EFFECT OF PHOTOPERIOD ONTO THE UPTAKE RATE OF PHYTOREMEDIATION OF DUCKWEEDS

by

SYAFIQAH BINTI ZULKEPLI

Thesis submitted in partial fulfilment of the requirement for the degree of Bachelor of Chemical Engineering

2021

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Syafiqah binti Zulkepli

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LIST OF ABBREVIATION

Symbol	Description
POME	Palm oil mill effluent
DOE	Design of experiment

ABSTRAK

Makrofit atau tumbuhan akuatik digunakan untuk penyingkiran nutrien untuk mengurangkan eutrofikasi dan meningkatkan kualiti produk sisa. Dalam kajian ini, fitoremediasi oleh Lemna sp. dan Spirodela polyrhiza dilakukan secara paksi atau satu spesis di simpan di dalam media yang mepunyai nutrien dalam air sisa sintetik dalam keadaan kawalan untuk menilai dengan tepat kecekapan penyingkiran nutrien NO_3^--N, PO_4^{3-}, NH_3-N dan pH dalam sampel air sisa sintetik dengan tempoh operasi terhadap cahaya yang berbeza. Hasil kajian menunjukkan bahawa penyingkiran amonia terpantas bagi *Lemna* sp. pada masa operasi 8:16 jam dan S. polyrhiza pada masa operasi 24: 0 jam dengan kecekapan masing-masing 87.8% dan 66.3% dalam masa 3 hari. Lemna sp. mampu mengurangkan 14.7% nitrat. *S. polyrhiza* pada masa operasi 16:8 jam mencapai pengurangan fosfat sebanyak 68.1% pada hari ke-3 kepada hanya 7.17 mg / L PO_4^{3-}. Kedua-dua tumbuhan akuatik menunjukkan kenaikan perubahan biomas. *L. minor* dan *S. polyrhiza* pada masa operasi 16:8 jam mengatasi masa operasi lain dalam penyingkiran nutrien. Dengan menggunakan profil pemulihan nutrien yang dikumpulkan, ia dapat dijadikan panduan untuk pemilihan tumbuhan akuatik dan tempoh operasi yang sesuai dalam rawatan air sisa dan sebagai penilaian aktiviti mikrobiol dalam sistem fitoremediasi bukan aseptikal.

ABSTRACT

Macrophytes or aquatic plants are utilized by their nutrient removal abilities to reduce eutrophication and improve waste product quality. In this study, phytoremediation by *L. minor* and *S. polyrhiza* were carried out axenically in synthetic wastewater under control condition to precisely evaluate nutrient removal efficiency of NO_3^--N, PO_4^{3-}, NH_3-N and pH in the medium sample with different photoperiod. The results showed that ammonia removal was rapid, significant for *Lemna* sp. at photoperiod 8:16 h and *S. polyrhiza* at photoperiod 24:0 h with efficiency of 87.8% and 66.3% respectively within 3 days. *L. minor* was capable of reducing 14.7% of the nitrate. *S. polyrhiza* at photoperiod 16:8 h achieved phosphate reduction of 68.1% at day 3 to mere 7.17 mg/L PO_4^{3-}. Both duckweeds showed biomass change increment. *L. minor* and *S. polyrhiza* at photoperiod 16:8 h outperformed other photoperiod in nutrient removal. By using the collected nutrient remediation profiles, it can be served as a guideline for the selection of suitable duckweeds and photoperiod in wastewater treatment and as microbiol activity assessment in non-aseptical phytoremediation system.

CHAPTER 1

INTRODUCTION

This chapter will introduce an overview about this research to study the effect of photoperiod onto the uptake rate and phytoremediation of duckweed. Therefore, this chapter will review the research background of duckweed and phytoremediation, the problem statement, and the objectives of this final project.

1.1 Background

Duckweeds are a small aquatic floating species consisting of 37 species scatter all over the world (Appenroth et al., 2018). Duckweeds belongs to the Araceae Family Lemmonoideae subfamilie. Araceae family consists of five genera which is Lemna, Wolffia, Wolffiella, Spirodela and Landoltia. Duckweed can survive and grow at range temperature 5℃ to 35℃. Moreover, duckweed biomass contains high protein, ranging 15% to 45% of dry weight. Duckweed can be utilized for the good value-added products, such as by using *Wolffia arrhiza* meal as diet drinking of Japanese quails (Suppadit et al., 2012), using duckweed species for meal of striped catfish (Da et al., 2013) and carp (Sharma et al., 2016).

The aquatic plants, duckweed holds big and huge potential as a highly demand as feedstock for biofuel production and food production help by sunlight. The several duckweed's characteristics make it working and perfect for waste to energy conversion. Furthermore, it has fast growth rate (up to 120 tonnes dry mass/hectare/yr) and able to survive and grow on wastewater sources. Duckweed also provide platform for sustainable biomass production.

Duckweeds are aquatic plants where importance technologies which combine wastewater treatment systems and protein production in feed resources for fish and animals (Lasfar et al., 2007). The specific operating condition for duckweed growth is very importance to manage duckweed crops (includes nutrient uptake efficiency, production of biomass and harvesting strategy). There are several parameters that affected duckweed growth, which are temperature, photoperiod, mat density and concentration of phosphorus “P” and nitrogen “N”.

The presence of duckweed can supply to the organic matter present in the water body. The layers of *L. minor* can evacuate amino acid and humic substances into the aquatic life. Therefore, it can provide nutrients to other organisms such as algae, bacteria, and indirectly to snails, isopods (*Asellus* sp.), and other microdetrivores. Under artificial lights, duckweed’s record growth rates far exceed growth rates under natural conditions.

