BIO-FLOTATION HARVESTING OF *CHLORELLA VULGARIS* MICROALGAE USING *JATROPHA CURCAS* L. PROTEIN-OIL EMULSION

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BIO-FLOTATION HARVESTING OF *CHLORELLA VULGARIS* MICROALGAE USING *JATROPHA CURCAS* L. PROTEIN-OIL EMULSION

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ABSTRACT

This study is a whole new approach to recover microalgae from aqueous medium using bio-flotation with its effectiveness examined. This new bio-flotation method involves utilizing a *Jatropha curcas* protein extract-oil emulsion mixed with sunflower oil for flotation removal of *Chlorella vulgaris* microalgae from its culture medium. Asia, particularly Japan and Taiwan are the largest producer country for *Chlorella vulgaris* in the world and different uses of this microalgae is being discover from time to time involving food, medical, aquaculturally, water treatment, skincare, and biofuel industry. Whereby, the dewatering stage of microalgae production is always a critical issue. Researches and comparison have been done among current common dewatering method including coagulation, flocculation, centrifugation, sedimentation, and many more to improvise this study. This project covered the process from microalgae cultivation, protein extraction of *Jatropha* seed, oil emulsion testing to flotation experiment under various parameters. The effectiveness of this method under various factors has been determined, including operating parameters such as pH, protein-oil emulsion dosage, and mixing time. A maximum flotation efficiency of 81% was achieved under protein-oil emulsion dosage of 20ml/L, pH 2, mixing time 4 min. Furthermore, the change of zeta potential of the microalgae is analysed and showed a mark difference in value affected by the protein-oil emulsion. This flotation method is not only simple, low cost, environmentally friendly, but also an efficient method for harvesting microalgae from culture medium. For the future scope, improvement should be focus on the pH limitations to enhance the feasibility of this method for large scale industrial implementation.

ABSTRAK

Kajian ini merupakan pendekatan baru untuk mendapatkan mikroalga dari medium berair menggunakan bio-flotation dengan keberkesanannya diperiksa. Kaedah bio-flotation yang baru ini melibatkan penggunaan emulsi minyak ekstrak buah *Jatropha curcas* bercampur dengan minyak bunga matahari untuk penyingkiran pengapungan mikroalgae *Chlorella vulgaris* dari medium berairnya. Asia, terutamanya Jepun dan Taiwan merupakan negara pengeluar terbesar untuk *Chlorella vulgaris* di seluruh dunia dan pelbagai kegunaan mikroalga ini sedang ditemui dari semasa ke semasa yang melibatkan bidang makanan, perubatan, akuakultur, rawatan air, penjagaan kulit dan industri biofuel. Dengan ini, peringkat penyahairan pengeluaran mikroalga sentiasa menjadi isu kritikal. Penyelidikan dan perbandingan telah dilakukan di kalangan kaedah penyahairan umum seperti koagulasi, pemberbukuan, sentrifugasi, pemendapan, dan banyak lagi untuk menambah baik kajian ini. Projek ini meliputi proses dari penanaman mikroalgae, pengekstrakan protein benih *Jatropha*, ujian emulsi minyak kepada percubaan pengapungan di bawah pelbagai parameter. Keberkesanan kaedah ini di bawah pelbagai faktor telah dikajikan, termasuk parameter operasi seperti pH, dos emulsi minyak-protein, dan masa pencampuran. Kecekapan pengapungan maksima sebanyak 81% dicapai di bawah dos emulsi minyak-protein 20ml / L, pH 2, masa pencampuran 4 min. Tambahan pula, perubahan potensi zeta mikroalgae dianalisis dan menunjukkan perbezaan nilai di bawah pengaruh emulsi minyak-protein. Kaedah pengapungan ini bukan sahaja mudah, kos rendah, mesra alam, tetapi juga kaedah yang efisien untuk menuai mikroalga dari medium berairnya. Untuk skop masa depan, penambahbaikan harus menumpukan kepada batasan pH untuk meningkatkan kemungkinan kaedah ini untuk pelaksanaan industri skala besar.

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LIST OF SYMBOLS

cm	Centimetre
°C	Degree Celsius
kg	kilogram
L	litre
m	Meter
µm	Micro meter
ml	Millilitre
mm	Millimetre
min	Minute
%	percent
mg	milligram
d	day
g	gram
h	hour
m^3	volume in meter
m^2	area in meter
v	volume
ppm	parts per million
Mpa	megapascal
rpm	revolutions per minute
nm	nanometre
µ	specific growth rate

LIST OF ABBREVIATION

USA	United State of America
PUFAs	poly-unsaturated fatty acids
SPT	Spiral plate technology
CO_2	Carbon dioxide
Fe_{3+}	Iron cation
Al_{3+}	Aluminium cation
JPOE	*Jatropha* protein-oil emulsion
C.vulgaris	*Chlorella vulgaris*
NA	Not available
BBM	Bold's Basal Medium
O_2	Oxygen
J.curcas	*Jatropha curcas*
H_2O	water
UMP	Universiti Malaysia Pahang
DI water	Deionized water
H_2SO_4	Sulphuric acid
KOH	Potassium hydroxide
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

1.1. PROJECT BACKGROUND

Increasing in population is good news for human community, the current average population increase is estimated at 83 million people per year (Worldometers, 2018). However, what come behind is the high demand of resources. Fear that we are running out of important resources is perpetual. Oil is a favourite thing to worry about; landfill space is another, and trees yet another (Lee, 2000).

Therefore, microalgae are gradually become more popular in choice of cultivation in multiple industry field, which provide much more benefits more than a regular cultivation crop. Microalgae is a type of single-celled organisms which able to store significant amounts of energy rich compounds used in biofuels production, such as biodiesel and ethanol. Secondly, they can grow up to twice as fast as conventional biofuels crops. Thirdly, they do not require as much resources, aside from a few nutrients, and water. And lastly, they are able to grow in non-arable lands such as waste-water, sea-water, and certain nutrient-deficient environments (HuLAB, 2012).

