EFFECTS OF FERMENTED SOY PULP ON THE GROWTH OF FRESHWATER MICROALGAE

By

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DECLARATION

I hereby declare that the work embodied in this report is the original research and has not been submitted for a higher degree to any universities or institutions.

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I certify that the Report of this final year project entitled **"Effects of Fermented Soy Pulp on the Growth of Freshwater Microalgae"** by Che Wan Nursyahidah Binti Che Wan Azam, matric number F14A0039 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry Science), Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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Effect Of The Fermented Soy Pulp On The Growth Of The Freshwater Microalgae Culture

ABSTRACT

The purpose of the study is to investigate the effect of fermented soy pulp (FSP) and commercial fertilizer (NPK) on the growth performance of the freshwater microalgae culture.The fermented soy pulp was obtained at Prima Mekar Enterprise. The pond water that already filtered were put into a conical flask with different concentration mixed with the commercial fertilizer NPK (3g,6g and 9g) and the fermented soy pulp (FSP) (1g, 2g and 3g) (three replicate for each conical flask). The data were collected every morning at 7 days using the spectrophotometer reading (650 nm) and for the statistical analysis of the absorbance (AU) and the growth rate of the microalgae were done by using one way ANOVA and post hoc Tukey test at $p<0.05$ (SPSS version 23). At the end of the experiment, the FSP has the highest peak absorbance (AU) at (1.02±0.043) and the highest reading of the growth rate at (0.52±0.028). As the conclusion, FSP can be applied as the fertilizer in the freshwater microalgae culture replaced with the commercial fertilizer such as the NPK.

Keyword: Fermented soy pulp, commercial fertilizer, growth rate, microalgae and concentration

Kajian Tentang Kesan Soya Yang Ditapai Kepada Pertumbuhan Mikroalge Air Tawar

ABSTRAK

Tujuan kajian ini adalah untuk mengkaji kesan soya yang ditapai (FSP) dan baja komersial (NPK) terhadap prestasi pertumbuhan mikroalgae air tawar. soya yang ditapai diperolehi di Prima Mekar Enterprise. Air kolam yang sudah ditapis dimasukkan ke dalam balang konikal dengan kepekatan yang berbeza dicampur dengan baja komersil NPK pada konsentrasi 3g, 6g dan 9g dan soya yang ditapai (FSP) pada kepekatan 1g, 2g dan 3g (setiap balang konikal mempunyai tiga pengulangan). Data yang dikumpul setiap pagi pada 7 hari dengan menggunakan bacaan spektrofotometer (650 nm) dan untuk analisis statistik penyerapan (AU) dan kadar pertumbuhan mikroalgae dilakukan dengan menggunakan satu cara ANOVA dan ujian pos hoc pada $p < 0.05$ (SPSS versi 23). Pada akhir eksperimen, FSP mempunyai penyerapan puncak tertinggi (AU) pada (1.02 ± 0.043) dan bacaan paling tinggi pada kadar pertumbuhan pada (0.52 ± 0.028). Sebagai kesimpulan, FSP boleh digunakan sebagai baja dalam budaya mikroalgae air tawar yang digantikan dengan baja komersial seperti NPK.

Keyword: Soya yang ditapai, baja komersial, kadar pertumbuhan, microalga dan kepekatan

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LIST OF ABBREVIATIONS

AU	Absorbance
CO_2	Carbon Dioxide
dH_2O	Distilled Water
d	Days
g	Gram
gL^{-1}	Gram per Litre
GR	Growth Rate
h	Hour
H_2O	Water
H_2O_2	Hydrogen Peroxide
H_3PO_4	Phosphoric Acid
i.e	That is
K	Potassium
mgL^{-1}	Milligram per Litre
$mg.m^{-3}$	Milligram per cubic meter
mL	Milliliter

N	Nitrogen
ųm	Nanometer
OD	Optical Density
P	Phosphorus
pH	Potential of Hydrogen
ppm	Part per Million
SGR	Specific growth rate

LIST OF SYMBOL

%	Percentage
<	Less than
ºC	Degree Celsius
>	More than
±	Plus-minus

CHAPTER I

INTRODUCTION

1.1 Research Background

Microalgae is generally existent in the freshwater environment, where they exist as the microorganism, that can be seen through the microscope. They are important in the freshwater environment which is essential ecology and in the relation to the human use of the natural resources. (Sigee & Belliger, 2010)

Edward & David, 2010, state that the microalgae size range from a small size with is <1 µm to the bigger size range that can reach 2000 µm that are the globular colonies of the blue algae such as the *Microcystis.* The planktonic algae are generally characterized by the size range which is the picoplankton < 2 µm, nanoplankton 2 – 20 µm, microplankton 20 -200 µm and the macroplankton > 200 µm.

1.2 Introduction to the Soy Pulp

The soy pulp as the main source of the study as the organic fertilizer that can affect the growth of the freshwater microalgae. The soy pulp forms the soybean residue that being thrown out. Mostly, the soy pulp has been used as the animal feed, as the fertilizer and majorities as the source of the human food. The soy pulp wealth with the

source of protein, fibre, protein, vitamin and another source of the nutrient (Li et al., 2012)

Fermented soy pulp (*okara*) have been using as the food in Japan and Asian. Okara is a pulp consisting or insoluble part of the soybean which continue from the soybean are refine in the production of soy milk and tofu. Okara contains 3.5 to 4.0% of the protein, 76 to 80% moisture content and 20 to 24% of solids, 8 to 15% fat, 12 to 14.5% crude fibre and 24% protein if the contain moisture from the okara were removed out (Li et al., 2012)

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The production of the soybean as the main source of the food production all around the world. Every year, there is almost 1.1 kg of the soybean waste knows soybean curd residue (SCR) has been producing as the waste. Treated okara consumed more balance and change the ratio from soluble to insoluble dietary fibre which benefits as the prebiotic (Perez-Lopez et al., 2017).

1.3 Problem Statement

There is a lot of use of the fertilizer from the animal waste such as the chicken manure and waste from the animal that can contaminate of the microalgae and can cause the hygiene issue. Meanwhile, the use of the chemical fertilizer can cause an increase of the chemical in the contagion of the microalgae. There is the limited research about the soy pulp as the organic fertilizer compares to other agriculture waste as the fertilizer. Thus, application of organic fertilizer derived from fermented soy pulp (FSP) can act as the alternative fertilizer for microalgae culture that can reduce the rise contamination of the culture.

1.4 Hypothesis

There is the effect on the growth of the freshwater microalgae by using the treated fermented soy pulp and chemical fertilizer at different concentration.

1.5 Objective

The objective of the present studied was to study the effect of the fermented soy pulp on the growth of the freshwater microalgae.

1.6 The Scope Of Study

Microalgae are known as the phytoplankton. The effect of the organic and inorganic fertilizer on the growth of the freshwater microalgae. Study the production of the freshwater microalgae and the nutrient content in the biofertilizer effect the production of the microalgae.

