PERFORMANCE OF *SPIRODELA POLYRHIZA, SALVINIA MOLESTA* AND *LEMNA* SP. IN PHYTOREMEDIATION OF FISH FARM WASTEWATER

by

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Thesis submitted in fulfilment of the

requirements for the degree of

Master of Science

April 2018

ACKNOWLEDGEMENT

First and foremost, I would like express my utmost gratitude to my supervisor, Dr. Derek Chan Juinn Chieh for his continuous support and unconditional help throughout my entire Master study. He is kind and helpful and has always been giving me guidance on my project, which allows me to learn and gain more insights into the study. He is also willing to spend his extra time for discussion, sharing his ideas and knowledge which has inspired me a lot. I would also want to thank him for giving me exposure to manuscript writing and conference presentation, which permits me to better understand the role as a researcher and fully experience the life as a postgraduate student. Not forgotten, the endless time that he spent in reviewing my papers and thesis.

I would like to express my sincere appreciation to Dean School of Chemical Engineering, Professor Dr. Azlina bt. Harun @ Kamaruddin and also to the Deputy Dean School of Chemical Engineering, Professor Dr. Ahmad Zuhairi Abdullah for their support towards my postgraduate affairs. I also appreciate the help given by the staffs and technicians in School of Chemical Engineering, that allowed my project to be carried out smoothly.

I am thankful to my friends and seniors, Bee Wah, Pey Yi, Lee Muei, Guat Wei, Choon Gek, Huey Ping, Jing Yao, Swee Pin, etc. for their accompany and advice during my study. They are willing to provide assistance and exchange ideas on the lab work and project when I needed to. Their support is priceless to me as well.

My acknowledgement also goes to a number of financial supports I received for this project including Fundamental Research Grant Scheme (FRGS) (Grant No. 203/PJKIMIA/6071271), Research University Grant (RUI) (Grant No.

1001/PJKIMIA/814209) and MyMaster scholarship from Ministry of Higher Education of Malaysia.

Lastly, deepest appreciation goes to my family members. Their love, support and sacrifice along the way are the prime factor and strength for me to strive for excellence.

-Ng Yin Sim, September 2017-

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LIST OF ABBREVIATIONS

BCA	Bicinchoninic Acid
BSA	Bovine Serum Albumin
COD	Chemical Oxygen Demand
CW	Constructed Wetland
DW	Dry Weight
N	Nitrogen
NTU	Nephelometric Turbidity Unit
P	Phosphorus
POME	Palm Oil Mill Effluent
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids

PRESTASI *SPIRODELA POLYRHIZA, SALVINIA MOLESTA* DAN *LEMNA* SP. DALAM FITOPEMULIHAN AIR SISA TERNAKAN IKAN

ABSTRAK

Air sisa ternakan ikan adalah tinggi dalam nutrien terlarut dan pepejal terampai hasil daripada pengumpulan makanan yang berlebihan dan perkumuhan ikan. Pemendapan mengurangkan pepejal terampai tetapi tidak efisien dalam menyingkirkan nutrient terlarut manakala rawatan termaju menghadapi kos yang tinggi dan permintaan tenaga yang besar. Penggunaan makrofit dalam fitopemulihan dapat menyelesaikan isu-isu tersebut disebabkan kos penyelenggaraan yang rendah dan penyingkiran nutrien yang ketara. Oleh itu, kajian ini bertujuan untuk menunjukkan keupayaan penyingkiran nutrient sebenar makrofit dalam keadaan aseptik serta menilai prestasi rawatan makrofit ke atas air sisa ternakan ikan. Dalam hal ini, makrofit *Spirodela polyrhiza*, *Salvinia molesta* dan *Lemna* sp. telah ditaksir dalam air sisa sintetik di bawah keadaan aseptik. Penyingkiran ammonia adalah pantas bagi *S. polyrhiza* dan *Lemna* sp., dengan kecekapan penyingkiran 60% dan 41% masing-masing dalam masa 2 hari. *S. polyrhiza* boleh mengurangkan 30% nitrat manakala *Lemna* sp. mencapai pengurangan fosfat tertinggi, sebanyak 86% pada hari ke-12. Profil yang diperolehi membolehkan pemilihan makrofit yang sesuai dalam rawatan air sisa ternakan ikan. *S. polyrhiza* dan *Lemna* sp. dipilih untuk merawat air sisa mentah ternakan ikan melalui rig kolam *raceway* (dalam sistem monokultur dan polikultur). Air sisa tersebut diperoleh dari ladang ikan keli tempatan, dengan tahap ammonia, fosfat, TSS dan COD sehingga 28.10 mg NH_3-N/L, 5.80 mg PO_4^{3-}/L, 175 mg/L and 322 mg/L masing-masing. Kolam *raceway* mempunyai dimensi 50cm x 25cm x 9 cm dan sistem boleh menakung 12 L air sisa. Sistem monokultur *S. polyrhiza* mengatasi sistem lain dalam penyingkiran nitrogen dan fosforus di mana 81% ammonia dikurangkan kepada 3.90 mg NH_3-N/L dalam 2 hari manakala aras nitrat, nitrit dan fosfat diturunkan secara ketara. Hal ini disebabkan keupayaan pengambilan yang tinggi terhadap pelbagai spesies nitrogen dan fosfat. Penurunan tajam paras TSS, kekeruhan dan COD (sehingga 75%, 88% dan 71% dalam 2 hari) dicatatkan. Semua sistem makrofit menunjukkan produktiviti biojisim yang tinggi (peningkatan sehingga 112%) dan keunggulan dalam kandungan protein (peningkatan sehingga 12%). Kesan *fed batch* dan penuaian secara berkala terhadap kapasiti dan prestasi rawatan sistem monokultur *S. polyrhiza* juga dinilai. Sistem dengan *fed batch* dan penuaian dapat merawat isi padu air sisa yang lebih banyak, menyingkirkan amaun bahan pencemar yang lebih tinggi sementara mencapai had efluen yang ditetapkan. Kajian ini mencadangkan sistem monokultur *S. polyrhiza* dengan *fed batch* dan penuaian yang optimum boleh dilaksanakan dan berkesan untuk merawat air sisa ternakan ikan dan menghasilkan biojisim yang berguna untuk pelbagai aplikasi seperti suplemen makanan ikan, diet unggas, baja dan biofuel.

PERFORMANCE OF *SPIRODELA POLYRHIZA, SALVINIA MOLESTA* AND *LEMNA* SP. IN PHYTOREMEDIATION OF FISH FARM WASTEWATER

ABSTRACT

Fish farm wastewater is high in dissolved nutrients and suspended solids due to accumulation of uneaten feed and fish excretions. Sedimentation reduces suspended solids but not efficient in removing dissolved nutrients while advanced treatment suffers from high cost and huge energy demand. Phytoremediation using macrophytes could solve these issues owing to low maintenance cost and significant nutrients removal. Therefore, this study intended to show the true nutrients removal capabilities of macrophytes under axenic condition and to evaluate the treatment performance of macrophytes on fish farm wastewater. In this regard, *Spirodela polyrhiza*, *Salvinia molesta* and *Lemna* sp. macrophytes were assessed axenically in synthetic wastewater. The ammonia removal was rapid for *S. polyrhiza* and *Lemna* sp., with 60% and 41% removal efficiency respectively within 2 days. *S. polyrhiza* could reduce 30% of the nitrate while *Lemna* sp. achieved the highest phosphate reduction, of 86% at day 12. The acquired profiles allow selection of suitable macrophytes in fish farm wastewater treatment. *S. polyrhiza* and *Lemna* sp. were chosen to treat raw fish farm wastewater via raceway pond rig (in monoculture and polyculture system). The wastewater was sourced from a local catfish farm, of ammonia, phosphate, TSS and COD levels up to 28.10 mg NH_3-N/L, 5.80 mg PO_4^{3-}/L, 175 mg/L and 322 mg/L respectively. The raceway pond had dimensions of 50cm x 25cm x 9 cm and the system could hold 12 L wastewater. *S. polyrhiza* monoculture system surpassed other systems in nitrogen and phosphorus removal where 81% ammonia was reduced to 3.90 mg NH_3-N/L in 2 days whilst the nitrate, nitrite and phosphate levels were significantly lowered. It was attributed to its high uptake capabilities of various nitrogen species and phosphate. Steep decline of TSS, turbidity and COD levels (up to 75%, 88% and 71% in 2 days) were recorded. All macrophyte systems demonstrated high biomass productivity (up to 112% increment) and superiority in protein content (up to 12% increment). The effect of fed batch and periodic harvesting on treatment capacity and performance of *S. polyrhiza* monoculture system were also evaluated. The system with fed batch and harvesting could treat more volume of wastewater, remove higher amount of pollutants while meeting effluent limits. This study suggested that *S. polyrhiza* monoculture system with fed batch and optimal harvesting is feasible and effective in treating fish farm wastewater and produces useful biomass for various applications such as fish feed supplement, poultry diet, fertiliser and biofuel.

