FED BATCH PHYTOREMEDIATION BY *SALVINIA MOLESTA* ON FISH FARM WASTE WATER

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2017

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by

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Thesis submitted in partial fulfilment of the requirement

for the degree of Bachelor of Chemical Engineering

May 2017

ACKNOWLEDGEMENT

First and foremost, I would like to convey my sincere gratitude to my supervisor, Dr. 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.

TABLE OF CONTENT

Page

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

S. molesta	**Salvinia molesta**
BOD	**Biological oxygen demand**
COD	**Chemical oxygen demand**
FB	**Fed batch**
MLVSS	**Mixed liquor volatile suspended solids**
TSS	**Total suspended solids**
VSS	**Volatile suspended solids**
TN	**Total nitrogen**
TA	**Total ammonia**
VFA	**Volatile fatty acid**
C-PC	**C-phycocyanin**
POME	**Palm oil mill effluent**
AOB	**Ammonia oxidizing bacteria**
NOB	**Nitrate oxidizing bacteria**
NUR	**Nutrient uptake rate**
ADP	**Adenosine triphosphate**
ATP	**Adenosine diphosphate**

FITOPEMULIHAN BERASASKAN PENAMBAHAN SECARA KUMPULAN MENGGUNAKAN KIAMBANG *SALVINIA MOLESTA* PADA AIR SISA LADANG IKAN.

ABSTRAK

Fitopemulihan memerihalkan pengolahan masalah persekitaran (biopemulihan) menerusi penggunaan tumbuh-tumbuhan untuk mengurangkan masalah tersebut tanpa menggali bahan kontaminasi dan melupuskannya di tempat yang lain. Dalam kertas ini, prestasi atas penambahan secara kumpulan (FB) dalam proses Fitopemulihan oleh kiambang *S. molesta* pada air sisa ladang ikan disiasat dengan mengubah kadar pertambahan sisa segar. Kajian ini bertujuan untuk menyiasat pengeluaran biojisim oleh *S. molesta* melalui berbeza penanaman peratusan penambahan bersama-sama dengan penyingkiran nutrien bertujuan untuk rawatan air sisa. Kualiti air selepas Fitopemulihan telah di uji sepanjang kajian 16 hari. Kecekapan penyingkiran nutrien adalah tinggi dalam peratusan yang lebih tinggi FB tetapi semua media mencapai kadar pelepasan air sisa. Ammonia dan fosfat menunjukkan penyingkiran sangat tinggi (<95%) 75% media FB dengan jumlah penyingkiran 66.55 mg/L dan 7.80 mg / L masing-masing. FB menunjukkan penurunan nilai nitrat dan 75% FB mempunyai tahap terendah 2.40 mg / L. Walau bagaimanapun jumlah nilai nitrogen dalam medium FB lebih tinggi menunjukkan nilai tertinggi 13.85 mg / L pada hari 16. Kekeruhan nilai susutan 75% adalah untuk menjadi tertinggi di mana berkurangan dari hari 8; 258.50 NTU untuk hari 10; 55.00 NTU dalam 2 hari. Penyingkiran COD dengan FB sederhana adalah tinggi di mana 100.50 mg / L dicapai. Kajian ini menunjukkan fitopemulihan *S. molesta* dalam kolam ikan beerpotensi tinggi dalam penyingkiran nutrien tetapi memberi kesan negatif kepada jisim, di mana jisim akhir tanpa FB adalah lebih tinggi daripada FB pada semua peratusan.

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ABSTRACT

Phytoremediation is a bioremediation process that uses various types of plants to remove, transfer, stabilize, and destroy contaminants in the soil and groundwater. In this study, the performance of fed batch(FB) phytoremediation by *S. molesta*, macrophytes on fish farm wastewater is investigated by varying percentage of fresh wastewater loading. This study was aim to investigate the biomass production of *S. molesta* through different FB percentage cultivation along with nutrient removal aiming to wastewater treatment. The water quality after phytoremediation were monitor throughout study of 16 days. The nutrient removal efficiency was high in higher percentage of FB but all the medium reach standard discharge limit. Ammonia and phosphate removal show very high (<95%) for 75% FB medium with total removal of 66.55 mg/L and 7.80 mg/L respectively. The removal of ammonia and phosphate by control set was 19.60 mg/L and 2.32 mg/L. Although the concentration of nitrate continues to increase throughout the study, FB cultivation minimized the increment where all percentage experience decrease in nitrate value and 75% FB has lowest level of 2.40 mg/L compare to control where 5.00 mg/L. However total nitrogen value in higher FB medium indicate highest value of 13.85 mg/L on day 16. MLVSS for FB medium is far below control. FB phytoremediation of *S. molesta* in fish pond water show high efficiency in nutrient removal but negative effect on its biomass, where the final biomass of non-FB is higher than the FB at all percentage. This study proves that FB phytoremediation of *S. molesta* have potential on nutrient removal from fish farm wastewater.