1.7 Signification Of Study

Fermented soy pulp (FSP) can act as a low-cost alternative fertilizer for the freshwater microalgae culture.

1.8 Limitation Of Study

There are few previous studies that have been carried out using the fermented soy pulp (FSP) as the fertilizer in freshwater microalgae culture.

CHAPTER 2

LITERATURE REVIEW

2.1 General Overview Of Microalgae

Microalgae generally described the photoautotrophic, simple microscopic, unicellular plant that converts the sunlight energy to the biochemical product (Munir et al., 2015). Microalgae freely use the carbon dioxide (CO_2) from the atmosphere and mostly 50% microalgae are responsible for the global carbon reduction (N. Munir et, al, 2015). Microalgae growth can go through four phase which is the lag, exponential, stationary and death phase (Belliger & Sigee, 2010).

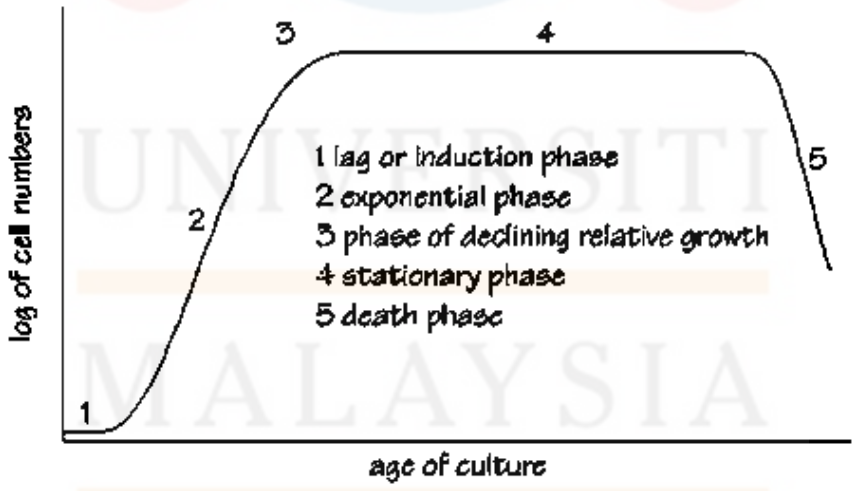

Figure 2.1: Five stages of the microalgae growth source from Lavens & Sorgeloos, (1996).

The growth mainly depends on the temperature, the source of the light and the species of microalgae. In the lag phase or induction phase, the growth is applied for the adaptation to the cell metabolism to growth. During the exponential phase, the cell density increase according to the growth of the population of microalgae. The growth of microalgae depends on many factors such as light, pH and temperature. Starting from the third phase which is the declining growth phase, in this phase, cell division will slowly decrease when the pH, nutrient source of light, CO_2 and another chemical factor slowly fully been absorbed by the microalgae. The stationary phase, the limitation factor and the growth rate are balance and for the death phase, the water quality is degraded and nutrient is depleted to inadequate of sustaining the growth and the cell density decrease (Lavens & Sorgeloos, 1996).

2.2 Important Component Of Microalgae

The microalgae contain 30 – 50% of the protein, 20 – 40% of the carbohydrate and 8 – 15% of the lipid and based on the study, the microalgae are the excellent source of the food to the other animal because of the protein contained in microalgae (Ren, 2014).

2.3 Microalgae Culture

As with any diverse group of organism, microalgae change in this requirement depending on the environment and habitat of microalgae, demanding different media and different handling techniques from culture to culture. Selecting the correct medium to culture is the key to success for microalgae to growth. (James, 2012).

2.4 Light

Microalgae need light as the source of the photosynthesis same as the plant. The source of the light depends on the density of the microalgae. Basically, microalgae require the light source to supplies the energy and for the photosynthesis process because microalgae are the autotrophic organism that only uses a part of the total sunlight spectrum 400-700 nm for the photosynthesis. (Bocsi at al., 2011). The source of light may from natural or can be supplied by the fluorescent tubes to discharge either in the blue or in the red light spectrum, and is very important the light source for the photosynthesis (Barsanti & Gualtieri, 2014).

2.5 Temperature

For the temperature, the culture should be controlled to the ideal as possible to the temperature which the organism was collected such as for the temperate 10 – 25^{o}C, and for the tropical should < 20^{o}C. mostly, the microalgae culture species can tolerate temperature between 16^{o}C and 27^{o}C. the temperature is below 16^{o}C will slow the growth of the microalgae and temperature higher than 35^{o}C, can be harmful to the species of microalgae.(Laura Barsanti & Paolo Gualtieri, 2014).

2.6 pH

The range pH of the microalgae species is between 7 to 9, the optimum range for microalgae between pH of 8.2 to 8.7. some species of microalgae can tolerance to the acidic condition that can reach to the pH 9 for the growth condition. (Lavens & Sorgeloos, 1996).

Table 2.1: Optimum condition for culture microalgae (Lavens & Sorgeloos, 1996).

Parameter	Range	Optimal condition
Temperature (°C)	16 – 27	18 – 24
pH	7 – 9	8.2 – 8.7
Source of light (lux)	1,000 – 10, 000 (depend on the volume and density)	2,500 – 5,000
Salinity ($g.l^{-1}$)	12 – 40	20 – 24

2.7 Aeration/ Mixing

According to the Laven et al., (1996), it is necessary to prevent the sedimentation of the microalgae and to ensure the cell population of the microalgae are equally be exposed to the nutrient and the source of light and also to improve the gas exchange between the culture with the air. The aeration contain carbon dioxide (CO_2) as the source for the photosynthesis and for a very dense culture, the CO_2 arising from the air bubble that containing about 0.03 % of CO_2 through the culture is limiting the growth of microalgae and the pure CO_2 can be supplemented to the air supply (Lavens & Sorgeloos, 1996).

2.8 Other Inorganic Nutrient Requirement

An optimal nutrient requirement for a specified microalgae might be not applied for another species in the same group. Generally, the nutrient that commonly a requirement for the microalgae such as the nitrogen (N), phosphorus (P), potassium (K). There is another macronutrient also important to the microalgae like magnesium (Mg), calcium (C), sulphur, but the macronutrient requires only in the small amount of concentration (Bocsi et al., 2011).

2.9 Analytical Method

Based on the study of Xin et al. (2011) the method for microalgae growth was determined by using the OD_{650} every 24 h. For the equation to determine the growth rate of the microalgae culture to an exponential function is followed by:

$$GR = (\ln OD_t - \ln OD_0) / t$$

Where OD_0 is the initial day and OD_t is the measured on the day t of the optical density (Wang, et al., 2010).

2.10 Soy Pulp Or *Okara* As Fertilizer

Okara basically develops to tofu or soymilk production process. Okara contains 25% protein, 10% lipids and another nutrient. (Li, Qiao, & Lu, 2012). The okara is the residue as a waste by-product in the soymilk and tofu processing industries. According to the Taruna et al, (2002), content 25.4 – 28.4% of protein, 9.3 – 10.9% of lipid, and

52.8 – 58.1% of fibre on the moisture-free basis.