CHAPTER ONE

INTRODUCTION

1.1 Macrophytes and Phytoremediation

Macrophytes refer to conspicuous aquatic plants. They prevail in the wetland, shallow lakes, and streams. They grow in or near water and are emergent, submerging or floating. They are important in ecosystem health by serving as primary producers of oxygen via photosynthesis, sheltering the fishes and numerous invertebrates, helping recycling of nutrients to and from sediments as well as assisting in stabilizing river and stream banks. They also act as food and are suitable nesting sites for the wildlife (Hebert, 2007). Certain macrophytes species has inherently high growth rate accompanied with enormous level of nutrients uptake rate, as the case in duckweed which could double their biomass in less than 2 days under optimal conditions (Leng et al., 1995) and remove most of the nutrients eg. ammonia, nitrate and phosphate from the water body (Hasan and Chakrabarti, 2009). Some of them may possess hyperaccumulating ability where they were capable of absorbing metals or trace metals rapidly and concentrating them in an extremely high levels in their tissues (Hossner et al., 1998, Rascio and Navari-Izzo, 2011) while some others could treat organic pollutants (Hughes et al., 1996, McCutcheon et al., 2003) as they contain high levels of organic-degrading enzymes. One or more from these attributes make phytoremediation possible (Salt et al., 1998, Pulford and Watson, 2003, Pilon-Smits, 2005). Phytoremediation is basically the use of plants to remove pollutants from the environment or to render them harmless (Salt et al., 1998). It utilizes ranges of plant biological processes and physical characteristics (Pivetz, 2001) to either partially or substantially remediate selected pollutants in the contaminated media like soils, water or air by containing, degrading or eliminating them from contaminated media. It could be applied to the waters or soils that have become polluted with inorganic and organic contaminants due to human activities. Examples of these contaminants include N and P that causing nutrient pollution in waters, and also metals, metalloids or non-metals (Cr, Cu, Fe, Mn, Mo, Zn, Cd, Co, F, Hg, As, Se, Pb, V, and W) that accumulating in elevated levels in soils and waters as well as radioactive isotopes (^{238}U, ^{137}Cs, and ^{90}Sr), man-made organic solvents, herbicides, explosives and petroleum hydrocarbons which polluting the aforementioned media (Horne, 2000, Lytle et al., 1998, Negri and Hinchman, 2000, Newman et al., 1997, Burken and Schnoor, 1997, Hughes et al., 1996, Pilon-Smits, 2005, Tu et al., 2002). As for the macrophytes, they have been employed to upgrade effluent quality from stabilization ponds (Pescod, 1992), mitigate eutrophication (Tyler et al., 2012) and are able to treat various types of wastewater. The examples include agricultural runoff or drainage water, industrial wastewater, sewage and municipal wastewater, mine drainage, landfill leachate and groundwater plumes (Reddy et al., 1982, Mitsch and Wise, 1998, Hadad et al., 2006, Nivala et al., 2007, Amon et al., 2007, Tyler et al., 2012, Shah et al., 2014).

1.2 Background of Research

According to The State of World Fisheries and Aquaculture 2016, world total fish production had reached 167.2 million tonnes in 2014 while aquaculture production alone accounted for about 44% of the total fish production. Provided that aquaculture comprised only 7 percent of fish for human consumption in 1974, this share had risen up to 26 percent in 1994 and 39 percent in 2004 (FAO, 2016b). It is not surprisingly that the figure will soon overtake the wild-caught fish production after 2014. Therefore, aquaculture would play a major role in world fish production now and future to ensure food security and nutrition to ever-growing human population. Malaysia was listed as one of the top 25 major aquaculture producers in the world with total production of 521.0 thousand tonnes in 2014, ranked 15th among the countries (FAO, 2016b). Its inland aquaculture covered an area of about 794.2 thousand hectares (Department of Fisheries, 2014). However, improper management of the aquaculture site in terms of effluent discharge would bring harm to the nearby water resources and environment.

