it and when the plants are harvested it will remove the suspended solids from the wastewater as well. Thus, this decreases the suspended solid as well.

A study by El-Shafai (2007) using a pilot-scale wastewater treatment system comprised a 40 L UASB reactor (6-h HRT) followed by three duckweed (*L. gibba*) ponds in series (total HRT 15 days) reported the treatment system achieved a removal value of 91% for TSS.

Furthermore, a three-year investigation by Dalu (2003) into the potential use of duckweed (*L. minor*) based wastewater stabilizations ponds for wastewater treatment was carried out at two small urban areas in Zimbabwe and the total dissolved solids have been constantly within an average level of 250 mg/l. When the influent and effluent levels were compared reductions of up to 90% were obtained from this investigation. In the same investigation, the turbidity achieved a reduction of up to 95% from an initial average of 125 NTU to final average of 7 NTU.

2.3.6 pH

The performance of the waste treating water bodies is affected by low or high pH levels and pH has effect on the living micro-organisms found in the water bodies. Duckweed plants can survive across a pH range from 6 to 9 and grows best in the 6.5 to 7.5 range. The change in pH were related to nitrate and ammonia uptake. Uptake of NH_4^+ will release H^+ ion to the surrounding wastewater, thus the pH declines. Meanwhile, uptake of NO_3^- will release OH^- ion, thus the pH will increase (Skillicorn, 1993).

A three-year investigation by (Dalu, 2003) into the potential use of duckweed (*L. minor*) based wastewater stabilizations ponds for wastewater treatment was carried out at two small urban areas in Zimbabwe reported that the pH levels have been within an average 7.5 which is the optimum operating pH for optimum pond performance.

2.3.7 Biomass growth

Duckweed systems distinguish themselves from other efficient wastewater treatment mechanisms in a way as they also produce a valuable, protein-rich biomass as a by-product other than treating the wastewater. Duckweed species have an inherent capability to exploit favorable ecological conditions by growing extremely rapidly and can double their mass in less than two days under ideal conditions of nutrient availability, sunlight and temperature (Skillicorn, 1993).

In a study by Mohedano (2012) the efficiency of two full-scale duckweed (*L. punctata*) ponds was evaluated considering nutrient recovery from a piggery farm effluent as well as the biomass yield. The two ponds together produced over 13 tons of biomass (68 t/ha year of dry biomass) after initially covering the water surface at a density of approximately 220 g/m^2 (fresh weight).

2.4 Physical treatment

In fish farm wastewater preliminary treatment is needed first as it focuses on the removal of large materials and the most common preliminary treatment is by running it through filters or coarse screening. Meanwhile, the objective of primary treatment is the removal of settleable organic and inorganic solids by sedimentation and floating materials are removed by skimming. Then, it is followed by secondary treatment which involves the removal of biodegradable dissolved and colloidal organic matter using biological processes such as submerged biofilters, trickling filters and activated sludge process which is employed for the oxidation of organic matter, nitrification or denitrification (Turcios, 2014).

A study by (Adhikari, 2015) reported that duckweed-based wetlands used for primary treatment had higher removal rates of COD and total nitrogen (TN) than those used for secondary treatment but no significant difference was observed for total phosphorus (TP) removal rates. COD removal rate in primary duckweed wetlands was significantly higher than COD removal in secondary wetlands with primary wetlands removing approximately 43% of the COD meanwhile secondary wetlands removed 12 %. Areal removal rates for nutrients in primary duckweed wetlands were 194.9 ± 18.9 g TN/m^2/yr and 13.0 ± 3.0 g TP/m^2/yr, while removal rates in secondary duckweed wetlands were 104.1 ± 13.1 g TN/m^2/yr and 9.3 ± 2.1 g TP/m^2/yr

CHAPTER 3

MATERIALS AND METHODS

3.1 Growth of *Spirodela polyrhiza*

The aseptic macrophytes are periodically subcultured and maintained in liquid Hoagland No. 2 medium with 30 g/L sucrose. The pH of all the culture media was adjusted to 5.8 using NaOH solution and autoclaved at 121 ^{o}C for 15 min. The healthy green fronds of *S. polyrhiza* were selected and after that each culture glass jar bottles containing 80 ml liquid medium was placed with similar amount of macrophytes. Finally all cultures were then incubated at 26±1 ^{o}C under the fluorescent tubes (1500 lux) with a 16 h light: 8h dark photoperiod for 14 days.