Table 2.2: The combination of fertilizer that can be used for the mass culture of marine microalgae (Lavens & Sorgeloos, 1996).

Fertilizer	Concentration ($mg.l^{-1}$)					
	A	B	C	D	E	F
Ammonium sulfate	150	100	300	100	-	-
Urea	7.5	5	-	10-15	-	12-15
Calcium superphosphate	25	15	50	-	-	-
Clewat 32	-	5	-	-	-	-
N:P 16/20 fertilizer	-	-	-	10-15	-	-
N:P:K 16-20-20	-	-	-	-	12-15	-
N:P:K 14-14-14	-	-	-	-	-	30

A : contain Ferric chloride ($FeCl_3$) (0.8 g), Manganous chloride ($MnCl_2$, $4H_2O$) (0.4 g), Boric acid (H_3BO_3) (33.6 g), EDTA, di-sodium salt (45.0 g), Sodium di-hydrogen orthophosphate (NaH_2PO_4, $2H_2O$) (20.0 g), Sodium nitrate ($NaNO_3$) (100 g)

B: contain Zinc chloride ($ZnCl_2$) (2.1 g), Cobaltous chloride ($CoCl_2$,6 H_2O) (2.0 g), Ammonium molybdate ($(NH_4)_6Mo_7O_{24}$, $4H_2O$) (0.9 g), Cupric sulphate ($CuSO_4$, $5H_2O$) (2.0 g), Concentrated HCl (10.0 ml).

C: contain Vitamin B_1 (0.2 g), Solution E (25.0 ml).

D: contain Sodium metasilicate (Na_2SiO_3, $5H_2O$) (40.0 g).

E: contain Vitamin B_{12} (0.1 g)

F: contain Sodium nitrate ($NaNO_3$) (200.0 g).

CHAPTER 3

METHODOLOGY

3.1 Sample Collection

The sample of the freshwater took at the fish pond at the back of AgroTechpark UMK campus Jeli, Kelantan district. The temperature and pH were measured by using the pH meter and the digital thermometer. The sample of the fermented soy pulp obtains from Prima Mekar Enterprise.The commercial fertiliser NPK and other chemical and also for the equipment during the experiment provided at the FIAT's laboratory in the UMK Jeli lab.

3.2 Experimental Design

The sample of pond water was obtained from the Agro Techno Park and was filtered by using the 100 ųm of the plankton net in the 1000 mL in order to avoid another organism that can contaminate the culture before being transferred into the 400 mL conical flask. All the apparatus and material such as 400 mL conical flask, 100 mL measured cylinder, 1000 mL of Schott bottle and distilled water (dH_2O) were autoclaved to be sterilised to avoid any contamination. the concentration of the fermented soy pulp (FSP) was measured into 1 g, 2 g and 3 g and for the commercial fertilizer were measured into 3 g, 6 g and 9 g. The N:P:K ration of the commercial fertilizer and fermented soy pulp were same at which at the ± 1 %. The fermented soy pulp was put into three different tea bag according to the weight and each tea bag was wrapped with

another three layer of the tea bag to avoid the residue from the fermented soy pulp spread into culture during the experiment and also can affect the data collection. 360 mL of distilled water (dH_2O) that have been autoclave were added in the 400 mL conical flask followed by the fertilizer that wrapped with the tea bag and commercial fertilizer into were put into the different conical flask. All the conical flasks with the fertilizer in each treatment were autoclave again to be sterilised and keep at the room temperature in the laminar flow.

40 mL of the filter pond water was added to the 400 mL conical flask with the fertilizer in each treatment and swirled gently to form a homogenizes mixture. Then all the mouths of the conical flask were closed tightly with the cotton wool to prevent contamination during the experiment. All the processes mentioned above were conducted in laminar flow (AHC – 301, ESCO Luminar Flow Cabinet, US).

All the conical flasks were placed on the rack and were provided with the artificial light (fluorescent bulb) and aeration for 42 h in 7 days. All the aeration tube were stuffed with the cotton wool to avoid evaporation of the treatment. the water parameter all treatment such as pH and temperature were monitored and record every morning by using the portable thermometer and pH meter. The reading of absorbance (AU) also was recorded every morning by using the spectrophotometer (Genesys 20, Spectro Nic Instrument, UK). All the treatment were carried out in the triplicate.

The experiment was carried out in the three cycle, which each of cycle had a duration of 7 days. The aeration tube was changed every and all the apparatus were autoclaved before started the new cycle to minimize the risk of the contamination. The pH of all treatment was maintained. If the pH of the culture in acidity condition, the lime

power were added to the culture to neutralize the mixture.

3.3 Growth Performance Of Freshwater Microalgae

According to Wang et al., (2010), for the measurement of the optical density (OD), this research using the OD_{680} using a spectrophotometer (Genesys 5, Spectronic Instruments, UK) as the algae density indicator. And for the equation to determine the specific growth rate of the microalgae culture to an exponential function as follow:

$$SGR = (\ln OD_t - \ln OD_0) / t$$

Where the OD_0 is the optical density at the initial day and OD_t is the optical density measured day *t* (Wang, et al., 2010).

3.4 Statistical Analysis

Data used to analyse using the one-way analysis of variance (ANOVA) and post hoc Tukey test at $p < 0.05$ using statistical package for the social sciences (SPSS) version 23. The graph is generated using the Microsoft Excel 2010 (Nabris, 2012; Ak, 2012; Yuan, 2013).

CHAPTER 4

RESULT

4.1 Correlation between Temperature, pH and Optical Density (OD) of the growth freshwater microalga.

Microalgae cultivation with a different type of fertilizer were carried out at the same volume of cultivation, temperature, light and the same environment in the laboratory.

Based on table 4.1, the relationship between optical density (OD) and pH was strongly related at 0.950 at significant level $p<0.01$. the correlation between temperature and pH have a correlation at 0.22 at significant level $p<0.05$.m.

Table 4.1: Correlation between temperature, pH and Optical Density (OD) for the growth of the freshwater microalgae culture.

	Temperature (^{o}C)	pH	Optical density (OD)
Temperature	1		
pH	0.292*	1	
AU	0.158	0.950**	1

Note: * $p <0.05$, ** $p < 0.01$

4.2 Optical Density (OD) vs Time of growth freshwater microalgae

The mean and stand deviation of optical density (OD) of the freshwater microalgae that were treated with different treatment fertilizer was demonstrated in Figure 4.1. Among of all concentration the of FSP fertilizer, the highest OD reading is microalgae culture treated with FSP at day 7 at (1.02 ± 0.043) and the lowest OD reading is the microalgae culture treated with the NPK 6 g at a value (0.05 ± 0.005). the moderate reading of microalgae culture OD for the FSP 2 g (0.81 ± 0.032), FSP 1 g (0.69 ± 0.006), NPK 9 g (0.07 ± 0.101) and NPK 3 g (0.08 ± 0.007).