In an enclosed, intensive inland aquaculture, the water used to culture the fish are generally easier to be concentrated with suspended solids and dissolved nutrients due to accumulation of by-products eg. uneaten feed, fish faeces and excretions (Pfeffer, 1990). In order to maintain the health and welfare of the fishes, water exchange need to be done regularly (Johansen et al., 2006). However, this effluent is normally either directly discharged into the nearby waterways or into sedimentation pond before released. Sedimentation may help reduce suspended solids, but not to remove dissolved nutrient, so eventually fish farm wastewater still poses risk of harming the receiving water. This phenomenon is attributed to rural farmers who are characterised as low capital cultivator, making advanced treatment system is too expensive for them to be installed and operated; whereas no clear provision made with regard to local aquaculture effluents (FAO, 2016a) also cause no further treatment of the effluents since the issue is not prioritised. Therefore, an affordable, efficient yet easy to implement treatment system for the fish farm wastewater is needed to ensure success of the system. The system will give the farmer a shot in the arm if it can generate valuable products or side income.

Phytoremediation is identified to be a treatment system which fulfils those criteria. It is is relatively low cost to maintain since it is solar-driven (LeDuc and Terry, 2005) and only a simple containment system is needed. It is cheaper than conventional treatment methods that rely on electricity, pumping, aeration or chemicals additions and usually need large concrete or steel vessels (Terry and Banuelos, 1999). Advanced treatment technologies for nutrient removal are costly, having high energy requirement and carbon footprint (Moore et al., 2009) whereas phytoremediation is cheap and sustainable. Moreover, it is the least harmful method as it uses naturally occurring organisms and preserves the environment in a more natural way, and it is aesthetically pleasing as well (Pradhan et al., 1998). The wastewater treatment technology for land based aquaculture is largely adapted from conventional/municipal wastewater treatment (Siddiqui, 2003). Thus, it has the drawbacks of sludge production, high energy demand and frequent maintenance requirement (Lin et al., 2002a). Furthermore, some of the adsorbents or coagulants added for water quality improvement may not be adaptable for treatment due to elevated costs, toxic residues, low treatment capacities, and high selectivity for variety of pollutants, which include alum, polyaluminium chloride, activated carbon, clay minerals, polymer hydrogel, and zirconia (Palacios and Timmons, 2001, Kioussis et al., 2000, Huang et al., 2000). Conventional biological processes are also designed to meet secondary treatment effluent standards and typically do not remove nitrogen and phosphorus to the extent of exceptionally low levels in protecting receiving water (Hranova, 2006, USEPA, 2017b, Headworks, 2017). Therefore, additional or enhanced treatment units are needed for further depurating the nutrient-rich wastewater (USEPA, 2017b). In spite of that, the macrophyte systems have shown to be efficient in removing significant amounts of pollutants eg. phosphate, ammonia, nitrate, nitrite, TP, TN, TSS and COD from variety of wastewater (Ozengin and Elmaci, 2007, Xu and Shen, 2011, Mohedano et al., 2012, Olguin et al., 2003, Lin et al., 2005, Effendi et al., 2015). The monoculture and polyculture types of macrophyte systems were also demonstrated to treat the wastewater in the study of Bashyal (2010). Periodic harvesting could be used to maintain optimal growth of the macrophytes colony (Hasan and Rina, 2009) as it avoids crowding of macrophytes (Skillicorn et al., 1993), which may indirectly assist in efficient removal of pollutants from wastewater. When the phytoremediation system is coupled with sedimentation pond, it will aid in removing the dissolved nutrients in the effluent as well as the suspended solids.

The macrophytes have their own potential uses. Traditionally, *Wolffia arrhiza* has been eaten in Myanmar, Laos, and northern Thailand (Bhanthumnavin and Mcgarry, 1971). King et al. (2004) showed that inclusion of *Salvinia molesta* in commercial fish feed diet will have higher fish weight on Nile tilapia (*Oreochromis niloticus*) compared to feeding with commercial feed alone and a significant effect is observed if feeding period is prolonged. Furthermore, biomass of *S. molesta* has the potential to be converted into organic fertilizer via vermiremediation (Hussain et al., 2016). Similarly, *Spirodela polyrhiza* can be promising substrate for biohydrogen production (Xu and Deshusses, 2015) and can also be included in fish meals (Cruz-Velásquez et al., 2014). Hence, the aquaculture farmers can earn extra income out from the valuable plant stock harvested besides being applied to remediate the fish farm wastewater.