CHAPTER ONE

INTRODUCTION

1.1 *S. molesta* species.

S. molesta is an aquatic fern (macrophytes) consist of spreading dense root system over water and its required less attention as it grows naturally. *S. molesta* is classified in *S. molestaceae* group which belongs to eastern Brazil. Kariba weed is another name for *S. molesta*. Temperature of 20 -30 ° C and pH range in between 4 and 9 is most favourable growth condition for *S. molesta* (Chandanshive et al. 2016) and also have potential in doubling it biomass about 16 h to s days (Ng and Chan 2017). They can grow in or near water and can be emergent, submerge or floating. Macrophytes contribute important role in healthy ecosystem which one of the source of oxygen via photosynthesis. It also serves as substrate for algae and shelter for fish and invertebrates. In recent study (Ng and Chan 2017), show that macrophytes play vital role in recycling nutrient along sediments and help in stabilizing stream and river. Some study shows that macrophytes have potential in dye removal (Chandanshive et al. 2016), detoxification of Cr(VI) (Prado et al. 2012), adaption to high NH_4^+ (Jampeetong et al. 2012) and also nutrient removal in treated POME (Ng and Chan 2017). The success of these adaption is because of the ability of *S. molesta* to growth in high level of nitrogen, phosphorous, potassium and concentrate the mineral and accumulates protein concentration in plant (Ng and Chan 2017). *S. molesta* help in stabilizing of sediments and serve as filter to prevent suspended solids along with prevention against corrosion by reducing turbulence and flow velocities of water bodies (Chandanshive et al. 2016, Ng and Chan 2017).

1.2 Problem statement.

Phytoremediation using microalgae is popular among the researcher regarding wastewater treatment. Although phytoremediation can achieve high levels of treatment, a major cost in operation of removal of microalgae wastewater treatment. This is because BOD of algae biomass in the effluent of treatment pond will be above standard discharged amount. Therefore, macrophytes is substitute in treatment pond since macrophytes (eg: *S. molesta*) biomass production is high with short period of time (Ng and Chan 2017). Some culture density is relatively lower which is 2-6 g m^{-3} and most algae have higher density which cause difficulty in harvesting and also costly. Therefore, use of floating fern like macrophytes is required as alternative choice to algae use because it is easy to harvest and no external cost is required. Floating fern can be remove through filtration process and study in feeding strategy is required to overcome the cost of treatment. Fed-batch feeding mode is said to be most economically friendly treatment (Ji et al. 2015, Markou 2015, Matsudo et al. 2009, Rodrigues et al. 2010).

1.3 Research objectives.

The research aim:

(i) To study the effect of fed batch cultivation on *S. molesta* biomass.

(ii) To study the performance of nutrient removal from fish farm wastewater.

1.4 Scope of study.

In this work, *S. molesta* from School of Chemical Engineering USM sample been used to investigate the nutrient removal of fish pond wastewater collected from Tanjung Piandang. Hoagland solution was used to cultivate *S. molesta* in laminar flow chamber. After 21 days of cultivation, *S. molesta* was harvest and introduce to wastewater with a constant mass distribution of 4.2g/L. Water sample of 12 mL is collected on every 2 days. At each 4 days' nutrient feeding to be happened through fed-batch(FB) mode where different percentage were FB (25%, 50% and 75%). The plant was not added or remove during the experiment. The collected sample were stored in cold room to prevent degradation and later tested for turbidity, suspended solid (TSS/MLVSS), total nitrogen (TN), nitrate, phosphate, pH and COD. At the end of experiment (day 16) the plant was harvest and weighted. The final weight is used to evaluate its biomass. All the test result is used to evaluate the performance of nutrient removal.

1.5 Organization of thesis.

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

Chapter 1 introduces *S. molesta* macrophyte, problem statement, research objectives and organization of thesis.

Chapter 2 discusses the literature review of this study which includes the effect of FB and studies conducted on *S. molesta* along with its responds.