Based on figure 4.1, the microalgae growth phase for the NPK 3 g, the lag phase starting from day 1 to day 3, and for the exponential phase starting from day 3 to day 4, the declining phase starting from day 4 to day 6 and for stationary phase started from day 6 to day 7. For the NPK 6g, the lag phase has been recorded starting at day 1 until day, for the exponential phase, started from day 2 to day 3, for the declining phase started at day 3 to day 4 and of the stationary phase, started at day 4 to day 5 and lastly, the stationary phase started on day 5 to the day 7. For the NPK 9 g, the lag phase started at day 1 until day 3 and continue with the exponential phase started at day 3 to day 4 and for the declining phase started at day 4 to the day 6 and at day 6 to day 7 started entered the stationary phase.

For the FSP 1 g, the lag phase started at day 1 until day 2 and started entered the exponential phase at day 2 until day 4. The declining phase started at day 4 until day 5 and the stationary phase started at day 5 until to day 7. For microalgae that supplied with FSP 2g at day 1 to day 2 entered the lag phase and from day 2 until day 4 at the exponential phase and at the declining phase started at day 4 until day 5 and for the stationary phase started at day 5 to the day 7. And for the FSP 3 g, the lag phase starting at the day 1 to day 3, from the day 3 to day 4 started entered the exponential phase, the declining phase started at day 4 and 5 and from day 5 to the day 7 started to enter the stationary phase.

There are significant differences between FSP 3 g with the FSP 2 g at $p<0.005$. for the FSP 2 g have significate different with the FSP 1 g at $p<0.005$ and FSP 1 g have significant differences with the NPK 9 g, NPK 6g and NPK 3g, but for the NPK 9 g, NPK 6 g and NPK 3 g there are no significant differences of this three fertilizer.

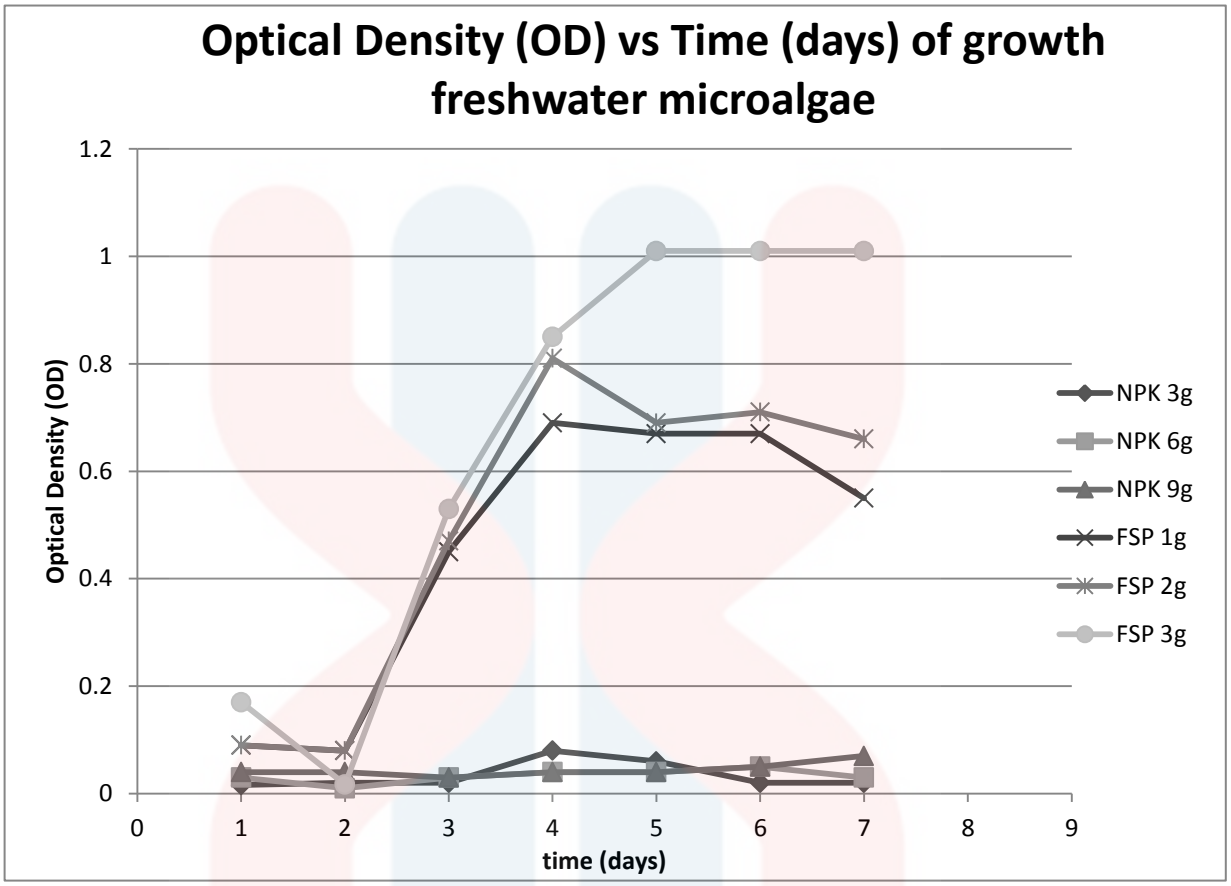

Figure 4.1: Graph of mean ± standard deviation of the optical density (OD) vs time (days) of the growth freshwater microalgae.

4.3 Specific Growth Rate (d^{-1}) vs Fertilizer Concentration

Based on Figure 4.2, the highest specific growth rate (SGR) is observed in microalgae culture that was supplied with 3 g of NPK with a value of 0.59 ±0.091 d^{-1}. The lowest SGR was recorded by microalgae culture that was treated with 6 g of chemical fertilizer (0.08 ± 0.046 d^{-1}). The rest of the treatments exhibited moderate growth, NPK 9 g at value (0.20 ± 0.046), FSP 1 g (0.21 ± 0.026), FSP 2 g (0.27 ± 0.028), and FSP 3 g (0.13 ± 0.013).

Even though the treatment NPK 3 g had the highest SGR, the microalgae concentration was still low (0.08 ± 0.007 OD) compared to microalgae that were treated with FSP. FSP 3 g showed the highest concentration of microalgae (1.02 ± 0.043 OD) at day 7 of the experiment although it had a lower SGR. For the rest treatment shown moderate growth for FSP 2 g at value (0.81 ± 0.032 OD), FSP 1 g (0.69 ± 0.006 OD), NPK 9 g (0.07 ± 0.101 OD) and NPK 6 g (0.05 ± 0.005).

The SGR, there is significate different between NPK 3 g with the FSP 2 g. For the FSP 2 g have are no significate different between FSP 1 g. For FSP 1 g also do not have significate different with the NPK 9 g and FSP 3 have significate different with the NPK 9g but do not have any significate different with the FSP 2 g.