Due to night and day phenomena, light (includes light intensity and photoperiod) and nutrition, both are play important role in environmental factors that can affect growth of the plant and nutrient uptake directly (Liu et al., 2018). Phytoremediation is defined as the method to alter contaminated environment which is commonly wastewater. It is a low cost, low impact, and environmentally sound remediation technology. Moreover, phytoremediation takes place in five mechanisms, which are rhizofiltration, phytostabilization, phytoextraction, phytovolatilization, and phytotransformation (Ansari et al., 2011).

Phytoremediation is one of technologies use of living green plants for situ removal, degradation, and containment in wastewater, soils, and groundwater. The phytoremediation method was used in this research is to reduce COD in all culture

medium (Palm Oil Mill Effluent (POME) dilution) due to the presence plant activity that also involve microorganisms (Kadir et al., 2020). With this, we can reduce the amount of contaminated and neutral the pH of wastewater that bring benefits to socio-economic development in the country. This will satisfy the requirement towards sustainable and eco-friendly future.

1.2 Problem Statement

Commonly, both nutrition and light (includes light intensity and photoperiod) are the important environment factors that affect the growth of plant directly. However, the interconnection of photoperiod and nutrition affects growths and nutrient uptake is less of study. In this study, duckweeds in medium solution will be used to show how photoperiod will affect the growth and nutrient uptake by the plants. On the other side, organic toxic was recently discharge into the water from industries every day. Thus, the removal of organic toxic from wastewater is a serious issue. Commonly, adsorption process is widely used for the organic toxic's removal since the cost is lower, availability and eco-friendly nature. In previous study, duckweed shows a good species for phytoremediation activities. In this study, we will find how photoperiod will affect phytoremediation of duckweed.

1.3 Objectives

i. To study the nutrient uptake rate affected by the different amount of light the plants received.

ii. To study the nutrient uptake rate of the plants affected in the dark.

iii. To predict the water treatment capacity (phytoremediation) of the plants during light and dark at constant concentration.

CHAPTER 2

LITERATURE REVIEW

This chapter will review article that had studied before. A literature review is a scholarly paper that presents the current knowledge including substantive findings as well as theoretical and methodological contributions to a particular topic.

2.1 Duckweeds

Duckweeds comprise a group of diminutive freshwater monocotyledonous plants (formerly place in the family lemnaceae) (Kutschera & Niklas, 2015). They are typically used as food by aquatic birds and can quickly build dense populations that cover the whole surface of lakes and slowly running rivers. *L. minor* represent **Figure 2.1** is a common duckweed and it is an invasive floating aquatic macrophyte with ecological and economic implication wherever the colony of the pant exist (Ekperusi et al., 2019). There are review of the ecological role of the plant as a crucial component of the aquatic ecosystem. While *S. polyrhiza* represent in **Figure 2.2** is the largest and purportedly the most of the 34 described species assigned to this group of plant. The individual fronds are up to 15 mm long. compared to other genera such as *Lemna, Landoltia, Wofffiella* and *Wolffia* are much smaller which is 5 mm to less than 1 mm long. In addition, the tiny and free-floating duckweeds need very little amount of lignin to support their growth (Pagliuso et al., 2018). They could instead preserve energy to synthesize more protein and carbohydrate. Most species of duckweed can double their biomass every 2 or 3 days. Therefore, species of Lemnaceae have great potential in agriculture.

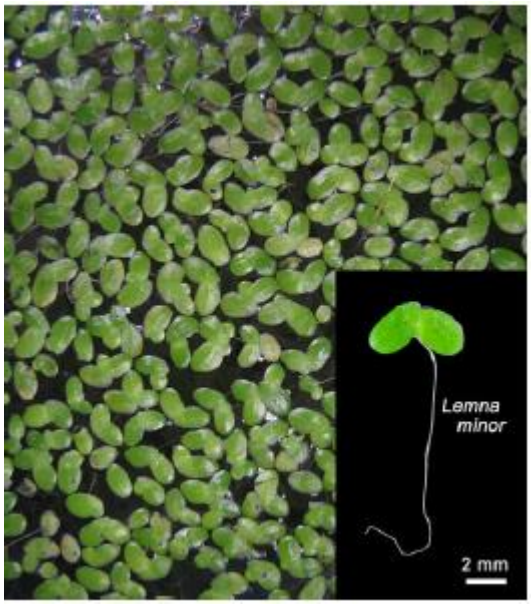


Figure 2.1 The above and side view (inset) of common duckweed *L. minor*

Figure 2.2 The above and side view (inset) of great duckweed *S. polyrhiza*

2.1.1 *L.* minor's Biology

L. minor is one of the smallest flowers in the plant kingdom. *L. minor* is a monocotyledons plant and can float in water. It can form thick layer in nutrient-rich fresh and brackish water. The angiosperm can produce one or several leaves known as fronds and a single root or rootlet with no steam. The plants reproduce by vegetatively which is it simply dividing to form each individual plants. In previous study, duckweed can grow with laboratory cultured with enough nutrients, light and water was supplied

then, the unlimited duckweed's specimen can be produces for use. It produces a several number of daughter fronds during their life cycle however, each six generation was producing the mother frond will dies (Sharma et al., 2016). (Kutschera & Niklas, 2015) dubbed the duckweed as 'Darwinian Demons' due to their ubiquitous reproductive capacity, sporadic development and ability to almost 'live forever'.

2.1.2 *S. polyrhiza*'s Biology

S. polyrhiza or greater duckweed is incredibly basic with only one leaf and steam and some roots in a tight form. Fronds grow vegetatively and can speedily increase biomass, reduce carbon dioxide in the air and reduce nitrogen and phosphorus in the water. However, *S. polyrhiza* has low amount of lignin (Pagliuso et al., 2018), which is contains 70.9% carbohydrate and 29.1% of protein in dry weight (Wang & Messing, 2011).