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. Further elaboration on the effect of several factors on nutrient removal, the results on nutrient removal are provided in this chapter.

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

CHAPTER TWO

LITERATURE REVIEW

This chapter discuss on exiting literature about the aspect and issue that going to be discuss in chapter three. Based on given tittle fed batch phytoremediation is one of remediation method that became popular among the researcher especial in wastewater treatment field where this method serves to be more economical and environmental sustainable.

2.1 Phytoremediation.

Since last decade phytoremediation became an emerging remediation technology due to its low cost high sustainability and very convenient technology for environmental improvement (Willscher et al. 2017). It been proving that post-treatment of phytoremediation show reduction in value of nitrate, pH, phosphate and phenolic compound. The pH slightly became more alkaline during the treatment. Nitrate show higher removal with a drastic reduction while phosphate reduction is said to be less than 10 % during phytoremediation. This technology is also noted to have improvement in colour and odour of wastewater over the treatment period (Ojoawo et al. 2015). Researcher investigate the phytoremediation impact not only for nutrient removal in wastewater but also on pH control and also heavy metal adsorption. This is because pH and heavy metal is said to crucial factor for the plant growth and the success of phytoremediation. The heavy material accumulation is to be found at different part of plants. The highest uptake in roots was measured for Fe (2.16 g/kg), Mn (1.68 g/kg) and Zn (0.85 g/kg). This show that heavy metal reduction in phytoremediation show positive result (Willscher et al. 2017).

2.2 Fed-batch cultivation.

Fed-batch (FB) is a strategy which been used in past decades by researcher for cultivation. There are a few studies been carried on bacteria, microalgae and macrophytes using this technique. These cultivation technique was not being carried out on *S. molesta* species yet. For past decades, wastewater been treated using bacteria and algae. Feeding strategy of fed-batch is almost being investigated on bacteria in order to remove nutrient and also enhance biodiesel production (Ji et al. 2015). Fed-batch cultivation strategy improves the removal of nutrient (eg; nitrogen and phosphorus) from wastewater. In these paper *S. molesta* was used for nutrient removal from fish pond wastewater using fed-batch mode as feeding strategy. Effect of FB on macroalgae has gain attention among the researcher in past decades. But *S. molesta* cultivation for FB mode in investigation the efficiency of nutrient removal is the less interest among researchers.

2.2.1 Effect on total biomass

Study demonstrate that FB cultivation can improve the growth rate and biomass (Craggs et al. 1997, García-Cañedo et al. 2016, Markou 2015, Patwardhan and Srivastava 2008, Wang et al. 2015). Researcher use microalga, *Scenedesmus incrassatulus* to investigate nitrogen limitation in carotenoid through batch and FB culture mode (García-Cañedo et al. 2016). Dry weight and cell concentration is the parameter use to evaluate the culture mode. Culture maximum biomass concentration as maximum dry weight and cell concentration result from fed-batch using higher nitrogen concentration which these values were 3 times higher than obtain in batch mode. It is also observed that maximum amount of biomass concentration was achieved by feeding the nutrient which show that FB culture mode favours cell division and dry weight accumulation. This study also proves the hypothesis of FB cultivation strategy which uses less or none CO_2 and yield higher maximum biomass accumulation. Higher biomass as dry weight and cell concentration more favour to FB culture, especially dry weight biomass in fed-batch culture is about 3 times more than in batch. These could mean that best strategy for biomass accumulation could be implementing FB cultivation with low CO_2 supplementation and high nitrogen concentration (García-Cañedo et al. 2016). Different feeding mode been studied (Patwardhan and Srivastava 2008) on nutrient where FB strategies was use for constant feeding of nitrogen and fructose. This strategy was compared with batch mode and resulting constant feeding of nitrogen and fructose gave a better polyhydroxybutyrate (PHB) production rate over batch cultivation. The study was carried out in fermenter which run as batch process first then feeding of nitrogen and fructose was started as per various nutrient feeding strategies. Based on this paper (Patwardhan and Srivastava 2008), FB strategy gave higher total biomass production when condition pH is controlled and fed-batch mode is applied for both nutrients (nitrogen and fructose). This feeding strategy ensured low nitrogen availability and low non-limiting fructose concentration in the broth, which was favourable for PHB production thus yield a higher value of total biomass production rate. In this paper (Markou 2015), researcher evaluate the influences of feeding strategy of nitrogen source (KNO_3 and NH_4Cl) which through FB mode and batch of either nitrogen source over cell concentration, cell productivity and nitrogen-to-cell conversion factor. Nitrogen source has its own benefit which KNO_3 was necessary to attain higher cell mass concentration while NH_4Cl will ensure 41% reduction in used of amount KNO_3 in the cultivation. In addition, ammonia also serve as identification mark for inhibitory or toxic levels of nutrient in medium. the maximum cell concentration obtained in presence of both nitrogen source via FB run was 55.8% higher than standard run. Effect of nitrogen source on biomass was investigate through few different components which chlorophyll, lipid and protein content. FB mode has higher KNO_3 amount and higher the concentration of chlorophyll. Investigation also proves that run with an average amount of KNO_3 show highest amount of chlorophyll because of influence by other factors such as cell concentration and light intensity. As the FB level increased, light absorption of the medium due to turbidity and dissolved coloured compounds of the wastewater also increased. Thus, photosynthesis rate increase along with light absorption which result in higher biomass. This study show that the biomass had a significant effect on feeding strategy which favour to fed-batch mode (Markou 2015, Matsudo et al. 2009). Besides, microalgae also have positive effect towards biomass using FB cultivation. It's found that marine microalgae *Nannochloropsis oculata* show that in FB culture was 1.25 times higher than in batch culture. Thus FB culture give positive effect on microalgae biomass and also overcome the inhibitory effect of CO_2 concentration on microalgae growth (Wang et al. 2015). In summary, FB method has positive effect towards macrophytes biomass content compare to standard phytoremediation. This is because FB mode increases the amount of nutrient in medium as time go on. Therefore, nutrient limitation that cause inhibition for production of biomass is not an issue. The biomass is continuing to build up when the environment is favour to the macrophytes.