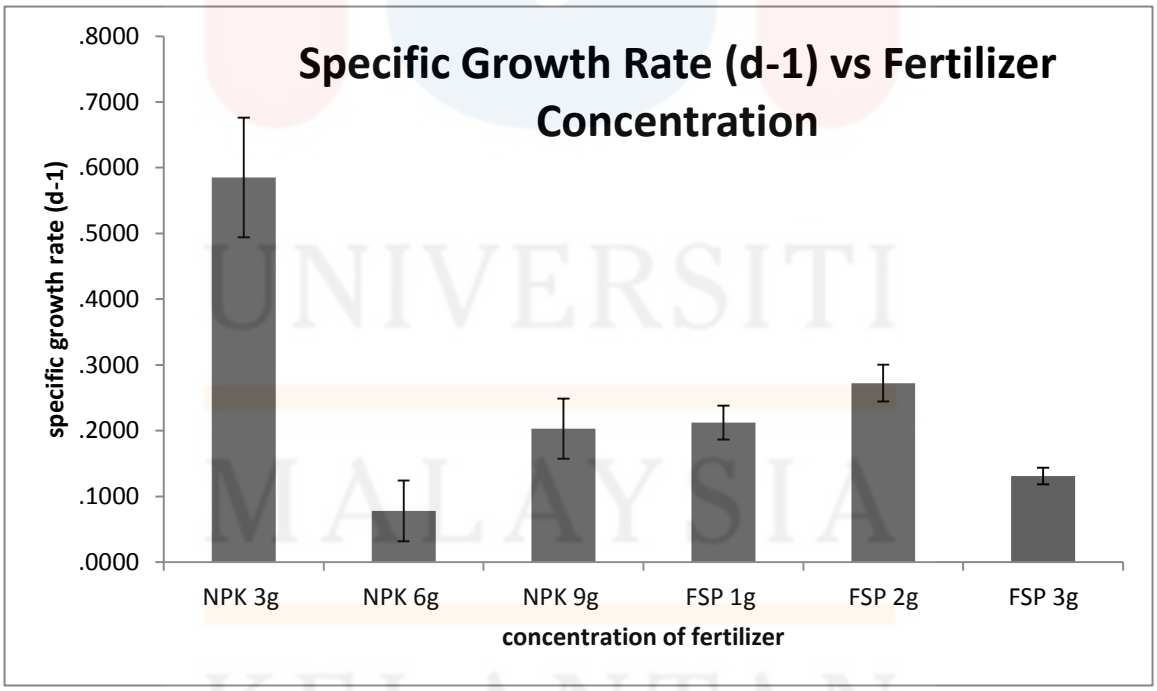

Figure 4.2: Graph specific growth rate (d^{-1}) vs fertilizer concentration

CHAPTER 5

DISCUSSION

Based of Singh & Singh (2015), the maximum growth was found the studies at a value (1.73 d^{-1}) for microalgae and the maximum growth rate was at a value (0.10 d^{-1}) of microalgae at temperature 25^{o}C were recorded in the studied. According to the previous study, recorded the specific growth rate (SGR) reported that the highest SGR was based on Figure 4.2, in microalgae culture that was supplied with 3 g of NPK with a value of 0.59 ±0.091 d^{-1}. The lowest SGR was recorded by microalgae culture that was treated with 6 g of chemical fertilizer (0.08 ± 0.046 d^{-1}). The rest of the treatments exhibited moderate growth, NPK 9 g at value (0.20 ± 0.046), FSP 1 g (0.21 ± 0.026), FSP 2 g (0.27 ± 0.028), and FSP 3 g (0.13 ± 0.013). This result was completely contrasting from optical density (OD) growth where the FSP recorded the highest reading at a value (1.02 ± 0.043 OD) and freshwater microalgae that supplied with the commercial fertilizer NPK recorded the lower reading at a value (0.05 ± 0.005 OD).

According to the Ak (2011), the best growth of the microalgae were determined at the organic agriculture fertilizer concentration at 0.01 mL/l and was silimar to the current studies, which is the microalgae that supplied with the fermented soy pulp (FSP) have best growth performance based on the optical density (OD) reading. Based on the previous studies, the FSP was better fertilizer compare to the commercial fertilizer based on the optical density (OD) of the freshwater microalgae, it can be proven that when the

brown colour of the FSP treatment turned into the greenish- brown which that showed the present the growth the microalgae. For the FSP 3 g have the highest reading of OD compare with the FSP 1 g and FSP 2 g.

Based on the Nabris (2012), showed that combination of agriculture fertilizer with such as ammonia, urea, calcium superphospate, micronutrient solution and vitamin solution show the higher performace of the growth microalgae because the nitrogen source, since the nitrogen is the major nutrient for microalgae cultivation compared with the previous studies, the commercial fertilizer show the highest specific growth rate compare with the fermented soy pulp (FSP), althought commercial fertlizer showed the highest growth rate, the microalgae concentration still lower compared with the fermented soy pulp (FSP) in the OD reading.

The optimum range at 8.2 – 8.7, though there are some species that can tolerate the acid or the based environment. (Barsanti & Gualtieri, 2014). In the previous studies, the pH of the freshwater microalgae culture were at range 3.88 ± 4.04 at the acid environment and there were still have the growth of the population of the microalgae and it has been proven by Barsanti & Gualtieri (2014), that some species of the freshwater microalgae can be tolerance to the acid-base environment.

From the previous studies, based on figure 4.1 optical density (OD) graph show the clear phase of growth freshwater microalgae of the treatment fermented soy pulp (FSP) which was showed the lag, exponential, declining, stationary and death phase compare with the commercial fertilizer which is not showed very clear phase the growth performance of the freshwater microalgae. According to the Lavens and Sorgelous

(1996), characterized the microalgae growth curve by 5 phase which is the lag phase, exponential phase, decline phase, stationary phase and death phase. For the lag phase for FSP treatment can be identified but for the NPK cannot be identified. For the death, the phase cannot be identified but still have the decrease the reading in the optical density (OD) reading.

The growth of the freshwater microalgae that supplied with the fermented soy pulp (FSP) was higher compared with the freshwater microalga that supplied with the commercial fertiliser lower that fermented soy pulp (FSP). Based on figure 4.1, the fermented soy pulp (FSP) have the highest peak reading at day 7 at value (1.02 ± 0.043 OD) and the lowest peak reading was the commercial fertilizer NPK at value (0.05 ± 0.005 OD) and the exhibit moderate growth of microalgae culture supplies with FSP 2 g (0.81 ± 0.032 OD), FSP 1 g (0.69 ± 0.006 OD), NPK 9 g (0.07 ± 0.101 OD) and NPK 3 g (0.08 ± 0.007 OD).