2.1.3 Growth Condition of Duckweed

Plant growth and reproduction is mainly affected by the availability of macronutrients such as nitrogen, phosphorus, and potassium in addition to micronutrients, temperature, light, wave action and plant density (Lyerly 2004). Previous study, duckweed is reported to be tolerant to a wide range of pH from 3 - 10 with an optimum range of 5 – 7 (Kesaano, 2011). The wide range for plant to grow from 6 to 33ºC with an optimum temperature range from 18 to 30ºC.

It is also known that duckweed growth is particularly sensitive to wind and wave activity because the wind blows the duckweed on the sides of ponds and thereafter dies. The effect of the wind on duckweed systems affects not only plant development but also plant biomass harvesting.

2.1.4 Composition of Duckweeds

Duckweed is composed of mineral elements, water, and organic matter. Fresh duckweed fronds have been reported to contain 87 to 97% water depending on the 7 species (Cross, 2013). Previous study, chemical analyses showed varying composition of crude protein, ash, fiber, water content, fat and mineral content depending on the harvest location, water source and species analyzed. **Table 2.1** Typical chemical composition of duckweed cultured on nutrient-poor and nutrient-rich water represent the nutritional value of duckweed increased with plants grown in nutrient rich waters (Kesaano, 2011).

Table 2.1 Typical chemical composition of duckweed cultured on nutrient-poor and nutrient-rich water (Kesaano, 2011)

	lagoon nutrient condition [a]	percent of dry wet									
		NFE[b]	crude protein	TKN[c]	Fat	Fiber	Ash	P	K	Ca	Mg
Spirodela punctata	Low		10.6	1.7	2.3	11.3	14.1	0.61	2.0	0.98	0.98
S.polyrrhiza	Low		13.1	2.1	2.5	16.1	13.3	0.56	2.4	1.21	0.76
Lemna gibba	Low		9.4	1.5	1.8	17	16.8	0.72	3.1	1.38	0.81
Spirodela punctata	High	33.2	36.8	5.9	4.8	9.7	15.2	1.50	2.8	1.75	0.84
S.polyrrhiza	High	31.8	39.7	6.4	5.3	9.3	12.8	2.10	3.4	1.28	0.92
Lemna gibba	High	31.1	36.3	5.8	6.3	10.1	15.5	2.60	4.4	1.81	0.88
[a]Low nutrient Lagoon contained less than 5 mg/L TKN. High nutrient lagoon contained greater than 30 mg/L TKN. Selected mean values from Culley et al. (1981)											
[b]NFE (Nitrogen Free Extract, an estimate of carbohydrates)											
[c]TKN (Total Kjeldahl Nitrogen)											

2.1.5 Distribution of Duckweeds

L. minor is easy to growth and the distribution is widely in different geographical regions from the tropics to temperature zone whether freshwater or brackish water. The flaying animals such as birds are important agent to disperse

duckweeds to new place. The duckweeds have sticky roots so the disperse rate will increase and can spread to different aquatic ecosystem (Ekperusi et al., 2019).

2.1.6 Cultivation of Duckweeds

Duckweed culture requires a water and nutrient supply from fertilizer consistently. Previous study has state that one ponds can produces until 10 generations of daughter plants over 10 days to several day before dying. The double time of duckweed is less than two days under ideal conditions. To harvesting the duckweed, the full attention is required to ensure the productivity and health of duckweed under monitor. The nutrients sources come from animal, manure and food waste can be used as culture purposes.

The duckweed requires ideal condition to growth which is a pH of 5 to 9 while the temperature is 6 to 33 ℃ and 0.5m of pond depth. The plants also need about 60 mgL^{-1} of nitrogen and a minimum of 1 mgL^{-1} of phosphorus to growth. During 1995, 10 to 30 tonnes of dried duckweed per hectare per year can be produced under ideals conditions. However, under laboratory conditions duckweed need a pH of 6 to 7.5 and appropriate amounts of nutrients.

2.1.7 Uses of Duckweeds

Increasing interest in duckweed has led to a series of international conferences in different parts of the world that offer researchers the opportunity to connect with and advance the study and application of the plant for human progress. L. Minor has a long history of use in aquaculture, livestock, manufacturing, poultry, pharmaceuticals, biofuels, toxicity checks, environmental monitoring and remediation of contaminated wastewater. However, duckweed has been confirmed to be human food too (K.-J. Appenroth et al., 2015). For farmer, the duckweed was useful as a feed sources for

animals (include pigs, rabbits and ducks) (Khan et al., 2014). Other researcher found that duckweeds have high content of essential amino acids. On the nutritional properties of duckweed as a potential food source for humans (K.-J. Appenroth et al., 2015). It has been stated that the quality of proteins and amino acids in duckweed is about the same as the WHO recommended importance for humans. *L. minor* also could be utilized for the production of butanol, alcohol and biogas (Cui & Cheng, 2015). The study is still ongoing to genetically use duckweed to increase lipid concentration for increased oil production for biofuels generation (Zhao et al., 2012). In a year, around 17 million tonnes or around 25% of the volume of fuels could be produced by duckweed in China stated by (Zhao et al., 2012). The sporadic distribution and invasive nature of the plant and the ability to thrive in diverse habitats increased the potentials of the plant to withstand harsh environmental conditions including polluted or degraded waters (Sukumaran, 2013).

More than two decades, duckweed has been used for treatment of municipal and industrial wastewaters. Furthermore, duckweed had update it application such as ability to phytoremediation a wide variety of wastewater setting by absorb high levels of Cd^{2+} and more than 3.0 g m^{-2} d^{-1} for growth rate (Appenroth et al., 2015). It effectively used for phytoremediation because able to grow a huge range of temperature, pH, and nutrient level. In previous study, duckweed genera, especially *L. minor*, were used as treating wastewater of both domestic origin and agricultural. When the duckweed is grown in the wastewater treatment ponds, the partitions and baffles was installed to prevent wind from blowing fronds to the surface of treatment pond.