3.2 Physical treatment using microfiltration

A total of 4 L of wastewater was collected from a local catfish fish farm in Nibong Tebal, Penang and was tested for its nutrients (nitrate, phosphate and ammonia) concentration, turbidity, pH, COD level and MLVSS. The wastewater was collected using buckets attached to the end of wooden rods so that the wastewater can be collected from various parts of the pond. The collected wastewater was then poured in plastic containers and stored in chiller at 4^{o}C.

The physical treatment was done by using filter paper of a specific size. Firstly a suitable pore size of filter paper was to be determined by testing three different pore sizes of Fendi (20 µm), Sterlitech (3.0 µm) and Sartorius (0.2 µm). Thus 100 mL of the wastewater was filtered using each of the filter paper respectively. The turbidity, pH, COD level, MLVSS and nutrients of the wastewater through the filter paper was measured for each of the filters and the most optimum filter paper for this experiment was then chosen to filter out 2 L of wastewater which was the batch that undergone physical treatment. After that the water quality of wastewater was tested for its pH, turbidity, MLVSS and nutrients.

3.3 Phytoremediation of fish farm wastewater.

This experiment was carried out to study the uptake of nutrients of *S. polyrhiza* in fish farm wastewater and the effect towards its biomass growth as well as water quality after phytoremediation. A total amount of 2 L of physical treated wastewater and 2 L of untreated wastewater was prepared. *S. polyrhiza* macrophytes were cultivated in a small scale constructed wetlands with internal circulation using the treated and untreated wastewater. The healthy *S. polyrhiza* were first selected from the cultivation jars and they are weighed first to determine the amount which will fill up the surface of the small scale constructed wetlands with the initial weight of 6g. The macrophytes were then placed evenly on the surface of the wastewater with a volume of 1 L. The wastewater is continuously circulated internally in the small scale constructed wetlands by a water pump at 0.1 LPM to provide aeration to the wastewater.

The experiment was run for 14 days incubated at 26±1^{o}C under the fluorescent tubes (1500 lux) with a 16 h light: 8h dark photoperiod for 14 days and two replicate batches was done to obtain an average result. A control group was then set up using the wastewater that did not undergo physical treatment and will be placed with *S. polyrhiza* as well. The schematic diagram of the small scale constructed wetlands with flow direction of wastewater is presented in Figure. 3.1.

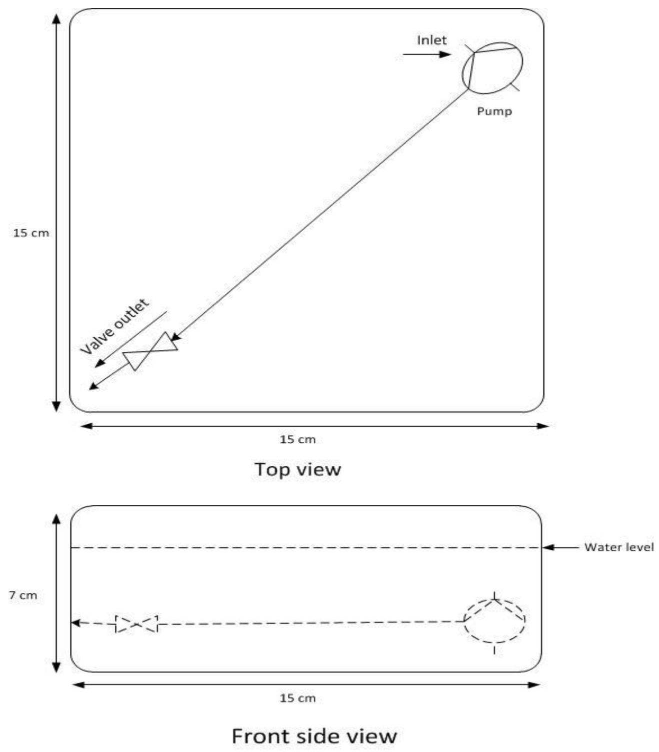

Figure 3.1 The schematic diagram of small scale constructed wetlands. Upper diagram is top view of the constructed wetlands while lower diagram is front side view of constructed wetlands. The arrows represent the flow direction of wastewater inside the small scale constructed wetlands for the cultivation of *S. polyrhiza.*

3.4 *S. polyrhiza's* nutrients uptake and water quality after phytoremediation

During the experimental period of 14 days, 35 ml of the fish farm wastewater sample was collected from each of the small scale constructed wetlands for each day 2 started from day 0 until day 14 in centrifuge tubes. The water level in the small scale constructed wetlands is ensured to be maintained at the initial marked level before collection by adding tap water if the water level dropped below the marked level due to evaporation. The wastewater sample will be tested for its nutrients (nitrate, phosphate and ammonia) concentration to determine respective nutrients uptake by *S. polyrhiza*. COD, turbidity, pH and MLVSS test were conducted on the sample to evaluate the water quality after phytoremediation.