During the experiment were conducted, there is some problem that has to face with was the monitoring and maintain the pH of the culture increased acidity to the neutral environment and showed that even the pH was acidic, there are still have the growth and increase the population of the freshwater microalgae. From the table 4.1 show that pH was highly correlated with the OD at level significant $p<0.01$. The pH of the fermented soy pulp was at 3.88 ± 4.04 in the acidity environment which excess from the optimum pH of the freshwater microalgae cultivation. The culture became acidity assumed the from the fermented soy pulp (FSP) fertilizer. According to the Chakraborty et al (2011), stated that the pH of the culture can affect the freshwater microalgae growth. Based of in this study, the pH of the freshwater increased from acidic to neutral

and can reach a maximum at 9.35 $mg.m^{-3}$ at the pH 8.15 and signify that green algae were influenced phytoplankton compared to cyanobacteria growth. It showed that the microalgae can adapt and survive in the acidic environment and also enhance to increase the population of the microalgae. When the pH increased to acidic, the lime power was added in the fermented soy pulp (FSP) treatment at different concentration and homogeneous the culture of freshwater microalgae until pH in a neutral environment.

Another problem was the contamination of the culture. Factor that caused the contamination of the culture were there is no proper place for the freshwater microalgae culture and the culture place was not properly disinfect. This can cause the growth of the fungi in the culture and another microorganism in the culture. As the precaution step to overcoming this problem was, all the apparatus and distilled water (dH_2O) were autoclaved prior to the experiment. Next, the pond water was filtered using the 100 µm plankton net to get rid of other microorganism and the impurities and the tube of the aerator were sprayed with 70% of ethanol.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

In the conclusion, the use of the fermented soy pulp (FSP) is the fertilizer that can affect the freshwater microalgae growth, FSP contains the more nutrient compare to commercial fertilizer that required by the freshwater microalgae. fermented soy pulp (FSP) is recommended by the farmer especially for those wants to reduce the cost using the commercial fertilizer in the freshwater microalgae culture and FSP is an eco-friendly fertilizer that is hygienic with reducing odour compared to other agriculture waste such as-as aminal manure. Fermented soy pulp (FSP) can easily earn at the market. FSP can use as the cheap alternative fertilizer to the commercial fertilizer that available in the market.

Another suggestion that in the future freshwater microalgae cultivation have the proper biosecurity with a suitable room for the cultivation of freshwater microalgae. This is the precaution measured to ensure the culture have the low risk of the contamination, compare with the previous studies, the experiment was conducted in the laboratory but do not have proper biosecurity procedure was apply which can lead to the risk of the contamination from another microorganism in the freshwater microalgae.

As the recommendation, have the future studied should be carried out by using the fermented soy pulp (FSP) as the fertilizer for the specific growth of freshwater microalgae species. And have the future research on the combination of the commercial fertilizer with the fermented soy pulp and the effect on the freshwater microalgae growth performance.

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REFERENCES

Abou-Waly, H., Abou-Setta, M. M., Nigg, H. N., & Mallory, L. L. (2017). Growth Response of Freshwater Algae, *Anabaena flos-aquae* and *Selenastrum capricornutum* to Atrazine and Hexazinone Herbicides. *Bulletin of Environment Contamination and Toxicology*, 223-229.

Agbede, M., T., Ojeniyi, S., & A.J., A. (2008). Effect of Poultry Manure on Soil Physical and Chemical Properties, Growth and Grain Yield of Sorghum in Southwest, Nigeria. *American-Eurasian Journal of Sustainable Agriculture*, 72-77.

Ak, i. (2012). Effect of an organic fertilizer on the growth of blue-green alga *Spirulina platensis. Aquaculture International*, 413-422.

Aneesh, C. N., Haridas, A., & Manilal, V. B. (2015). Role of Nutrients Input Pattern on the Growth Dynamics of Common Freshwater Microalgal Community. *American Journal of Plant Sciences*, 2481--2491.

Apel, A. C., & Weuster-Botz, D. (2014). Engineering solutions for open microalgae mass cultivation and realistic indoor simulation of outdoor environments. *Bioprocess and Biosystems Engineering*.

Aroge, S. O. (2005). Evaluation of the nutritional composition, sensory and physical properties of a potential weaning food from locally available food material - Breadfruit (*Artocarpus altiilis*) and soybean (*Glycine max*). *Polish Journal of Food and nutrition sciences,* 411-415.

Asele, O. D.-O. (2006). The induced growth of phytoplankton using two fertilizers (NPK and agrolyser) under laboratory conditions. *African Journal of Biotechnology*, 373-377.

Bhola, V., Swalaha, F., Kumar, R. R., Singh, M., & Bux, F. (2012). Overview of the potential of microalgae for CO2 sequestration. International Journal of Environment Science and Technology.

Bocsi, R., Horvath, G., L.Hanak, & Z.Hodai. (2011). Extraction examination of microalgae propagated for biodiesel additives. H*ungarian Journal of industrial chemistry*, 45-49.

C.Butterwick, Heaney, S., & J.F.Talling. (2005). Diversity in the influence of temperature on the growth rates of freshwater algae, and its ecological relevance. F*reshwater Biology,* 291-300.

Chakraborty, P., Acharyya, T., Babu, P. V., & Bandhyopadhyay, D. (2011). Impact of salinity and pH on phytoplankton community in a tropical freshwater system: An investigation with pigment analysis by HPLC. *Journal Environment Monitor*, 614-620.

Flynn, K. J., & Butler, I. (Applied Biochemistry and Biotechnology). Nitrogen sources for the growth of marine microalgae: the role of dissolved free amino acids. *Marine Ecology*, 281-304.

Gouveia, L., Graça, S., Sousa, C., Ambrosano, L., Ribeiro, B., Botrel, E. P., Silva, C. M. (2016). Microalgae biomass production using wastewater: Treatment and costs Scale-up considerations. *Algal Research*, 167-176.

Grobbelaar, J. U. (2012). Microalgae mass culture: the constraints of scaling-up. *Journal of Applied Phycology*, 315-318.

Gualtieri, L. B. (2014). Algae Anatomy, biochemistry, and Biotechnology Second Edition. New York: *Taylor & Francis group.*

James, D. E. (2012). Culturing Algae. US: *Carolina Biological Supply Company.*

Kirroliaa, A., Bishnoia, N. R., & Singh, N. (2011). Salinity as a factor affecting the physiological and biochemical traits of *Scenedesmus quadricauda. Journal of Algal Biomass Utilization*, 28-34.

Kumar, M. S., Miao, Z. H., & Wyatt, S. K. (2010). Influence of nutrient loads, feeding frequency and inoculum source on the growth of *Chlorella vulgaris* in digested piggery effluent culture medium. *Bioresource Technology,* 6012-6018.

Lam, M. K., & Lee, K. T. (2012). Potential of using an organic fertilizer to cultivate *Chlorella vulgaris* for biodiesel production. *Applied Energy*, 303-308.

Lavens, P., & Sorgeloos, P. (1996). Manual on the Production and Use of Live Food for Aquaculture. Ghent, Belgium: *Fao Fisheries Technical Paper.*

Li, B., & Lu, M. &. (2012). Composition, Nutrition, and Utilization of *Okara* (Soybean Residue). *Journal Food Review International*, 231-252.