Furthermore, duckweed can cover treatment ponds by reduce the growth of algae and nitrogen in the effluent through denitrification and ammonia uptake. Also,

duckweed can be constructed as wetland system. Either as a component that receive wastewater or treated effluents (Ansari et al., 2011).

2.2 Phytoremediation

Due to the rapid growth and increased complexity of the global chemicals industry in this last century, complex environmental effluent, particularly aquatic ecosystems, have become progressively liberated. Since 1950 more than 140,000 synthetic chemicals and pesticides have been synthesized, with over 5,000 large-scale products being commonly distributed throughout the atmosphere and uniform human exposures. For the remediation of different chemical contaminants, L minor has been commonly used. As an ecology-based emissions control technology, the plant is used independently or in conjunction with other marine macrophytes. The following types of emissions will be considered for separate treatment technologies as below **Figure 2.3**.

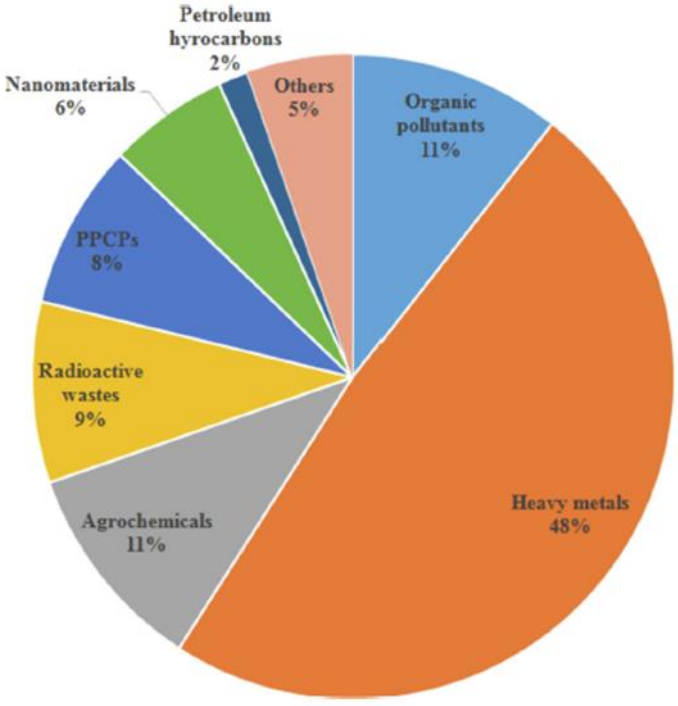


Figure 2.3 Categories of pollutants remediated by *L. minor* (Ekperusi et al., 2019)

2.2.1 Phytoremediation of Organic Emission/Pollutant

The production and growth of aquatic pollutant phytoremediation began with the need to treat residential and industrial wastewater effluents. Organic load, smell, and colour elimination to enhance the consistency of the water before drainage into lakes, waterways or groundwaters spurs a wide variety of studies from wastewater treatment plants into natural and human or manufactured wetlands. Other study had found that *L. minor* is a very efficient floating macrophyte for the phytoremediation (Mohedano et al., 2012).

2.2.2 Phytoremediation of Toxic Organic Compound

Duckweeds species can accumulate toxic organic compounds such as phenols, chlorinated phenols, pharmaceuticals, and surfactants (Ansari et al., 2011). Duckweed species can directly or indirectly accumulate through microbiota living on frond surfaces. Duckweed can take up fluorinated agricultural chemicals (Reinhold & Saunders, 2006) and detoxify chlorinated phenols. Therefore, the ability of duckweed can be used as phytoremediation of industry wastewaters as they can perform reductive dichlorination. Duckweed species are certainly capable of contributing to natural bioremediation systems.

P removal efficiencies by duckweed systems were reported to range from 14 – 99% (Şekerdağ et al., 2003). The duckweeds able to uptake phosphorus depends on the harvesting frequency, growth rate and the available orthophosphate. There are many study of duckweed on phosphate removal from wastewater such as impact of harvesting duckweed on phosphate removal from secondary effluents (Öbek & Hasar,

2002) and efficiency of *L. minor* in a secondary clarifier tank of a conventional biological treatment plant (Şekerdağ, 2008).

2.2.3 Phytoremediation of Heavy Metals

Nowadays, application of duckweed in the uptake nutrient and remediation of heavy metals form wastewater a very popular case to investigated in the useful of macrophytes or duckweed for removal of pollutants in aquatic media. The pollutions include heavy metals can caused a serious risk for body health's and environments because they are toxic and easy to enter the food chain through marine life. It will lead to poisoning and damage for tissues and organ (Adesiyan et al., 2018). But is some case, deaths also can be happened. In previous study, duckweed has shown that they are efficiently remove heavy metals for both domestic and industrial by bioaccumulate heavy metals over 100,000 times higher.

2.3 Photoperiod

2.3.1 Duckweed Photoperiod

The operating condition such as temperature and photoperiod give a different effect. In previous study, the ideal value of duckweed growth is 26℃ for temperature and 13 h for photoperiod time. The temperature ranges from 23 to 28℃ and photoperiod ranges from 11 to 14 h, the relative intrinsic growth rate is less than 5% (Lasfar et al., 2007). These parameter's value gave effectively ideal ranges for duckweed growth. The duckweed growth inhibited when temperature lower than 10℃ or higher than 35℃ while for photoperiod, duckweed can grow within 2 to 20 h.