In the context of a sustainable bio-economy, microalgae are promising candidates to compensate for greenhouse gas emissions due to their high capability for fixing carbon dioxide. Microalgae are generally eukaryotic organisms, although cyanobacteria, such as spirulina, which are prokaryotes, are included under microalgae due to their photosynthetic and reproductive properties. Microalgae range in size from about 5 μm (*Chlorella*) to more than 100 μm (*spirulina*).

The commercial cultivation of microalgae began in Japan with the cultivation of *Chlorella* in the 1960s, followed by the cultivation of spirulina in Mexico and the USA in the 1970s. Since then, the industrial biotechnology of microalgae has grown tremendously. The immense chemical diversity of microalgae provides numerous

applications in the food, feed and pharmaceutical industries. Microalgae are cultivated for the production of whole biomass and valuable substances such as nutraceuticals, carotenoids, phycocyanin and poly-unsaturated fatty acids (PUFAs), which are utilized in the food and feed (notably aquaculture) industry. The production of biofuel from lipid- or carbohydrates-rich microalgae is under way (Feedipedia - Animal Feed Resources Information System, 2017).

Commercial production of microalgae already has at least 30 years of history. Main species of microalgae which being grown are *Chlorella, Spirulina, Dunaliella salina* and *Haematococcus pluvialis*. Initially these species of microalgae were grown commercially for purpose of food supplement and pharmaceutical industry and currently developing towards renewable fuel source (Sathe).

Microalgae cultivation is the stage of growing microalgae which will contribute up to 30% of total production cost. In commons, there is two major system of microalgae which is open system and closed system. Open system, also named as open-pond system a more common and low-cost method which normally used in mass cultivation. However, it has a major drawback which is easily contaminated by bacteria and unpredictable environmental factors including evaporation, light intensity, pH levels and temperature which bring negative effect of biomass concentration. Therefore, the second cultivation method is by using closed system. Closed system grows microalgae inside a photobioreactor. This allow the control of environmental factors for a more efficient growth. Despite that, this method will have required cost in bioreactor construction and energy demands for operation (HuLAB, 2012).

For the harvesting, microalgae are needs to be concentrated and separates from the growth medium. Harvesting can be divided into two-step process which first is bulk harvesting and followed by thickening process. In the bulk harvesting, microalgal biomass is separated from the bulk cultures. While in the thickening process, it is to concentrates the algal slurry. Generally, method of harvesting microalgae is divided into 4 based methods involving mechanical, chemical, biological and electrical methods.

Mechanical technique is including centrifugation, filtration, gravity sedimentation, flotation, and foam separation. For chemical dewatering, this method is mostly flocculation induced by either inorganic or organic polyelectrolyte (polymer)

flocculants. While for electrical dewatering, this method is based on electro-coagulation process. For biological dewatering technique, it includes auto-flocculation which occurring at high pH, flocculation by secreted biopolymers, and microbial flocculation.

Nowadays, extensive research has been done on different microalgae species in regards of the economic and technical limitation on microalgae harvesting. Separation of microalgae from their aqueous medium is a critical steps which accounts up to 30% of total biomass production cost (Salim, Bosma, Vermuë, & Wijffels, 2011). Current harvesting methods is energy cost extensive, toxicity from the flocculants and not feasible in the large-scale production. However, in the current technology, different type of technique has shown a remarkable potential to be carry out for harvesting and dewatering microalgae.

This technology is complicated due to the both physical and chemical properties of dilute algal solution. By using a high impact performance single technology, or combination technique in sequence, harvesting and dewatering microalgae can be carrying out. The effectiveness from the combination technique in sequences is relying on the individual performances for each unit. The first combine technique performances will affect the following technique in the combination. Example of the current available technology for harvesting and dewatering for microalgae is centrifugation, spiral plate technology (SPT), pressure filtration, vacuum filtration, membrane filtration, sedimentation, chemical flocculation, drum drying, spray drying and solar drying.

For solid-liquid separation, it can using different method such as sedimentation, disc stack centrifugation, dissolved air flotation, dispersed air flotation, micro bubble generation organic flocculation, inorganic flocculation, vacuum filtration, cross flow filtration, pressure filtration, decanter centrifugation, bio-flocculation, auto-flocculation and electrolytic coagulation, electrolytic flocculation and electrolytic flotation (Al Hattab, Ghaly, & Hammoud, 2015)

Jatropha curcas is a species of flowering plant in the spurge family, *Euphorbiaceae*, native to the American tropics most likely Mexico and Central America and has been spread throughout the world in tropic and subtropical regions around the world and make the cultivation of *Jatropha* uncomplicated (Ab van Peer, n.d.) .The plant can grow in wasteland and grow on almost any terrain even on gravelly, sandy and saline

soils. The plant flourishes well in different climatic zones in the tropical world. It requires moderate rainfall and soil fertility. It leaves are not edible for animal and used for fencing in crop fields. It does not require large doses of fertilizer and once planted, it can produce seed crop more than 50 years. Each fruit contains 2-3 oblong seeds those are matured when the capsules changes from green to yellow (Maroid, Mohamed Elwathig Saeed Mirghani, & Ismail Hussein, 2017).

A plant of about 7 years of age produces about 2-5 kg seed per year with 30-35% oil content (Hareesh Chandra, 2013). *Jatropha* oil is traditionally used for soap and lamp oil, in the last decades, it has received high attention as it is considered a very promising feedstock for the production of biodiesel. The kernel also rich in protein content 27-32wt% while the pressing residue after oil extraction has relatively high protein content around 53-58wt% (A.I Hamarneh, 2010).

Even though *Jatropha* cannot be used as human food because of its toxicity, it provides highly nutritious and protein sources for animal feed if the toxins are removed. Several studies showed that both the oil and the seed cake are toxic due to the presence of phorbol ester, certain protein(curcines) and other antinutrients which is trypsin inhibitor, lectin and phytate which limit the uses of *Jatropha* for food and feed application without further detoxification (Harinder PS Makkar, 2008). Despite this, the relatively high protein content of *Jatropha* can be advantageous since this rich source of protein does not compete with protein obtained from food crops such as soy and wheat (A.I Hamarneh, 2010).