Li, B., Qiao, M., & Lu, F. (2012). Composition, Nutrition, and Utilization of *Okara* (Soybean Residue). *Food Reviews International,* 231-252.

Li, Y., Chen, Y.-F., Chen, P., Min, M., Zhou, W., Martinez, B., Ruan, R. (2011). Characterization of a microalga *Chlorella sp*. well adapted to highly concentrated

municipal wastewater for nutrient removal and biodiesel production. *Bioresource Technology*, 5138-5144.

Makara, A., & Kowalski, Z. (2015). Pig manure treatment and purification by filtration. *Journal of Environmental Management,* 317-324.

Markou, G., & Georgakakis, D. (2011). Cultivation of filamentous *cyanobacteria* (blue-green algae) in agro-industrial. *Applied Energy*, 3389-3401.

Maryam Al-Qasmi, N. R.-R.-B. (2012). A Review of Effect of Light on Microalgae Growth. *Proceedings of the World Congress on Engineering* 2012.

Mulbry, W. W., & Wilkie, A. C. (2001). The growth of benthic freshwater algae on dairy manures. *Journal of Applied Phycology,* 301-306.

Munir, N., Asghar, M., Tahir, I. M., Riaz, M., Bilal, M., & Shah, S. M. (2015). Utilization of Agro-Wastes for Production of Ligninolytic Enzymes in Liquid State Fermentation by *Phanerochaete Chrysosporium*-Ibl-03. In*ternational Journal of Chemical and Biochemical Sciences,* 9-14.

Nabris, K. J.-A. (2012). Development of Cheap and Simple Culture Medium for the Microalgae *Nannochloropsis sp*. Based on Agricultural Grade Fertilizers Available in the Local Market of Gaza Strip (Palestine). *Journal Al Azhar University-Gaza (Natural Sciences)*, 61-76.

Nayak, M., Thirunavoukkarasu, M., & Mohanty, R. C. (2016). Cultivation of freshwater microalga *Scenedesmus sp*. using a low-cost inorganic fertilizer for enhanced biomass and lipid yield. *Journal of General and Applied Microbiology,* 7-13.

Pleissner, D., Lam, W. C., Sun, Z., & KiLin, C. S. (2013). Food waste as nutrient source heterotrophic microalgae cultivation. *Bioresource Technology*, 139-146.

Priyadarshani, I., & Rath, B. (2013). Commercial and industrial applications of microalgae – A review. *Journal of Algal Biomass Utilization,* 89-100.

Quinn, J., Winter, L. d., & Bradley, T. (2011). Microalgae bulk growth model with application to industrial scale systems. *Bioresource Technology,* 5083-5092.

Riano, B., Hernández, D., & García-González, M. (2012). Microalgal-based systems for wastewater treatment: Effect of applied organic and nutrient loading rate on biomass composition. *Ecological Engineering,* 112-117.

Rodriguez, E. G., & Maestrini, S. Y. (1984). The use of some agriculture fertilizer for the mass production of marine algae. *Aquaculture*, 245-256.

Sigee, E. G. (2010). Freshwater Microalgae Identification and Use as Bioindicators. UK: *Wiley-Blackwell.*

Taruna, I., & Jindal, V. K. (2002). Drying of soy pulp (*Okara)* in a bed of inert particles. *An International Journal*, 1035-1051.

Wang, H., Zhang, W., Chen, L., Wang, J., & Liu, T. (2013). The contamination and control of biological pollutants in mass cultivation of microalgae. *Bioresource Technology*, 745-750.

Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., Ruan, R. (2010). Cultivation of Green Algae *Chlorella sp*. in Different Wastewaters from Municipal Wastewater Treatment Plant. *Applied Biochemistry and Biotechnology,* 1174-1186.

Xin, L., Hong-ying, H., & Yu-ping, Z. (2011). Growth and lipid accumulation properties of a freshwater microalga *Scenedesmus sp*. under different cultivation temperature. *Bioresource Technology*, 3098-3102.

Xin, L., Hong-ying, H., Ke, G., & Ying-xue, S. (2010). Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus sp*. *Bioresource Technology*, 5494-5500.

Yuan, L. Z. (2013). Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. *Water Research,* 4294-4302.

APPENDICES A

Table A.1: Correlations pH, Temperature and Optical Density (OD)

		pH	°C	AU
pH	Pearson Correlation	1	.292*	.950**
	Sig. (2-tailed)		.032	.000
	N	54	54	54
°C	Pearson Correlation	.292*	1	.158
	Sig. (2-tailed)	.032		.253
	N	54	54	54
AU	Pearson Correlation	.950**	.158	1
	Sig. (2-tailed)	.000	.253	
	N	54	54	54

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table A.2: ANOVA table for the Optical Density (OD) of the growth freshwater microalgae FSP and NPK.

		Sum of Squares	df	Mean Square	F	Sig.
DAY1	Between Groups	.142	5	.028	425.368	.000
	Within Groups	.003	48	.000		
	Total	.145	53			
DAY2	Between Groups	.159	5	.032	453.752	.000
	Within Groups	.003	48	.000		
	Total	.162	53			
DAY3	Between Groups	2.810	5	.562	861.439	.000
	Within Groups	.031	48	.001		
	Total	2.841	53			

DAY4	Between Groups	7.363	5	1.473	1398.103	.000
	Within Groups	.051	48	.001		
	Total	7.414	53			
DAY5	Between Groups	8.191	5	1.638	1771.504	.000
	Within Groups	.044	48	.001		
	Total	8.235	53			
DAY6	Between Groups	8.332	5	1.666	1362.201	.000
	Within Groups	.059	48	.001		
	Total	8.391	53			
DAY7	Between Groups	7.701	5	1.540	2459.083	.000
	Within Groups	.030	48	.001		
	Total	7.731	53			

Table A.3: One way ANOVA of the peak Optical Density (OD)

ANOVA

peaked

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.589	5	1.718	3331.043	.000
Within Groups	.025	48	.001		
Total	8.614	53			

Table A.4: Post Hoc Test of peak of Optical density (OD)

peaked

Tukey HSD[a]

FERTILIZER	N	Subset for alpha = 0.05			
		1	2	3	4
npk6g	9	.0458			
npk9g	9	.0718			
npk3g	9	.0751			
fsp1g	9		.6898		
fsp2g	9			.8074	
fsp3g	9				1.0176
Sig.		.086	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

Table A.5: Mean ± standard deviation of the Optical density (OD)

Concentration of fertilizer	Peak optical density (OD)
NPK 3g	0.08 ± 0.007[d]
NPK 6g	0.05 ± 0.005[d]
NPK 9g	0.07 ± 0.010[d]
FSP 1g	0.69 ± 0.006[c]
FSP 2g	0.80 ± 0.032[b]
FSP 3g	1.02 ± 0.403[a]

a: highest peak OD, b & c: moderate peak OD, d: lowest peak OD

Table A.6: One way ANOVA specific growth rate (SGR) of freshwater microalgae

ANOVA

SGR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.440	5	.288	122.255	.000
Within Groups	.113	48	.002		
Total	1.553	53			