2.3.2 Impact of Photoperiod on Net Photosynthetic Rate of Duckweed

Previous report plant cultivated in short days condition showed more chlorophyll per unit leaf area or biomass weight than plants cultivated under 24-h photoperiod. At the beginning of growth, the net photosynthesis was higher under longer photoperiod (24 h d^{-1}) because longer illumination time can lead to faster development of chloroplast (Liu et al., 2018). However, after a certain period, photosynthesis higher under shorter period (16 h d^{-1}). This is because extended growth period at shorter illumination time made the chloroplast develop more thoroughly (Yunze & Shuangsheng, 2014). In this study, although the 24-h photoperiod decreased the chlorophyll content, it seems to be more positive to the accumulation of biomass (Liu et al., 2018).

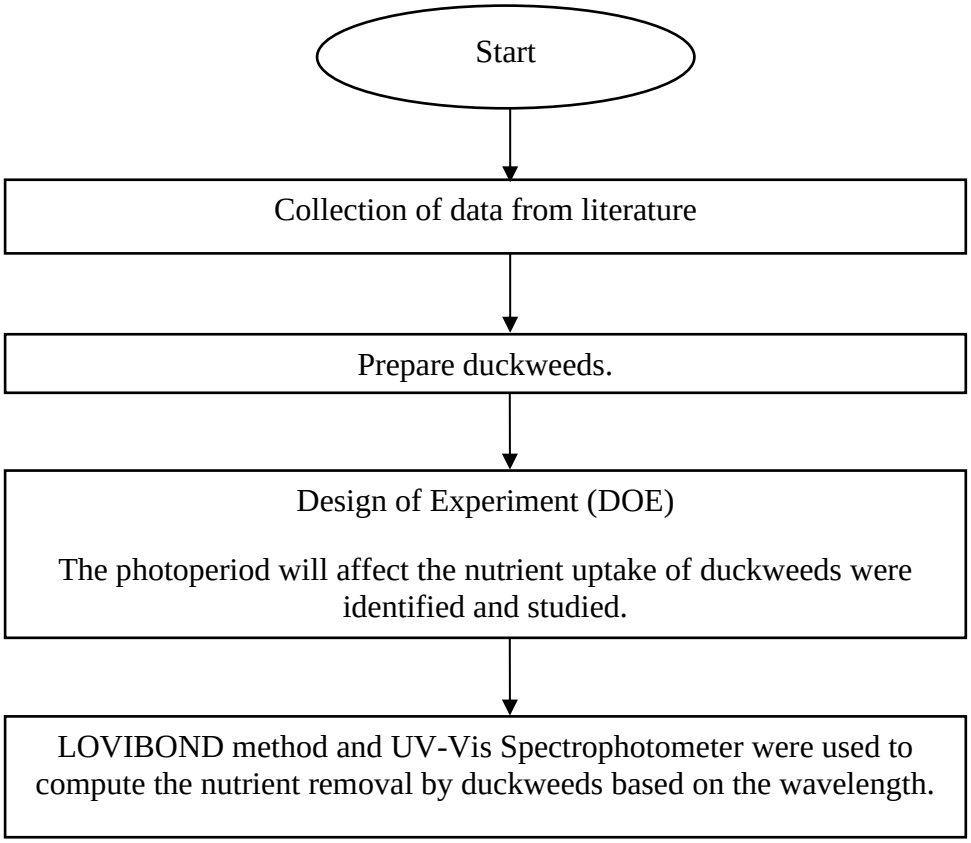
CHAPTER 3

METHODOLOGY

This chapter will list an overview of the final year project. Therefore, the overall experiment, method preparation of duckweed and sample analysis for light effect and phytoremediation will study in this chapter.

3.1 Overview of Research Methodology

Overall, this final year project focused on effect of photoperiod onto the uptake rate of phytoremediation of duckweeds. The experimental design and statistical analysis duckweeds to study the effect of photoperiod on various nutrient uptake and phytoremediation were discussed. **Figure 3.1** shows the overview of the activity of this research.