Due to high protein content, many researches have been conducted in order to extract the protein from *Jatropha* seed cake in combination with detoxification process to produce protein concentrate or protein isolates for animal feed (Dianika Lestari, 2011). Various methods of extracting *Jatropha* oil have been performed, whether by thermo-chemically processing, mechanically using a press or chemically extracting with solvents. Many studies have been done to evaluate the optimal condition for *Jatropha* oil extraction. However, different extraction methods have affected the maximum oil produced (Jinjuta Kongkasawan, 2012).

1.2. PROBLEM STATEMENT

Microalgae are gaining wide attention in the recent times energy scenario as an alternative renewable source of biomass due to the increased awareness of finite fossil-fuel resource and the associated problems. From the environmental aspects, algae could be used to produce biofuel to remove nutrients and other pollutant wastewater to maintain water quality as indicators of environmental change and as CO_2 sequester. At the same time, microalgae are also widely used in industry for example in pharmaceutical and health related product, animal and fish feed and used in cosmetic.

Another current main problem of faced by this planet is the land use for cultivation. In this aspect, microalgae have an overwhelming advantage because it does not compete with agriculture. Algae cultivation uses both land that in many cases is unsuitable for traditional agriculture, as well as water sources that are not useable for other crops, such as sea-, brackish- and wastewater. As such, algae-based fuels complement biofuels made from traditional agricultural processes (Algae Basics, n.d.).

Generally, there is no single best method for harvesting microalgae and reducing their water content and 20-30% of the total biomass production cost are consist of biomass recovery from culturing medium in algal harvesting (Uduman, Qi, Danquah, Forde, & Hoadley, 2010);(Mata, Martins, & Caetano, 2010). One of the major challenges for full scale production of algal biofuel is to lower the energy cost in harvesting algae.

Typically, microalgae harvesting employs methods which can be technically and economically challenging when considering in larger production scales. For thickening process, the bulk harvesting method plays and important role for the energy requirement. The initial harvesting stage is generally costly and determines the following downstream processing. Besides that, strain selection also is an important consideration since certain species are much easier to harvest than others. It is highlighted that in order to make the whole biofuels production process economical, cost-effective and energy efficient harvesting methods criteria is require.

By referring to the difficulties faced on microalgae harvesting, there are numerous methods is established and for each of the method has its own advantages and limitations. Each method of harvesting has different advantages and drawbacks. Centrifugation has a

high energy demand and is expensive. For filtration method, it requires high operating and maintenance costs. For sedimentation method, due to the small size of microalgae and their negative surface charges, processing time using this method taking too long to make it feasible as an option for harvesting microalgae. For coagulation method, selection of an appropriate coagulant is critical for low-cost and effective microalgal harvesting. Coagulant such as Fe_{3+} and Al_{3+} reduce the quality of the microalgal biomass for food, feed, and fertilizer. Besides that, it also increases the complexity of lipid extraction and the residual from the coagulant may affect microalgal growth which cause problems with recycling the culture medium.

In present study, technique that proposed for harvesting method is simple floatation with aid (*Jatropha curcas*) seed cake as bio-flotation method. *Jatropha* is a promising choice because it is easy to cultivate. *Jatropha* can grow on all the climatic conditions and soils hence it is cultivated in most of the places. It is less expensive to cultivate Jatropha and most of the *Jatropha* seed varieties are available of less cost.

The percentage of yield is high, and the extraction of oil is also maximum. *Jatropha* also can be used for biogas production from its press cake formed during oil production. The press cake contains high amount of protein and there is no important application for the press cake. Studies have shown that the nutritional value of *Jatropha* seed cake equals that of soya bean, and better than sunflower and cotton seed meal, making it an ideal stock feed, if detoxified.

Jatropha is famous for being a potential source of raw material for biodiesel. Its popularity stems from the widespread general knowledge that it is a non-edible, oil-yielding tree, well adapted to marginal areas with poor soil and low rainfall, where it grows without competing with annual food crops. Several methods for oil extraction have been developed. In all processes, about 50% of the weight of the seeds remain as a press cake containing mainly protein and carbohydrates. The protein quality of the *Jatropha* seed cake is high. Levels of essential amino acids (except lysine) are higher in *Jatropha* seed cake (A. Wakandigara, 2013).

Investigations have shown that this residue contains toxic compounds and cannot be used as animal feed without further processing (Biogas production from *Jatropha* Curcas Press Cake, n.d). *Jatropha* can also be used to prevent and/or control soil erosion,

to reclaim exhausted land (Benge, 2006), as a medicinal plant, be planted as a commercial crop, grown as a natural fence, especially to contain or exclude farm animals. The fresh *Jatropha* fruit contains about 35–40% shell and 60–65% seed (by weight) of which 40–42% husk/hull and 58–60% kernels which consists of about 50% oil. The fruit shell is reported to contain about 34% cellulose, 10% hemi cellulose and 12% lignin and is good in minerals (Singh et al., 2008)

1.3. OBJECTIVE

The focus of this report will be emphasized on objective number one. Covered the growth monitoring process and continuously supply fresh microalgae for flotation experiment.

1. To cultivate *Chlorella vulgaris* microalgae in lab scale with controlled environment using Basal's Basal Medium until exponential growth stage.
2. To perform protein extraction on *Jatropha curcas* seed and produce functioning *Jatropha* protein-oil emulsion.
3. To develop a new bio-flotation method for harvest *Chlorella vulgaris* microalgae from its culture medium using JPOE and evaluate its performance.

1.4. SCOPE OF STUDY

The scope of this study was focused on investigating the effectiveness of *Jatropha* protein-oil emulsion (JPOE) in flotation removal of *Chlorella vulgaris* microalgae. The cultivation of microalgae and protein extraction of *Jatropha* seed was done continuously along the project to enable fresh material available all the time for flotation experiment. By trial and error method, the suitable ratio for protein-oil emulsion was being identified by adding sunflower oil. The performance of removal efficiency using protein oil emulsion with optimum ratio has been evaluated under different pH, dosage, and mixing time. Characterization was done by zeta potential and flotation removal efficiency was measured by biomass dry weight comparison and UV-vis spectrophotometer.