2.2.1.1 Effect of pH on the growth and biomass production.

Every one of the species indicate highest biomass generation between pH 6.5 and 7.5. Macrophyte demonstrate ideal development at pH 7.5 through *Arthrospira sp.* show highest biomass at pH 7.0. It shows that no much variety changing pH effect for cyanobacteria in biomass production. The pH of the medium assumes an essential part in refined as it decides the solubility of CO_2 and minerals in the medium which thus specifically or by implication impact the digestion of a species varieties. A few works have been accounted for about pH resistance of cyanobacteria. The life forms have been accounted for to develop well in the pH extend 6.5-10 (Lavery et al. 1991).

2.2.2 Nutrient uptake.

In recent years, researcher take a closer view on nutrient removal alternative. Nutrient removal is dependent to nitrogen and other component. Nutrient uptake rates (NUR) increases as amount of nitrogen in cultivation increases. Nitrogen consumption is nearly 100% which these indicate that at low or depleted nitrogen concentration, carotenoid accumulation is considerable. Study prove that phytoplankton can use internal nitrate reserve to synthesize macromolecular even depletion of nitrate in the medium happened. Therefore, stored nitrogen can be utilized for growth or carotenoid accumulation even after depletion. Therefore, nutrient uptake in fed-batch mode is higher compare to its batch mode. But this hypothesis is based on microalgae *Scenedesmus incrassatulus* (García-Cañedo et al. 2016). Researcher also use cynabacterium and microalga to prove hypothesis of increasing in total ammonia concentration through fed-batch mode causes decreases in biomass composition (Markou 2015). This is because due to nutrient limitation causes accumulation of carbohydrates which tend to decreases nitrogenous compound and lowering biomass production. Nutrient limitation also leads to accumulation of lipids which leaves no effect on biomass production either in cyanobacteria or microalgae. But accumulation of carbohydrates and lipids is very usable in microalgae biomass for the production of biodiesel which now become a vision for these centuries researcher (Markou 2015). However, the quantity of ammonia loss is not only determined by pH of the culture and ammonia concentration but also by the agitation type used which this prove by writing in (Markou

2015) that aeration of cultures result in higher ammonia losses than stirring. They also show that intracellular phosphate gradually increases as the FB of total ammonia (TA) addition level increases. Literature state that nutrient uptake is depend on nitrogen level where nutrient uptake rate increase as the amount of nitrogen in the medium increases because excess stored nitrogen been utilized for growth even after depletion in medium.