1.3 Problem Statement

However, the available studies on the nutrient removal performance by macrophytes were carried out outdoor and their data do not show the true uptake or removal by the aquatic plant itself. It is because those measured data or levels in nitrogen and phosphorus species (ammonia, nitrate and phosphate) were resulted from the assimilation by macrophytes and algae, nitrification, denitrification and other available processes. The precise evaluation of removal performance by macrophytes and comparison between them, thereby cannot be done accurately. In addition, the comprehensive performance data of macrophytes in phytoremediation of fish farm wastewater with complete set of water quality parameters, presentation of data in profile and kinetics and followed by detailed analysis and inference are limited. Most of the studies were reported in efficiency on pollutant removal (mere application or performance), but lacking strong evidence to show the fate and removal of the studied pollutant by the macrophytes. The systems examined by other co-workers can be too complex in which they may include sand and gravel or extra other units in the study, as in the treatment wetland, even making macrophytes contribution in removal more hardly to be traced. They are also mostly absent in addressing the discharge effluent to the standard limit and restricted to certain macrophytes species. The experiment with raw wastewater is also limited as most studies generally used pretreated wastewater. Besides that, only few studies are conducted on monoculture and polyculture of the macrophyte systems in treating the wastewater. Although the routine harvesting is known to have allowed optimal growth of the macrophytes in the system, the subsequent effect on the treatment performance of the wastewater is not found.

1.4 Research Objectives

In view of the context mentioned previously, the objectives of this research study are as follows:

1. To assess nutrients removal performance of *Spirodela polyrhiza*, *Salvinia molesta* and *Lemna* sp. in terms of ammonia (NH_3-N), nitrate (NO_3^--N) and phosphate (PO_4^{3-}) under axenic condition in synthetic wastewater.

2. To evaluate the performance of the selected macrophyte systems in phytoremediation of fish farm wastewater with regard to ammonia (NH_3-N), nitrate (NO_3^--N), nitrite (NO_2^--N), phosphate (PO_4^{3-}), TSS, turbidity and COD removal as well as the corresponding changes in biomass, total carbohydrate and protein contents of the systems.
3. To determine the effect of fed batch and periodic harvesting on the treatment capacity and performance of the selected macrophyte system (ammonia (NH_3-N), nitrate (NO_3^--N), nitrite (NO_2^--N), phosphate (PO_4^{3-}), TSS, turbidity and COD removal) and the corresponding changes in biomass, total carbohydrate and protein contents.)

1.5 Scope of Research Study

The study was divided into three major parts/sections. The first section of the study was conducted to assess the true nutrients removal performance of the commonly used macrophytes of *Spirodela polyrhiza*, *Salvinia molesta* and *Lemna* sp.. They were done in the synthetic wastewater under axenic and controlled condition to eliminate the interference due to microorganisms on nitrogen and phosphorus. Among water quality parameters being evaluated during phytoremediation included ammonia (NH_3-N), nitrate (NO_3^--N), phosphate (PO_4^{3-}), chemical oxygen demand (COD), total carbon (TC) and pH. The biomass increment in fresh weight of the macrophytes was also determined at the end of the study.

In second section of the study, the best two macrophytes in nutrient removal in first section, namely *Spirodela polyrhiza* and *Lemna* sp. were utilised as substrate of the real case remediation study for the raw, untreated fish farm wastewater. Monoculture and polyculture systems of the macrophytes were set up in a raceway pond rig to evaluate their performance in phytoremediation of fish farm wastewater. The water quality assay included ammonia (NH_3-N), nitrate (NO_3^--N), nitrite (NO_2^--N), phosphate (PO_4^{3-}), chemical oxygen demand (COD), turbidity, total suspended solids (TSS) and pH. The changes in biomass (fresh weight) and biochemical content (total carbohydrate and protein) of the macrophytes were determined to find out the extend of phytoremediation towards biomass, carbohydrate and protein accumulation.

In last section of the study, fed batch and periodic harvesting were carried out on *Spirodela polyrhiza* monoculture system, which was the best macrophyte system in fish farm wastewater treatment in second section of the study. Its effect on treatment capacity and performance of the system were determined. Similar water quality, growth and biochemical tests as in second section of the study were performed.