CHAPTER 2

LITERATURE REVIEW

Research was started by looking at the market value and suitability of this study to be implemented in large scale for industry in Malaysia. There will be three main direction during research which is microalgae, *Jatropha*, and current common dewatering method use in relevant industry. This was done to check if this project will be contributing significant benefit to current industry and environment.

2.1. MICROALGAE

Spolaore et al (2006) study reviewed that the human already started to use microalgae from 2000 years ago and eventually develop into numerous commercial applications. Nowadays, microalgae are cultivated as a high value molecules sources and believed that microalgae products will be more diversified and economically competitive by future research work effort. Mata, Martins, & Caetano (2010) review stated that microalgae are abundant in earth ecosystems, in both aquatic and terrestrial, representing a big variety of species living in a wide range of environmental conditions. It is estimated that more than 50,000 species exist, but only a limited number, of around 30,000, have been studied and analysed. In Figure 2.1, there is a schematic figure which summarized different uses and potential of microalgae contribution to the industry and its major products.

As mentioned before, figure 2.1 below shown the importance of microalgae in different industry.

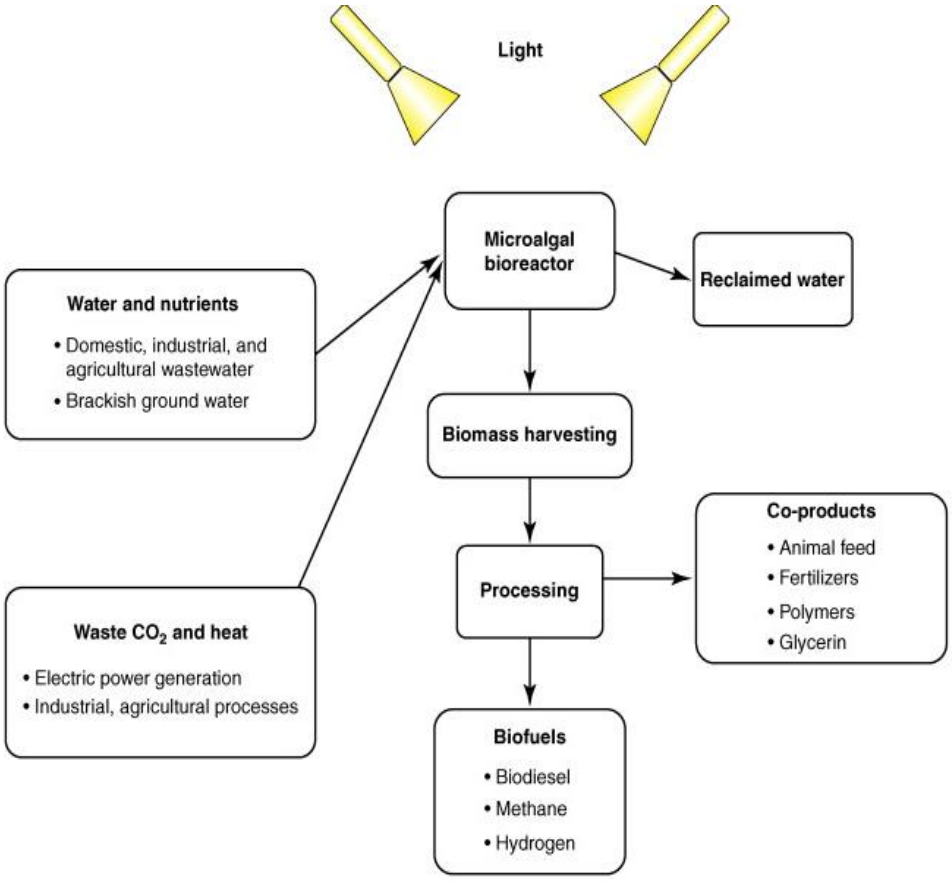

Figure 2.1 Different product and uses of microalgae grow in bioreactor.

Source: Kumar et al. (2010)

2.1.1. Microalgae Market Value

Biofuel Production

Teresa (2010) stated that, any microalgae species can be induced to accumulate substantial quantities of lipids thus contributing to a high oil yield. The average lipid content varies between 1 and 70% but under certain conditions some species can reach 90% of dry weight. Based on Widjaja, Chien, & Ju (2009), has shown that the lipid content of *Chlorella vulgaris* microalgae can be up to 50% when undergoes manipulated CO_2 supply and nitrogen depletion. Higher lipid productivity is achievable by varying length of nutrient starvation and length of normal nutrition. This prove that microalgae can be much more feasible for further application in biodiesel production.

Also, Feng et al. (2011) works shown that the *C. vulgaris* cultured in a column aeration photobioreactor has achieved a lipid content of 42% and lipid productivity of 147 mg/l d^{-1}. This percentage is comparatively very high because based on Wang et al. (2007) study, soybean which considered as the most important seed crop among oil crops, only hit about 13% to 22% oil contain. As cost analysis done by Yujie et al (2011), the microalgae biomass is already able to compete with petroleum at US$63.97 per barrel along with other potential functions of microalgae in various fields. They concluded that the microalgae have a good future in lipid production in terms of economical technology (Feng et al., 2011). The different type of biofuel product by microalgae after downstream processes is shown in Figure 2.2 below.