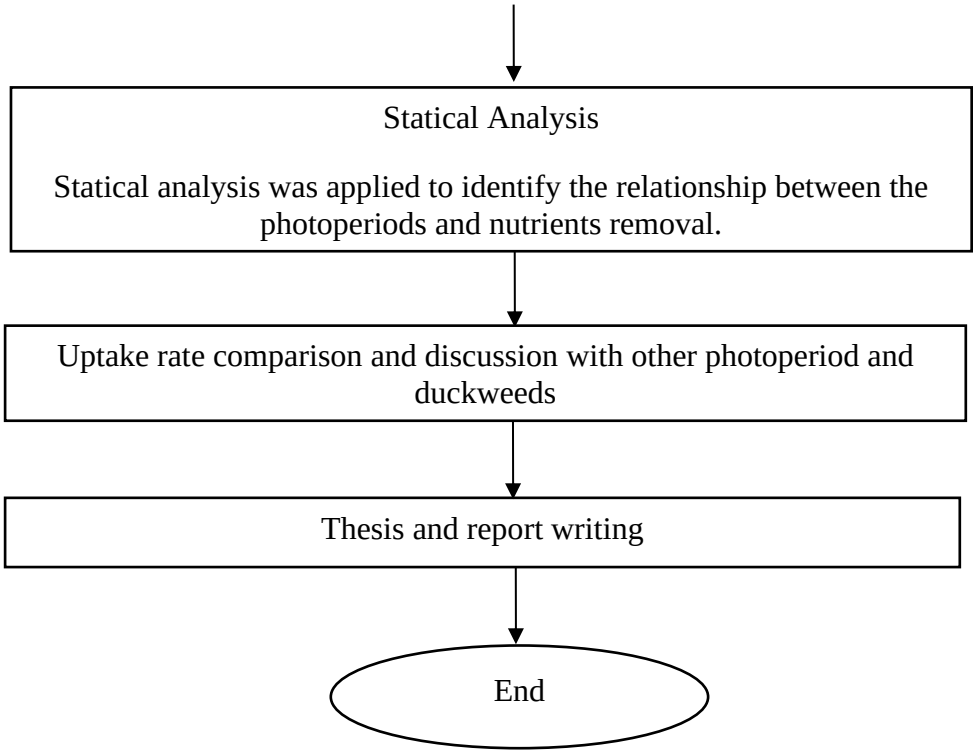


Figure 3.1 Flow diagram of research project on duckweeds

3.2 Plant Isolation and Establishment

First, the duckweed shall be prepared and cultivated in the laboratory. Duckweed need wash slowly with tap water to remove adhering mud particles, algae and other undesired organisms, and then placed on the tissues for five minutes before carrier the experiment. When the liquid medium (Hoagland No.2 medium with addition of sucrose) for growth was clear and not muddy and there was no unpleasant smell, the sterility of the culture of the macrophytes was ensured. The cultured media were adjusted to pH 5.8 using NaOH solution. Then, *L. minor* And *S. polyrhiz*a were periodically subcultured and stored in liquid Hoagland No. 2 medium with 15g/L sucrose for the required plant stock of the study before autoclaving all the bottle at 121ºC for 15 min. Each species of duckweeds was subcultured in five culture glass jar

bottles contain 150ml liquid medium. All cultures were the incubated in a growing room at 25 $\pm$ 2ºC under the light intensity of 1,600 lux with a 24:0 h light: dark photoperiod for 7 days. The solution consisting of all nutrients needed by the duckweed to maintaining the culture stock prior to the experiments. After 7 days, the fresh and healthy duckweeds can be use in experiment.

3.3 Phytoremediation of Duckweeds in Medium

This test was conducted under axenic condition by using medium to determine the nutrient removing capabilities of two duckweeds, *L. minor* and *S. polyzhiza* for several nutrients including phosphate, nitrate, and ammonia. Medium will be prepared in laboratory. The samples will store at room temperature, 15-20 ℃. The medium consisted of 94.34 mg/L $(NH_4)_2SO_4$, 246.4 mg/L $MgSO_4.7H_2O$, 27.22 mg/L KH_2PO_4, 153.49 mg/L $Ca(NO_3)_2.4H_2O$, 243.96 mg/L K_2SO_4, 0.74 mg/L $MnCl_2.2H_2O$, 1.43 mg/L H_3BO_3, 2.51 mg/L $Fe.SO_4.7H_2O$ and 3.37 mg/L $Na_2.EDTA.2H_2O$ was prepared in a 2 L SCHOTT DURAN® GLS 80® wide neck glass bottle. After the medium was prepared, each glass jar bottle was filled with 150ml of the medium solution. All 32 glasses jar was filled with 150ml of the media solution. All the cultured media were adjusted to pH 5.8 using NaOH solution before autoclaving at 121ºC for 15 min.

In laminar flow cabinet, all plants (*L. minor* and *S. polyhiza*) were taken from Hoagland medium will placed on the tissues for five minutes before carrier the experiment. It is to remove adhering mud particles, epiphytes and reduce the amount of water contains in plants. After dry, 1g of fresh weight *L. minor* and *S. polyrhiza* will placed into each glass jar contains synthetic medium. The experiment for each species was carried out in 4 replicates. Water sample for day 0 was collected before inoculation. All the samples were subsequently placed on the culture rack and

cultivated at 25 ± 2ºC under the light intensity of 1,600 lux. The samples were cultured for 3 days under photoperiod of 24 light:0 h dark, 16 h light:8 h dark, 8 h light:16h dark and 0 h light:24 h dark. The water sample was collected once every photoperiod time starting day 0 to day 3 to determine the water quality of medium during phytoremediation period. The parameter of water quality examined included phosphate (PO_4^{3-}), nitrate (NO_3^--N), ammonia (NH_3-N), pH and biomass change. For each water samples collection was carefully carry out in the laminar flow to avoid contamination. The sample was taken 10ml for each glass jar and was stored in small bottle before undergo analysis. The duckweeds were harvested at the end of experiment and weighed for fresh weight analysis.

3.4 Analytical analysis

3.4.1 Determination of Nitrate 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 500nm with a detection range between 0.3 to 30.0mg/l NO_3^--N.

3.4.2 Determination of Phosphate Concentration for Water Samples

3.4.2(a) Ammonium Molybdate Solution

In approximately 150 ml warm water, 1.7081 g ammonium molybdate has been dissolved. The solution was somewhat milky and refrigerated to room temperature. It was put to a volumetric bottle of 250 ml and was diluted with water (Shyla et al., 2011).

3.4.2(b) Thiourea

A weighed amount, 2 g of thiourea (Shyla et al., 2011) was transferred into a clean 100 ml beaker. It was dissolved in about 50 ml water. It was transferred into a 100 ml volumetric flask and diluted to the mark with water.

3.4.2(c) Sulfuric Acid

Sulfuric acid (0.25 N) was prepared by diluting the concentrated sulfuric acid (≈36 N) with water.

3.4.2(d) Absorption Spectrum of Reduced Phosphomolybdate Complex

The absorption spectrum of thiourea reduced phosphomolybdate complex solution is having its maximum absorption at 840 nm (Shyla et al., 2011).

3.4.2(e) Procedure

0.5 ml of 5.54 x 10^{-3} M ammonium molybdate solution, 0.5 ml of 0.25 N sulfuric acid and 1 ml of 2% thiourea solutions are too be added into 2 ml of the water sample corresponding to 0.5-10 $\mu g ml^{-1}$. After 20 minutes, the solutions are to be measured at 840 nm against water. Calibration graph is to be obtained by plotting absorbance values of the solutions against their phosphate concentration.

3.4.3 Determination of Ammonia Concentration for Water Samples

3.4.3(a) Salicylate Catalyst Solution

440 g sodium salicylate ($C_7H_5NaO_3$) and 0.28 g sodium nitroprusside ($Na_2[Fe(CN)_5NO]$ were dissolved to 1000 mL ammonia-free water (Le & Boyd, 2012). The solution was store in a brown glass bottle at 5ºC.