2.2.3 Ammonia toxicity.

Since KNO_3 and NH_4Cl is used as nitrogen source (Rodrigues et al. 2010). The main role of NH_4Cl is to reduce the usage of KNO_3 which contribute for biomass yield. Besides being a cheaper source compare to conventional nitrate, it also consumes by *Arthrospira sp.* and end up toxic at high levels. Ammonia toxicity can be eliminated through feeding strategy; fed-batch mode which increases the feeding rate exponentially. Based on these paper (Markou 2015), fed-batch cultivation modes in ammonia-rich waste water effect on biomass were investigated using cyanobacterium, *Athrospira* platensis and the microalga Chlorella vulgaris were used. They prove that cyanobacterium show no lag phase while microalga displayed lag phase up to day 4 for all ammonia addition. For cyanobacterium, the biomass production increases as the ammonia addition increases until it reaches the optimum. In this study, they have used four different ammonia concentration. At low ammonia concentration, yellowish macroscopic is visible which show bioflocculation of cell due to insufficient of nutrient. However, at high ammonia concentration, there is no significant changes were happening to its biomass. These paper (Markou 2015) state that 20 mg-N/(L d) is said to be optimum ammonia concentration for production of biomass in cyanobacterium and for microalgae show a same growth pattern and almost same final biomass density for all ammonia concentration. Therefore, this show that microalga (C. vulgaris) is mixotrophic. These mean the presence of organic compound induced the biomass synthesis by providing energy from volatile fatty acid (VFA) instead of photosynthesis which promote to mixotrophic growth. Ammonia toxicity promote limitation for growth and also decrease the efficiency for nutrient removal. Thus FB mode can eliminate ammonia toxicity in which the feeding rate increases exponentially.

2.2.4 Removal of wastewater constituents.

Phosphate removal process by macroalgae research been carried out by many researchers in these centuries. Based on (Srivastava and Srivastava 2008), FB mode is the suitable feeding strategy for better phosphate removal. This method is proven by using kinetics/inhibition data, and a mathematical model been proposed during this research. Based on the paper, FB strategy achieve better, quicker and simpler phosphate removal compare to batch mode. Based on (Markou 2015), researcher measure the removal at the end of each cultivation. Both cyanobacteria and microalga has same capability which almost 99% removal of NH_4^+-N in all fed-batch mode. In this study nitrogen(N) up take were measured based on Protein content. Both species show a trend of decreasing in protein content as ammonia concentration adding via fed-batch mode increases. This also may influence by ammonia losses to the atmospheric which not only determine by pH of the culture and ammonia concentration but also agitation type used. Both show PO_4-P removal of 99% and total phosphate removal of 96% based on its dry biomass. The trend also showed that the intracellular phosphate increases as the fed-batch addition of ammonia increased. These shows that some microalgae can grow on organic composition liquor such as monosaccharides, organic acids, amino acids and glycerol. FB cultivation been tested on *Desmodesmus sp.* by (Ji et al. 2015). Nutrient removal tested on the removal of TN, NH4-N and PO4-P where FB mode removal is higher compare to batch mode. It also states that biomass production via this mode is 3 times higher than batch mode because of higher lipids production were recorded in FB mode. In addition, researcher also state that FB provide an effective control of nutrient limitation and ammonia inhibition on microalgae cultivation as well as growth. FB cultivation gives effective in nutrient removal in cyanobacterium, microalgae as well as macroalgae where this method give positive effect on removal of TN, NH4-N and PO4-P.