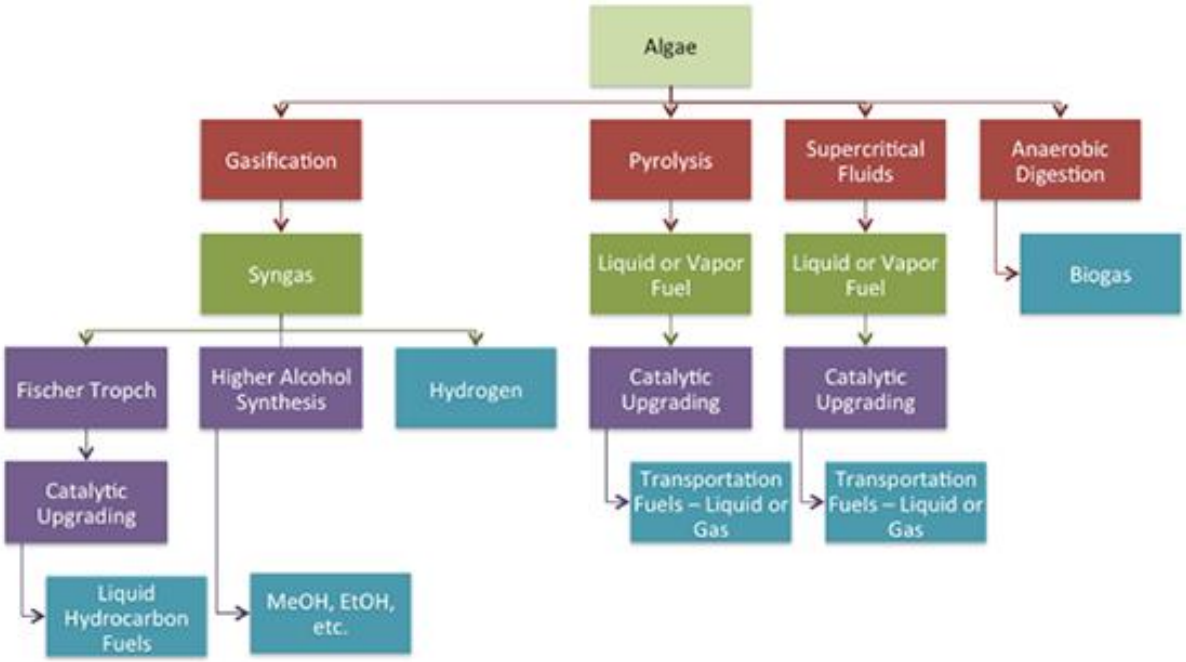

Figure 2.2: Fuel products from various processes of algae.

Source: The Pennsylvania State University (2018)

Carbon Dioxide Fixation

Murakami & Ikenouchi (1997) works have proven that microalgae able to provide up to 1gCO_2/1/day carbon dioxide fixation at 24h illumination. The continuous overwhelm emission of carbon dioxide (CO_2) to the atmosphere has become a global

problem. This is because the industry production is crucial to keep the developing and surviving of humanity for continuous supply or resources and product.

Some technologies have been developed for enhanced biological carbon dioxide fixation using microalgae. Several species of microalgae have been introduced flue gases directly and that's shown a promising carbon fixation rate especially for *Chlorella vulgaris* sp. in bioreactor, membrane reactor, and highest in open pond reactors which able to generate up to 50% removal efficiency in Kumar et al., (2010) study. The effectiveness of different species microalgae in carbon fixation is summarized by Hirano et al. (1977) study as shown in Table 2.1.

Table 2.1 Performance of microalgae in carbon fixation.

Reactor type	**Microalga species**	**CO_2 feed gas (%)**	**CO_2 fixation rate ($g/m^3/h$), or removal efficiency (%)**	**Specific growth rate (/h), or biomass productivity ($g/m^3/h$)**
Open pond reactors	*Nannochloropsis salina*	15	NA	4.1
	Chlorella sp.	6-8	10-50%	NA
	N. salina	5	NA	1.25
Bioreactors	*Phorphyridium sp.*	2-3	3-18	NA
	Botryococcus braunii			
	Euglena gracilis	11	3.1	4.8
	Chlorella vulgaris	1	128 and 141	NA
Membrane bioreactors	*C. vulgaris*	1	NA	4
	Nannochloropsis sp.	1	NA	4.2-5.8
			NA	0.8-41.7
	C. vulgaris	1	80-260	NA
	C. vulgaris	1	43 and 275	NA
	C. vulgaris	0.045	148	NA
	S. platensis	2-15	38.3-60	3-17.8

Source: Hirano, Ueda, Hirayama, & Ogushi, (1997)

Food Supplement

In Spolaore et al. (2006) finding, microalgae chemical composition is not only able to enhance the nutritional content of conventional food processing, but it also brings able to boost human and animal's health. The protein, carbohydrate, and lipid composition are surprisingly high compare to convenient food that was consumed daily. Iwamoto finding in Handbook of microalgal culture said that a rare substance β-1,3-glucan, which is an active immunostimulatory, a reducer of blood lipids and a free-radical scavenger can be found inside Chlorella microalgae. Currently, *Chlorella* is produce in about 70 companies in the world and the largest company named Chlorella manufacturing and Co. is located in Taiwan which produce 400 ton of dried biomass microalgae per year (Spolaore et al., 2006).

Ethanol Production

Hiranoe et al. (1997) study stated that microalgae able perform intracellular ethanol production from accumulated starch under dark and anaerobic conditions. This has made microalgae with high starch content as a very good raw material for ethanol production. Some strains productivity shown up to 20-30g/m^2/day and starch content can be as high as 50%. *Chlorella vulgaris* with 37% starch content chosen as raw material for conventional ethanol-fermentation process. (Hirano et al., 1997)

Minimum Land Use

According to Chisti (2010), microalgae is relatively easy to cultivate as it does not encroach on cropland and able to growth with minimum land area occupation. That will help reducing deforestation. Mata et al. (2010) stated that microalgae cultivation is not directly linked to human consumption, and more importantly have low space requirements for its production.

2.1.2. Parameters Affecting Microalgae Growth

The growth of microalgae can be affected by many environmental factors such as light intensity, pH value, temperature, salinity, nutrients, toxic elements, aeration, and carbon dioxide supply. Also, some biological factors such as viruses, predation, competition, and growth of epiphytes. Lastly, for microalgae growth in reactor will affect

by operating conditions for example, hydraulic residence time, gas transfer, harvesting rates, and mixing methods (Kumar et al., 2010).

Temperature

Temperature can stimulate cellular, physiological and morphological responses of a microalgae. A higher temperature environment will increase the metabolic rates of microalgae, and low temperature will inhibit growth of microalgae. The optimal temperature requirement is different for all types of microalgae species which also will be influenced when involved other environmental parameters for example the light intensity. Optimal growth temperatures of 15–26°C have been reported for some species, with maximum cell densities obtained at 23 °C. Only daytime higher temperatures were observed to have clearly favourable effects on microalgal growth rates due to photosynthesis, except when the night temperature was as low as 7 °C (Kumar et al., 2010).