3.4.3(b) Alkaline Citrate Solution

18.5 g sodium hydroxide (NaOH) and 100 g sodium citrate ($C_6H_5O_7Na_3.2H_2O$) were dissolved in 1000 mL ammonia-free water (Le & Boyd, 2012). Stable indefinitely.

3.4.3(c) Alkaline Hypochlorite Solution

10 mL of sodium hypochlorite solution was added to 90 mL of alkaline citrate solution. The solution was prepared fresh daily.

3.4.3(d) Procedure

0.1 ml water sample was diluted with 9.9 ml of deionized water. The diluted samples and reagent blank (10 ml deionized water) were pipet to each glass bottles. Then, 1.2 mL of salicylate catalyst solution and 2.0 mL of alkaline hypochlorite solution were added. Mix well. The diluted samples were placed in a low light area (dak box) for 1 hour. After 1 hour, by using spectrophotometer at 640 nm, set absorbance with the reagent blank. Read the absorbance of the sample. Then the concentrations of ammonia removal were computed.

3.4.4 Determination of pH

pH measurement was performed using Hanna Edge® pH meter HI-2020(Ng & Chan, 2018). The water sample has been swirled continuously with the pH sample until the pH is stabilized. The pH reading was collected during day 0 and 3.

3.4.5 Determination of Mass

Both *L. minor* and *S. polyrhiza* will collect after experiment for dry biomass analyses. Before weighting the plants, the plants collected will rinse three times with distilled water, then filter the plants using 1mm filter paper and left dripping and rest

the plants in tissue to reduce the amount of moisture for 5 minutes. Then, weight the dry plants.

CHAPTER 4

RESULT AND DISCUSSION

This chapter presents the results obtained from the work as described in Chapter 3. All the data and results are thoroughly discussed to meet the outlined research objectives. The results of the effect of photoperiod onto the uptake rate of phytoremediation of duckweeds will be presented in separate sub sections.

4.1 Effect of Nutrient Removal in Medium

The nutrients monitored for removal studies in this experiment included nitrate (NO_3^--N), phosphate (PO_4^{3-}) and ammonia (NH_3-N).

4.1.1 Effect of Nitrate Removal by Photoperiod on *L. minor*

Nitrate removal by the *L. minor* with different photoperiod and existence of sucrose during 3 days of the study is presented in **Figure 4.1**. The study was shown the nitrate removal under photoperiod of 24 light:0 h dark, 16 h light:8 h dark, 8 h light:16h dark, and 0 h light:24 h dark. A 10.0%, 14.6%, 7.9%, and 6.8% decrement in nitrate was observed at the end of the experiment. On the other hand, nitrate level decreased much of it in photoperiod of 16 h light:8 h dark throughout the experiment with 14.66% nitrate removal achieving a concentration of 24.75 mg/L on day 3. It showed the highest removal efficiency when compared to others photoperiod. Therefore, photoperiod 16 h light:8 h dark is the best candidate for treating nitrate. Duckweed use nitrates as a source of food. Phosphate and nitrates are needed for eutrophication for excessive growth of algae.

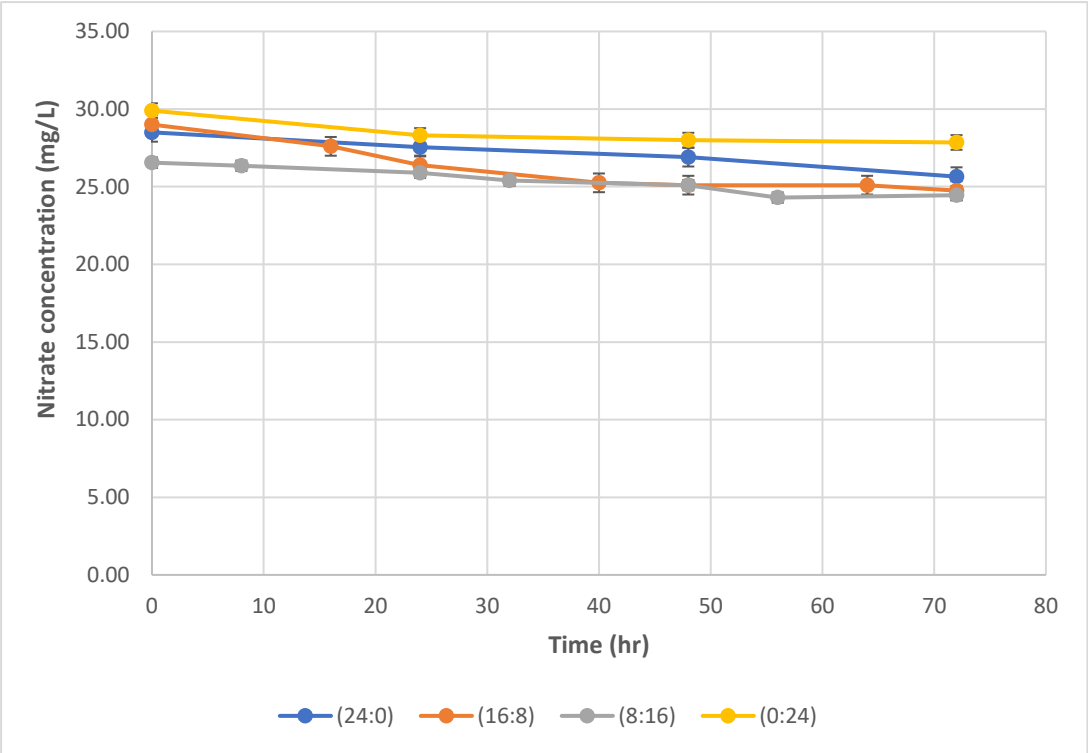


Figure 4.1 The nitrate (NO_3^--N) concentration versus time of phytoremediation by *L. minor*