3.5 Effect towards *S. polyrhiza's* growth

At the end of 14 day evaluation the harvested *S. polyrhiza* will be collected and weighed.

3.6 Analytical Analysis

3.6.1 Determination of nitrate and phosphate concentration for water samples

The nitrate was determined by Cadmium Reduction Method (HACH method 8039) using NitraVer®5 Nitrate Reagent Powder Pillows by HACH DR2800 spectrophotometer at 500 nm with allowable detection of high range (0.3–30.0 mg/l NO_3^--N). The phosphate was determined by Ascorbic Acid Method (HACH method 8048) using PhosVer®3 Phosphate Reagent Powder Pillows by HACH DR2800 spectrophotometer at 880 nm with detection range of (0.02–2.50 mg/l PO_4^{3-}). This phosphate determination was in accordance to USEPA method 365.2 and Standard Method 4500-P-E for wastewater. Each method will consume 10 ml of wastewater sample.

3.6.2. Determination of ammonia concentration and COD for water samples

The ammonia was determined by Salicylate Method (Lovibond method 66) using VARIO Am tube test Reagent, Set HR, F5 (VARIO Ammonia Salicylate, F5 and VARIO Ammonia Cyanurate, F5 powder packs and VARIO Am Diluent Reagent High Range reaction tube) by LOVIBOND Maxidirect MD600 photometer at 660 nm with detectable range of (0–50 mg/l NH_3-N). This method will consumes 0.1 ml wastewater sample. The COD was determined by Dichromate/H2SO4 Method (Lovibond method 131) using COD VARIO 0–1500 mg/l tube test Reagent by LOVIBOND Maxidirect MD600 photometer at 610 nm with detectable range of (0–1500 mg/l COD/CSB). This method consumes 2ml of wastewater sample. This method complies with Standard Methods for the Examination of Water and Wastewater.

3.6.3. Determination of turbidity of water samples

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

3.6.4. Determination of MLVSS

The centrifuge tube is shaken vigorously to ensure the water sample is well-mixed. 50 ml water samples were filtered through a 47 mm diameter weighed Whatman^{TM} glass microfiber filters with mini air pump and the retained residues were dried in an oven to a constant weight at 105°C for 1 h. The increase in weight of filter represents the total suspended solids. The filters with the residues were ignited in muffle furnace to constant weight at 550°C for 30 min. The remaining solids represent the fixed solids while weight lost on ignition was the volatile solids. This MLVSS test was carried out based on APHA 2540D and APHA 2540E.

3.6.5. Determination of biomass (fresh weight)

The fresh *S. polyrhiza* biomass is carefully dried by blotting them with a clean cloth before being weighed.

CHAPTER FOUR

RESULT AND DISCUSSION

4.1 Physical treatment

The initial water quality of the collected fish farm wastewater can be seen from Table 4.1. The nitrate and phosphate level was lower than the acceptable conditions of sewage discharge of standard A body (standard A is applicable to discharges into any inland waters within catchment areas which is the most stringent limit), 10.00 mg/l and 5.00 mg/l respectively for enclosed water body. Moreover, the pH value of 7.89 was in the range of discharge limit standard A as well of 6.00 to 9.00 but for ammonia the initial value of 36.30 mg/l was above the standard A, 5.00 mg/l ammonia-N. The COD level of 248.00 was above the standard A as well of 120.00 mg/l. Furthermore, the total suspended solids valued at 220.00 mg/l were above the Standard A discharge limit of 50 mg/l (Environment, 2010). Meanwhile, the initial turbidity of 203.00 NTU was above the class IIA (water supply II) of 50 NTU which passes for conventional treatment to produce clean drinking water (Salleh, 2018). From the initial analyses only nitrate, phosphate and pH passes the discharge limit standard A.

Table 4.1 Chemical analyses of initial and filtered fish farm wastewater by different pore size of filters

Parameter	**Initial**	**Fendi (20 µm)**	**Sterlitech (3.0 µm)**	**Sartorius (0.2 µm)**
Nitrate-Nitrogen (mg/l)	0.00	0.00	0.00	0.00
Phosphate (mg/l)	1.17	0.93	0.46	0.42
Ammonia-Nitrogen (mg/l)	36.30	49.00	40.20	38.50
COD (mg/l)	248.00	166.00	145.00	81.00
pH	7.89	7.93	7.97	7.94
Turbidity (NTU)	203.00	95.30	53.60	19.10
MLSS (mg/l)	220.00	140.00	80.00	40.00
MLVSS (mg/l)	188.00	132.00	62.00	22.00