Culturing Medium

BBM medium will be using to cultivate *Chlorella vulgaris*. This widely used medium is an inorganic salts medium. Most of the time, BBM is used for the culture of free-living planktonic freshwater algae and is used at the University of Reading for culturing Chlorella vulgaris as food for Daphnia magna. It is recommended for culturing such organisms by the Culture Centre of Algae and Protozoa, Cambridge (CCAP 1992). Table 2.2 contains information regarding the chemical composition of the medium dissolved in water along with the concentrations of its components. We can note at this point that the medium formed in the manner described above was subsequently subjected to sterilization in an autoclave. When the medium is prepared in aqueous solution from a single stock solution containing all chemicals some constituents precipitate, because of this stock solutions of each chemical should be prepared separately. All solutions should be prepare using distilled (RO) water.

Table 2.2: Chemical composition of common BBM medium.

Stock Solution No.	Chemical name	Formula	Weight(g)	Distilled Water(mL)
1	di-potassium hydrogen orthophosphate	K_2HPO_4	1.875	250
2	Potassium di-hydrogen orthophosphate	KH_2PO_4	4.375	250
3	Magnesium sulphate	$MgSO_4.7H_2O$	1.875	250
4	Sodium Nitrate	$NaNO_3$	6.250	250
5	Calcium chloride	$CaCl_2.2H_2O$	0.625	250
6	Sodium Chloride	NaCL	0.625	250
7	EDTA tetrasodium salt Potassium Hydroxide	$EDTA – Na_4$ KOH	5.000 3.100	100
8	Ferrous sulphate Sulphuric acid conc. (we per mL = 1.84g)	$FeSO_47H_2O$ H_2SO_4	0.498 0.1mL	100
9*	Boric acid	H_3BO_3	1.142	100
10	Zinc sulphate	$ZnSO_4.7H_2O$	0.353	25
11	Manganese chloride	$MnCl_24H_2O$	0.058	25
12	Cupric sulphate	$CuSO_45H_2O$	0.063	25
13	Cobaltous nitrate	$Co(NO_3)_26H_2O$	0.020	25
14	Sodium molybdate	$Na_2MoO_42H_2O$	0.048	25

Source: Connon, (2007)

pH Value

Kumar et al. (2010) said that, in general microalgae species prefer neutral pH value. Some like Spirulina able to tolerate higher which is pH 9. The carbon supply to microalgae can affect pH value of microalgae in a bioreactor, higher carbon concentrations will lead to higher biomass productivity but lower down pH. This increase in pH can be beneficial for inactivation of pathogens in microalgal wastewater treatment

but can also inhibit microalgal growth. Similarly, the speciation of NH3 and NH4+ in microalgal bioreactors is strongly dependent on pH – NH3 uncouples electron transport in the microalgal photosystem and competes with water molecules in oxidation reactions, thus leading to release of O2.

Carbon Supply

In Yoo et al (2010) works, they successfully cultivate Chlorella by only providing normal ambient air with 2% CO2 concentration. Whereas de Morais & Costa (2007), study identified that microalgae grew when exposed to 6%, 12% or 18% CO2 (v/v) and the Chlorella strain showed maximum growth of 0.60 g/L in the presence of 10% CO2.

Toxic Compounds

Elements and compounds that may be toxic to microalgae include heavy metals and various gases, such as CO2, NOx, SOx, O2 and NH3. Optimal CO2 concentrations vary greatly among microalgal species, as discussed previously. Common freshwater microalgae exhibit changes in photosynthetic characteristics when grown under high CO2 concentrations [i.e. above 5% (v/v)]. These changes include lower affinity to CO2, higher photosynthetic sensitivity to O2, higher CO2 compensation points and lower activity of carbonic anhydrase. The effect of trace acid gases (NOx and SOx) on microalgal growth has been determined using both model gases and actual flue gases. Maeda et al. have examined the tolerance of a strain of Chlorella to NOx and SOx and found that the strain can grow under trace element addition. Furthermore, Yoshihara et al. have reported that Nannochloropsis sp. can grow with 100 ppm NO, whereas Matsumoto et al. have observed that Tetraselmis sp. can withstand flue gases that contain up to 185 ppm SOx and 125 ppm NOx, as well as 14.1% (v/v) CO2. When the concentration of SO2 is high (>400 ppm), the pH of the medium will decrease, thus resulting in low productivity. By contrast, NO at ∼300 ppm does not directly influence microalgal growth because NO absorbed by the cultivation medium is changed to NO2–, and thus can be further utilized as a nitrogen source (Kumar et al., 2010).

Light Intensity

Based on Qitao et al (2014) study, the cell density of *Chlorella vulgaris* microalgae after a period of growth under standard condition reach the highest amount for growth under light intensity 3960. At the same time, Lukasz et al (2016), has

successfully cultivate *Chlorella vulgaris* microalgae in a laboratory with only 1500 lux provided and capable to achieved highest productivity of 40 mg/(dm3h) in the first hour.

2.1.3. Microalgae Growth Determination

Absorbance Measurement

As explain by Sutton (2006), absorbance measurement is a light scattering technique to monitor the concentration of pure cultures which have enormous advantages of being rapid and non-destructive. However, it does not measure cell numbers. Light scattering is most closely related to the dry weight of the cells. Light is passed through the suspension of a solution, and all light that is not absorbed is re-radiated. There is a significant amount of physics involved in this and related to optical treatises and Huygens' Principle. It is enough to say that light passing through a suspension of solution is scattered, and the amount of scatter is an indication of the biomass present in the suspension. In visible light, this appears "milky" or "cloudy" to the eye. It follows from this that if the concentration of scattering particles becomes high, then multiple scattering events become possible. When specified for a given wavelength, optical absorbance is equivalent to optical density. (NanoHybrids, 2018)