2.2.5 Effect on photosynthesis composition

Researcher (Xie et al. 2015) also have study strategy to enhance photosynthetic light-harvesting and come up that fed-batch mode cultivation improve the production of C-phycocyanin (C-PC) which is a water-soluble phycobiliprotein and protein component. These component is essential for photosynthesis process where found in cyanobacteria, red algae and cryptomonads. Biomass production is related to photosynthesis process where this highly depend on C-PC. C-PC production is depended on nitrate concentration of wastewater. In these study (Xie et al. 2015) researcher used *Arthrospira (Spirulina)* to investigate effect of initial biomass concentration on C-PC production via fed-batch cultivation mode. These cultivation strategies been used to maintain nitrate concentration where pulse-feeding started when the nitrate concentration in the culture medium depleted. One of the parameter measured was microalgae biomass concentration by measuring the optical density of the sample (Xie et al. 2015). Based on (Xie et al. 2015) study, high C-PC content and production rate achieve during initial stage of nitrogen depletion. Since it shows that C-PC production is highly depend on residual of nitrogen concentration, therefore it is necessary to consider nitrogen concentration. Fed-batch operation is often used as an effective way in controlling nutrient concentration during cultivation. Thus on this study fed-batch operation used to control nitrogen concentration in order to promote C-PC generation and also biomass production. According to (García-Cañedo et al. 2016, Ji et al. 2015, Markou 2015, Matsudo et al. 2009, Rodrigues et al. 2010, Xie et al. 2015) control nutrient concentration in order to enhance biomass production or number of cell by applying fed-batch operation. The researcher shows that C-PC content and C-PC productivity in low nitrate fed-batch mode feeding is 15% higher than of nitrate by batch mode feeding. But for high nitrate feeding, the production rate was same for both feeding strategy. This is because based on (Chen et al. 2013) C-PC production rate start to decrease when the initial nitrogen concentration was higher than 45 mM which therefore nitrogen concentration maintain at lower level via fed-batch is more favourable for C-Pc production while lutein content also tend to favour lower nitrogen concentration. Based on (Xie et al. 2015) finding nitrate consumption start to decrease while biomass production increases when use fed-batch mode feeding where about 98% higher than that obtain from batch feeding. FB mode is suitable way to control nitrogen level and enhance the photosynthesis. This is because nitrogen level play vital role in most of micro and macroalgae. Therefore, it is necessary to control the level. Photosynthesis rate is solely depending on C-PC production rate at which itself depending on nutrient concentration. Photosynthesis is essential for biomass production. FB mode is best way to control the nutrient among which been prove through literature that FB has higher C-PC production compare to batch mode. Thus Fb is viable method for nutrient control.

2.2.6 Effect towards the cost.

It is proven that FB cultivation yielded a 77% cost reduction in production of poly(3-hydroxybutyrate) using cane sugar (Wisuthiphaet and Napathorn 2016).

2.3 *S. molesta*

There are different species of *S. molesta* available but mostly *S. molesta* is been used in past decade in phytoremediation due to its widely spread root system. Dye effluent from textile industry waste rich in organic compounds, metals, salt that affect water colour, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solid (TDS), total suspended solid (TSS) and also varies pH (Chandanshive et al. 2016). Normally activated carbon is used to alter the colour and absorb organic compound. Few study shows that phytoremediation of textile dyes using plants from different habitats. In India researcher use *S. molesta* for decolourization and detoxification of textile industry effluent. *S. molesta* is an aquatic fern where it has a potential of degrading azo dye Rubine GFL about 97 % at a concentration of 100 mg/L within 72 h usng 60 ± 2 g of root biomass. The trend (Chandanshive et al. 2016) show that *S. molesta* has ability to use for treating dye mixture and textile effluent in 72 hour. Stem analysis shows the dye absorb spread to neighbouring cortical cells at 24 hour of dye exposure. These was prove during anatomical analysis of stem tissue of *S.molesta*. Since *S. molesta* became popularity on organic compound removal, a study on *S. molesta* response toward ammonia been carried out. Even though FB mode feeding strategy minimize the effect of ammonia toxicity, behaviour of *S. molesta* towards ammonia ion concentration is necessary. *S. molesta* show positive growth with low ammonium (NH_4^+) concentration (Jampeetong et al. 2012). But when concentration of NH_4^+ increases (above 5mM), plant growth suppressed and exhibit NH_4^+ toxicity which had low growth rate, short root, low number of roots and showed chlorosis. It also shows the plant had a high uptake rate even at NH_4^+ toxicity level, which had negative effect on K uptake but the plant increases the N content (Jampeetong et al. 2012). Behaviour of other component of *S. molesta* is essential to be investigated in order to fully utilized *S. molesta*. Researcher investigate the mechanism of *S. molesta* minima against Chromium(VI) (Cr(VI)) toxicity upon different climate conditions. Chromium (Cr) is most toxic pollutant commonly happen in the environment which upon for long time accumulation could increases contamination of soils and water bodies. Thus behaviour of Cr towards plant is necessary to understand (Prado et al. 2012). In that study, they were manipulated climate upon summer and winter and investigate Cr(VI) concentration at submerged and floating leaves. Based on (Prado et al. 2012), Cr(VI) accumulation in both leaf type (floating and submerged) depends on metal concentration and grown season. Highest metal accumulation is to be found during summer in both type leaves. But the Cr(VI) concentration during summer and winter for submerged leaf is higher than floating leaf. This is because accumulation of Cr(VI) at submerged leaves indicate the ability of S. minima to withstand Cr-induced oxidative stress. In recent studies, floating macrophytes is identified as a potential way for removal of nutrient in wastewater. Past decade floating macrophytes being used to treat effluent from stability pond and also to attain secondary treatment effluent quality from primary effluent. Based on research conduct by (Ng and Chan 2017) *S. molesta* was used as floating macrophytes for phytoremediation of treated Palm Oil Mill Effluent (POME). The researcher state that *S. molesta* has ability to remove phosphate about 95% from wastewater at lower concentration. Thus, to attain higher efficiency, the concentration of nutrient should be control and the suitable strategy which already establish in other species is fed-batch. This paper (Ng and Chan 2017) aimed to investigated the nutrient removal by *S. molesta* from treated POME and also the effect towards fresh weight of plant and total carbohydrate and protein content. *S. molesta* have effective removal on phosphate, turbidity and total suspended solid concentration and show high nutrient uptake. The roots of *S. molesta* has capability to adsorb the suspended solid which the turbidity can reach to low level in short period. Phytoremediation of *S. molesta* gives positive effect on its biomass and biochemical content (Ng and Chan 2017). The literature show that FB mode has establish for cyanobacteria, algae and also microalgae and macrophytes for nutrient control method and has improvement in biomass production. Even though FB mode on *S. molesta* has less attention among the researcher, *S. molesta* has been used for pass decade in nutrient removal of various type of wastewater which POME is popular among the researcher. *S. molesta* is easily adapt and the rate of nutrient uptake is constant as the environment is not disturb.