4.1.2 Effect of Nitrate Removal by Photoperiod on *S. polyrhiza*

Nitrate removal by the *S. polyrhiza* with different photoperiod and existence of sucrose during 3 days of the study is presented in **Figure 4.2**. The study was shown the nitrate removal under photoperiod of 24 light:0 h dark, 16 h light:8 h dark, 8 h light:16h dark, and 0 h light:24 h dark. A 18.0%, 16.8%, 18.6%, and 8.4% decrement in nitrate was observed at the end of the experiment respectively. On the other hand, nitrate level decreased much of it in photoperiod of 8 h light:16 h dark throughout the experiment with 18.6% nitrate removal achieving a concentration of 21.80 mg/L on day 3. It showed the highest removal efficiency when compared to others photoperiod. Therefore, photoperiod 8 h light:16 h dark is the best candidate for treating nitrate by using *S. polyrhiza*. The nitrate uptake is slow at the first 60 hours as the removal ammonia at higher rate than nitrate. However, after ammonia concentration decrease

to a specific threshold concentration, it will continue to be taking up huge value nitrate for fulfilling its nitrogen requirement for growth. **Figure 4.2** shown the drastically nitrate concentration decrease between day 2 to day 3.

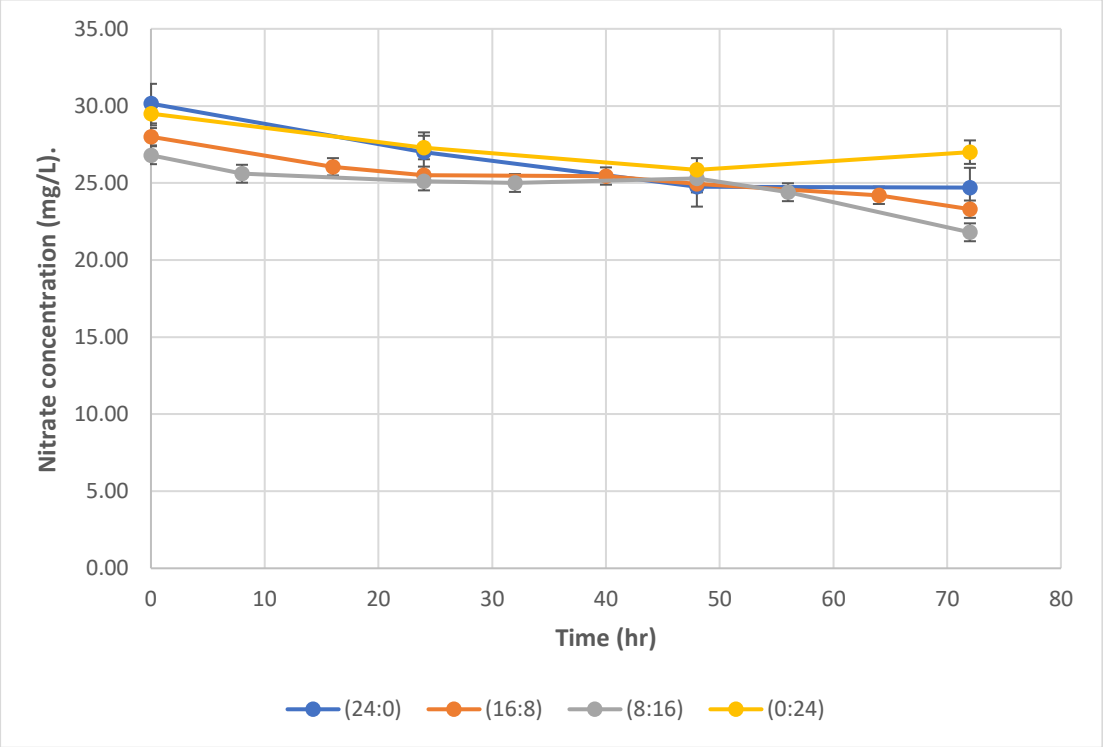


Figure 4.2 The nitrate (NO_3^--N) concentration versus time of phytoremediation by S. polyrhiza

Between the *L. minor* and *S. polyrhiza* uptake, it was found that removal by *S. polyrhiza* was higher than *L. minor* Therefore, *L. minor* at photoperiod 18:6 h and *S. polyrhiza* at photoperiod 8:16 h are the best candidate for nitrate removal since it has the highest removal efficiency among the tested species. *L.minor* with 14.66% nitrate removal achieving a concentration of 24.75 mg/L while for *S. polyrhiza* with 18.66% nitrate removal achieving a concentration of 21.80 mg/L.

4.1.3 Effect of Phosphate Removal by Photoperiod on *L. minor*

Phosphate removal by the *L. minor* with different photoperiod during 3 days of the study is presented in **Figure 4.3**. In all samples, the phosphate concentration decreases significantly during the experimental run. Since the duckweeds were very efficient in phosphate absorption for their growth. Plants need phosphorus for their normal development and timely maturity. Phosphorus is crucial for the ATP component. ATP is formed during photosynthesis and contains phosphorus as part of its structure (Tajer, 2016). It in the plants until achieve maturity as for their process nutrition and development. Phosphate is needed for formation of ADP and ATP and also as the building block for nucleic acids, nucleotides, sugar phosphates and many more (Nelson et al., 1993) especially during the active growth of plants. As for *L. minor*, it achieved the highest phosphate removal among the duckweeds with 86.43% at photoperiod 8:16 h removal efficiency at day 3 and capable of reducing phosphate concentration to a mere 3.45 mg/L. For *L. minor*, phosphate removal increment order in water samples in different photoperiod was as follows, 0:24 h < 24:0 H < 16:8 h < 8:16 h.