Spectrophotometer

The spectrophotometer method measures turbidity directly. The best case (i.e. most sensitive) would be to have a narrow slit and a small detector so that only the light scattered in the forward direction would be seen by the detector. This instrument would give larger apparent absorption readings than other instruments. As should be obvious, each spectrophotometer used must be independently calibrated for use in estimating suspension concentrations. Not only is the apparent absorption affected by the width of the instrument's slit, the condition of the filter, and the size and condition of the detector, but also each time the lamp is changed the calibration needs to be repeated as different bulbs may vary in total output. The correlation of absorption to dry weight is very good for dilute suspensions of microorganism, and this relationship seems to hold regardless of cell size. However, in more concentrated suspensions this correlation (absorption to dry weight) no longer holds. (Sutton, 2006)

2.2. *JATROPHA CURCAS*

(Harinder PS Makkar, 2008) stated that *J.curcas* is known as a physic nut, belongs to the *Euphorbiaceae* family. It is hard plant, thrives on degraded land and requires limited amount of nutrient and water. *J.curcas* is a tree that can grow up to 6m in height, matures within 5-6 years and can live for more than 50 years.

In good condition, small 2-4year-old trees may currently yield between 1-4kg of fruits a year with very large single mature trees in optimal places known to yield up to 25-33kg. *J.curcas* can growth rapidly in optimal conditions and it is a tropical plant/small bush that can grow in low to high rainfall areas. Jinjuta Kongkasawan (2012) reported that the trees shed the leaves in dry season and blossom in wet season.

Two flowering peaks often appear during summer and autumn. It will take approximately 3 – 4 months after blooming to seed maturity. The seeds are black with the seed weighing about 1,375 seeds per kilogram (Jinjuta Kongkasawan, 2012).

Again, in Harinder PS Makkar (2008), it reported that *Jatropha curcas* can be planted in the form of hedges to reduce erosion and to protect enclosed area from animal such as goat and cattle because it contains phorbol ester and antinutrient such as saponin, trypsin inhibitor, lectin and phytate that considered toxic to human and animals. (Dianika Lestari, 2011) stated that the seed and also seed cake also contain this toxic which limit the uses of *J.curcas* seed cake for food and feed application.

However, the toxic compound in the seed cake can be removed by further detoxification. (A.I Hamarneh, 2010) review stated that due to the high oil content in the seed, *J.curcas* is classify as high potential energy crop. An oil content of the kernel reported between 40-66% and the kernel has a protein content of 27-32 wt% while pressing residue after oil extraction has a relatively high protein content around 53-58 wt%.

2.3. CURRENT METHOD OF MICROALGAE DEWATERING

In general, microalgae are one of the important sources for oils and other biomolecules because of the uses in the production of biofuels and high-value products. Recovery of biomass can be a significant problem because of the small size (3–30μm diameter) of the algal cells and the growth of algae in dilute suspension at around 0.02–0.05% dry solids poses considerable challenges in achieving a viable energy balance in micro-algal biofuel process operations (Milledge & Heaven, 2013).

Culture broths is generally relatively dilute (<0.5 kg m−3 dry biomass in some commercial production systems) and thus, large volumes of need to be handled to recover the biomass of microalgae. Microalgae biomass can be harvested through one or more solid-liquid separation steps. Any suitable harvest method must be able to process the large volumes typical of algal biomass production processes.

The acceptable level of moisture in the product is one of the acceptable level of in selecting a suitable harvesting method (Molina Grima, Belarbi, Acién Fernández, Robles Medina, & Chisti, 2003). According to previous study by Milledge & Heaven (2013) states that there is a numbers of method to harvesting algae which including the combination of any of sedimentation, flocculation, centrifugation, flotation, and filtration method.

The combination of two dewatering techniques has also been found to significantly improve the process by reducing the energy demands and/or total emissions (Soomro et al., 2016). Most of the method for microalgae harvesting possessing their own advantages and disadvantages. High operational and capital cost is the major challenges for harvesting microalgae.

CHAPTER 3

METHODOLOGY

This chapter will briefly explain about the procedure and steps on how the experiment is being done. Besides that, this chapter will also provide the equipment and material used to complete the experiment.

3.1. MATERIALS

3.1.1. Microalgae Strains

The microalgae *Chlorella vulgaris* pure strain as shown in Figure 3.1 was originally obtained from the collection of an undergraduate student, Mok Yok Leng, from School of Fisheries and Aquaculture Science, Universiti Malaysia Terengganu. Microalgae is an active microorganism, but under condition with no aeration, low temperature and sunlight exposure, it will undergo hibernating state. The *C. vulgaris* strain is kept in a clean H_2O medium before the experiment is start.

Figure 3.1: Photo of *Chlorella vulgaris* sp. strain

3.1.2. Bold's Basal Medium (BBM) Powder

This product was produced and sold by a company named PhytoTechnology Laboratories which located in United State of America. After a series of discussion, the product was brought into Malaysia by its Malaysia distribution company, Euroscience SDN BHD. A bottle of BBM powder as shown in Figure 3.2 was mailed to UMP and then kept inside laboratory chiller at 2^{o}c-6^{o}c within a tight closed container.

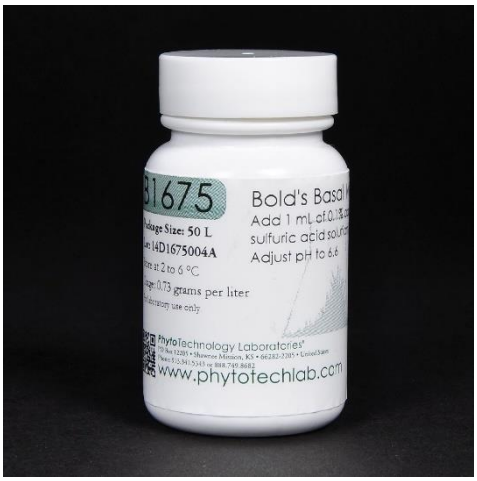

Figure 3.2: Photo of BBM powder.

According to its production descriptions, BBM was a freshwater algae medium that has been used to grow a variety of green algal cultures (e.g. Trichosarcina, Chlorococcum, and Chlorella) without the need for soil-extract or vitamins. The predominantly inorganic nature of this medium facilitates itself as an axenic-culture maintenance medium. The BBM powder formula and composition of each chemicals are shown in Table 3.1.