From this chapter it’s clear that FB phytoremediation indicate good nutrient removal and biomass production for most of the macrophytes. But study on *S. molesta* is less interest among the researcher. However, *S. molesta* indicate good removal on phytoremediation.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Plant species cultivation, cultivation media and equipment

The macrophyte, *S. molesta* platensis was obtained from the School of Chemical Engineering, University Science of Malaysia. Table 3.1 show the medium of Hoagland Solution (Science 2015) as nitrogen source was used for cultivation media and were cultivated in closed glass bottle containing 70 mL of culture medium.

Table 3.1 Hoagland Solution.

Stock A(i)	g in 250 mL
KNO_3	12.638
NH_4NO_3	2.000
$MgSO_4.7H_2O$	12.320
KH_2PO_4	3.403

Stock A(ii)	g in 250 mL
$Ca(NO_3)_2.4H_2O$	23.610

Stock C	g in 100 mL
$MnCl_2.2H_2O$	0.148
H_3BO_3	0.286
$ZnSO_4.7H_2O$	0.022
$CuSO_4.5H_2O$	0.008
$Na_2MoO_4.2H_2O$	0.003

Iron	g in 100 mL
$FeSO_4.7H_2O$	0.251
$Na_2.EDTA.2H_2O$	0.337

All the macrophytes were placed in closed room with fluorescent lamp (as sunlight) and room temperature were maintaining at 25 °C. After 14 days of cultivation (exponential growth phase), the resulting suspension were collected, filtered and wash to remove any medium attached. This experiment was conducted to study the effect of fed batch cultivation of *S. molesta* in fish farm wastewater on its biomass and biochemical content along with nutrient removal. This fish farm wastewater was collected from USM environmental lab and *S. molesta* were cultivated in container which stimulated as small scale pond. The healthy floating macrophyte were chosen and weight of 8.4 g were placed with even distribution in the container. The experiment was conducted for 16 days and three replicate batch was conducted and average result is considered.

3.2 Fed batch cultivation condition

An initial biomass of 8.4 g was always used at early stage of each fed batch culture is used. Four sets of culture were prepared with different fed batch mode percentage which no FB, 25% FB, 50% FB and 75% FB which labelled as 0%, 25%, 50% and 75% respectively. The medium is added every 4 day of cycle with total duration of 16 days. At each cycle fed batch mode, the mention percentage of medium were remove and added with fresh medium. In order to kept constant, the total volume, 700 mL of medium were ensured for each sets.

3.3 *S. molesta* nutrient uptake and water quality after phytoremediation

A 12 ml of treated wastewater test was gathered for each day 2 started from day 0 until day 16 in rotator tubes. The water level in the bottle was ensure to kept constant. Tap water was added to the bottle if the water level dropped underneath the checked level due to evaporation. The water tests were done for its nitrate, phosphate and ammonia to study nutrient up take by *S. molesta*. COD, turbidity and MLVSS test were conducted on the specimens to investigate the water quality after phytoremediation.