Table A.7: Post Hoc Specific Growth Rate (SGR) of freshwater microalgae

SGR

Tukey HSD[a]

treatment	N	Subset for alpha = 0.05			
		1	2	3	4
fsp2g	9	.0780			
fsp3g	9	.1311			
npk9g	9		.2029		
fsp1g	9		.2125	.2125	
fsp2g	9			.2724	
npk3g	9				.5851
Sig.		.207	.998	.112	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

Table A.8: Mean ± standard deviation of specific growth rate (SGR) of freshwater microalgae

Concentration of fertilizer	Specific growth rate (d^{-1})
NPK 3g	0.59 ± 0.091^{a}
NPK 6g	0.08 ± 0.046^{d}
NPK 9g	0.20 ± 0.046^{c}
FSP 1g	0.21 ± 0.026^{b}
FSP 2g	0.27 ± 0.028^{b}
FSP 3g	0.13 ± 0.013^{c}

a: highest SGR, b & c: moderate SGR, d: lowest SGR

APPENDICES B

Figure B.1: Collect the pond water.

Figure B.2: Fermented soy pulp used as the fertilizer in the freshwater microalgae culture.

Figure B.3: Mixed the pond water into the conical flask.

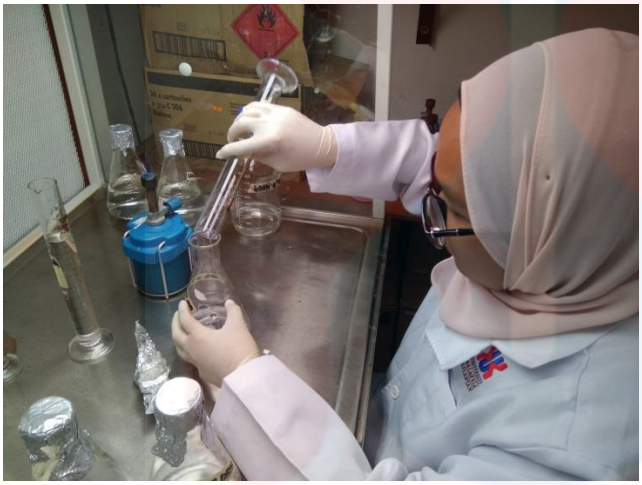

Figure B.4: Distilled water (dH_2O) were autoclaved were put into the 400 mL of conical flask.

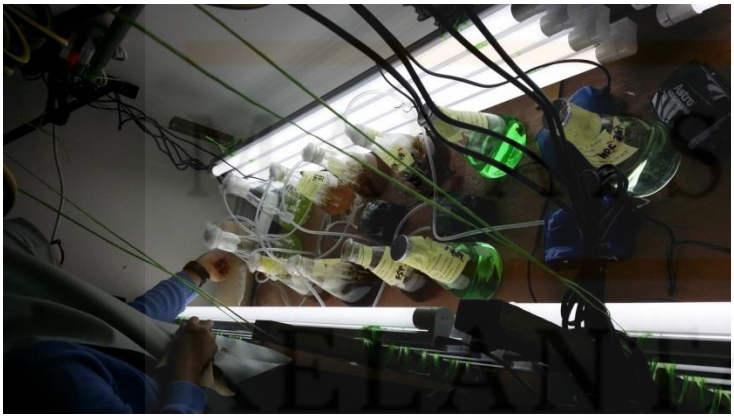

Figure B.5: Setup the place of freshwater microalgae culture.

Figure B.6: Reading were recorded using the spectrophotometer.

Figure B.7: The ammonia test to determined pH in the freshwater microalgae culture.

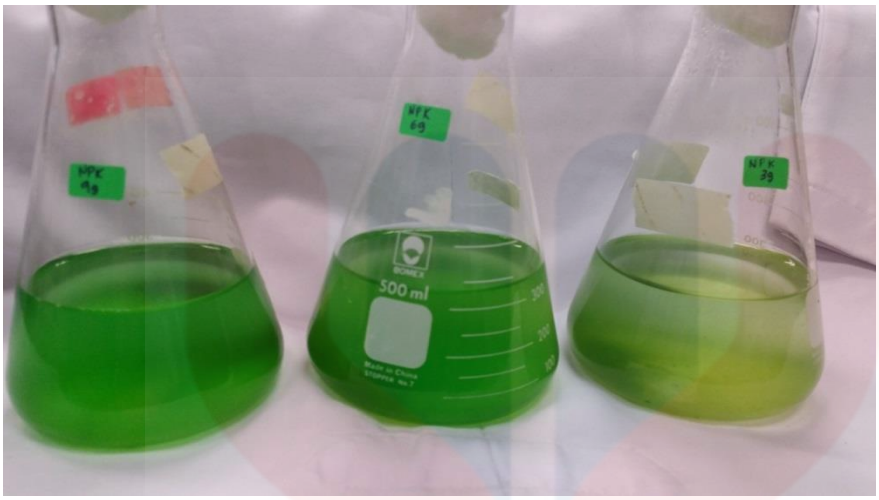

Figure B.8: Sample of freshwater microalgae that supplied with the commercial fertilizer.

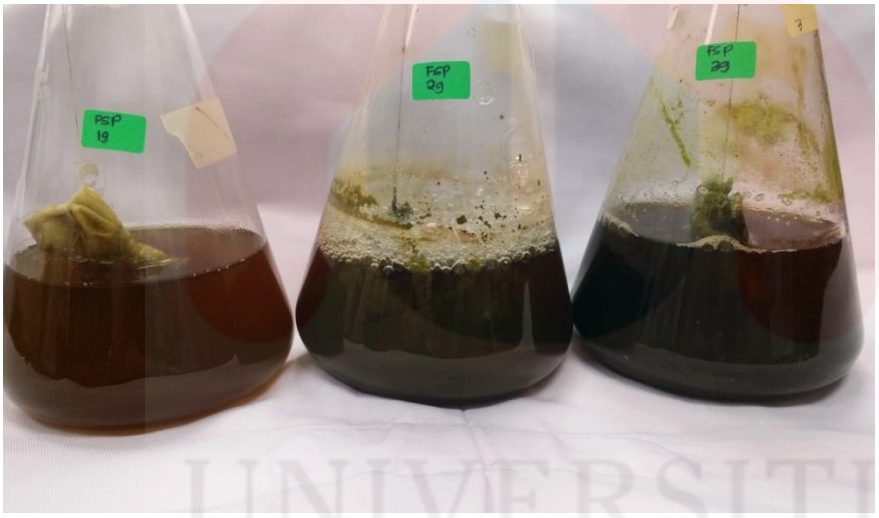

Figure B.8: Sample of freshwater microalgae with supplied fermented soy pulp (FSP).