3.4 Analytical analysis

3.4.1 Determination of nitrate and phosphate concentration for water sample

The supernatant of water tests was achieved by centrifugation at 10000g for 15 min. The supernatant was utilized for nitrate as well as, phosphate content determination. The nitrate was resolved by Cadmium Reduction Method (HACH strategy 8039) utilizing 5 Nitrate Reagent Powder Pillows. The phosphate was identified by Ascorbic Corrosive Method (HACH technique 8048) utilizing 3 Phosphate Reagent Powder Pillows. This phosphate assurance is in understanding to USEPA technique 365.2 and Standard Method 4500-P-E for wastewater. Every technique expended 10 ml of wastewater tests.

3.4.2 Determination of ammonia concentration, total nitrogen and COD for waste water with Vario Tube Test.

All the test conducted were using MD 600 photometer and its respective reagent. For ammonia the detectable range was 1 – 50 mg/L NH3-N with usage of 0.1 mL sample. The method used was VARIO Am tubetest reagent. Set HR, FS (ammonia salicylate along with ammonia cyanurate). The reagent required 20 min of reaction period before it ready for test. While COD test was conducted by dichromate/H2SO4 method by LOVIBOND method 131with sample usage of 2 mL and detectable range of 0 – 1500 mg/L O2. Total nitrogen use hydroxide LR digestion vials along with persulfate and three TN reagent powder pack. The detectable range is 0.5 – 25 mg/L N with consume 2 mL of water sample.

3.4.3 Determination of turbidity of water sample

Turbidity was measured using HANNA HI 93703 microprocessor turbidity meter by 890 nm along with 0-1000NTU. Centrifuge tube is used for ensure perfect mixing the clean cuvette were filled with water sample. In order to remove bubble in water sample degasser is used.

3.4.4 Determination of MLVSS

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

3.4.5 Determination of biomass (fresh weight)

The macrophyte were harvest and dried before weighed.

3.4.6 Determination of pH

The collected water sample were tested for pH value using pH meter.

CHAPTER FOUR

RESULT AND DISCUSSION

This chapter presents the experimental results and discussion consisting of four main sections. The first section illustrates the experiment design applied for fed batch (FB) feeding strategy cultivation of *S. molesta* in fish farm wastewater as well as optimization of parameters used. The second and third section discusses the nutrient uptake along the experiment duration on respective FB percentage and wastewater quality test. The forth part is on FB effect toward *S. molesta* biomass (fresh weight).

4.1 Experimental Overview.

This experiment been carried out to determine *S. molesta's* nutrient uptake namely nitrate, phosphate, ammonia and nitrogen in fish farm wastewater using FB mode cultivation. Along with this, effect towards its biomass on FB mode is also been investigate. The fish farm wastewater after phytoremediation was examined for its COD level, turbidity, nutrient concertation (ammonia, nitrate, phosphate, and nitrogen) and MLVSS in order to check on its water quality. Two replicate were repeated along this experiment and an average result is obtained. Control group is set up to compare the nutrient uptake level without applying FB feeding strategy (labelled as 0% FB) throughout 16 days of experiment. The following is set up on day 0 where 6 g/L *of S. molesta* is used for cultivation where 0%, 25%, 50% and 75% indicate percentage of FB respectively. 0% act as control which no FB mode is applied. The following are experiment set up. Figure 4.1-4.2 show that the number of plant multiply throughout phytoremediation 16 days after each FB cycle.

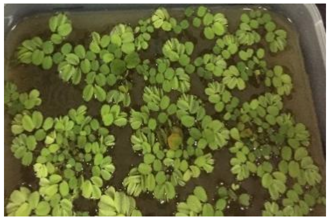
	Control (0%)	25% FB	50% FB	75% FB
Day 0				
Day 4				
Day 8				
Day 12				

Figure 4.1 *S. molesta* growth of Replicate 1.

	Control (0%)	25% FB	50% FB	75% FB
Day 0				
Day 4				
Day 8				
Day 12				

Figure 4.2 *S. molesta* growth of Replicate 2.

This prove that *S. molesta* is easily adapt to the environment (fish farm wastewater). (Jampeetong et al. 2012, Ng and Chan 2017, Prado et al. 2012). Based on figure 4.1 and 4.2, the distribution of plant on surface of wastewater is poor where uneven distribution is to be observer on day 0. This would lead to inconsistent result on analysis. Therefore, every data is plotted were average value of both replicate with standard error. The plant growth indicates positive result on nutrient removal from wastewater. Further water analysis is done on the wastewater to understand on efficiency of nutrient removal from wastewater.