Table 3.1: Chemical composition of BBM powder.

Chemical name	Quantity (mg/L)	Chemical Name	Quantity (mg/L)
Boric Acid	11.42	Manganese Chloride•$4H_2O$	1.44
Calcium Chloride, Anhydrous	18.87	Potassium Hydroxide	31.0
Cobalt Nitrate•$6H_2O$	0.49	Potassium Phosphate, Dibasic	75.0
Cupric Sulfate•$5H_2O$	1.57	Potassium Phosphate, Monobasic	175.0
EDTA, Disodium Salt	63.61	Sodium Chloride	25.0
Ferrous Sulfate•$7H_2O$	4.98	Sodium Molybdate	1.19
Magnesium Sulfate, Anhydrous	36.63	Sodium Nitrate	250.0
		Zinc Sulfate•$7H_2O$	8.82

Source: PhytoTechnology Laboratories® (2017)

3.1.3. Aeration Pump and tubing

A common plastic aquarium air pump (JIY-666A, 5W,2x4L/min, 2x0.02Mpa) as shown in Figure 3.3 and rubber tubing with suitable size and length was bought from a fish shop located at Taman Tas. The power of the air pump is 4L/min with 2 separated output head. For tubing, originally 1m length was the cut into several parts with suitable length use to connect the pump to Erlenmeyer flask and also the output from the flask which the end of tube is covered with an air filter.

Figure 3.3: Aquarium air pump use for aeration

3.2. EXPERIMENTAL METHODOLOGY

3.2.1. Overall *Chlorella vulgaris* sp. cultivation process

The cultivation stages and process of *Chlorella vulgaris* in lab scale were detailly arrange in a flowchart and summarized in Figure 3.4 below.

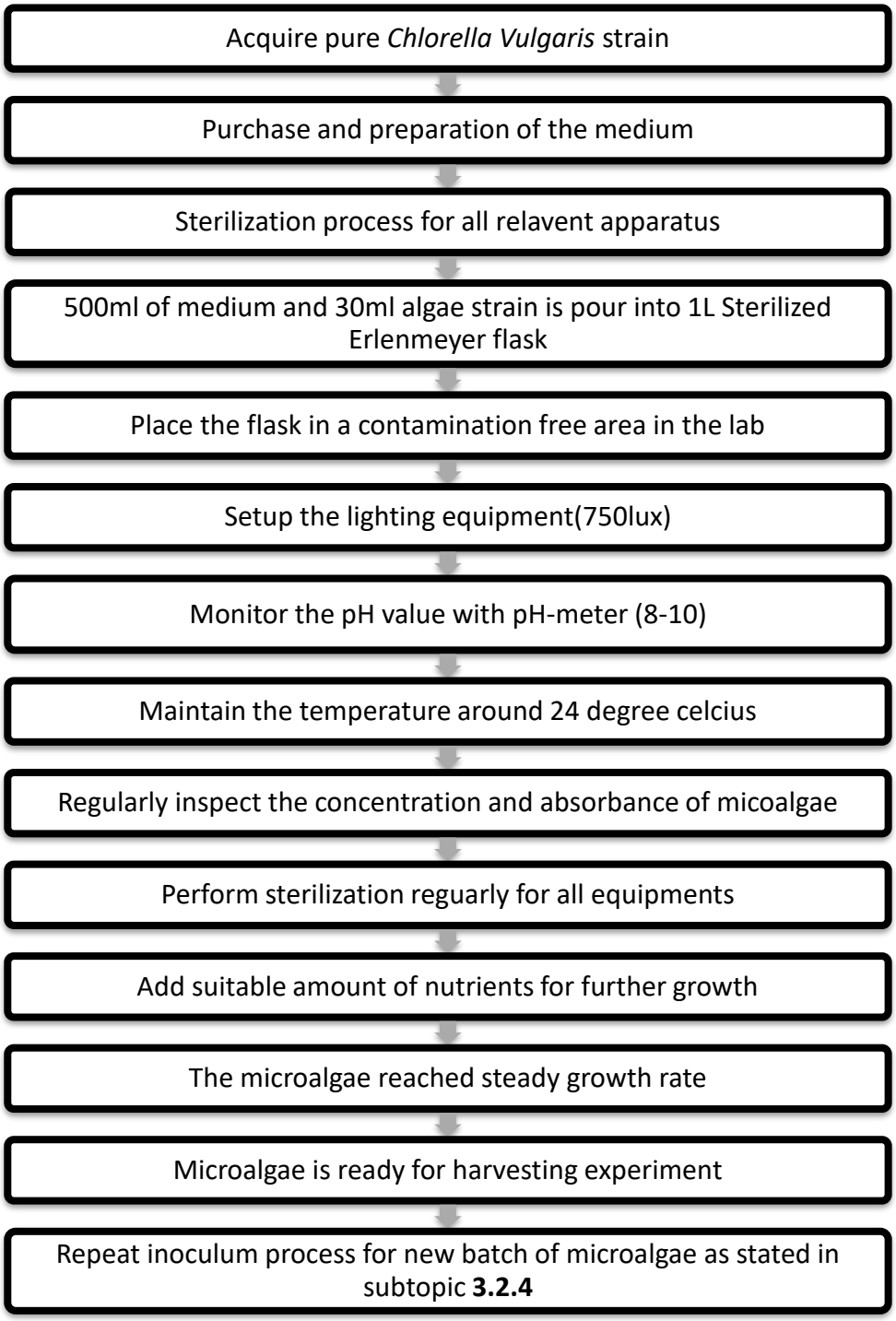

Figure 3.4: Overall process for microalgae preparation until harvesting

3.2.2. Preparation of Culture Medium

The standard medium preparation required optimum amount of deionized (DI) water and two 1Liter Erlenmeyer flask. A beaker containing 1Liter DI water was placed on a magnetic stirrer with heating function. Then, 0.705g of BBM powder was weight using an electronic balance and then pour into the beaker. The magnetic stirrer was switched on with 120^{o}c and 150rpm to ensure the powder was well mixed in the DI water as shown in Figure 3.5.

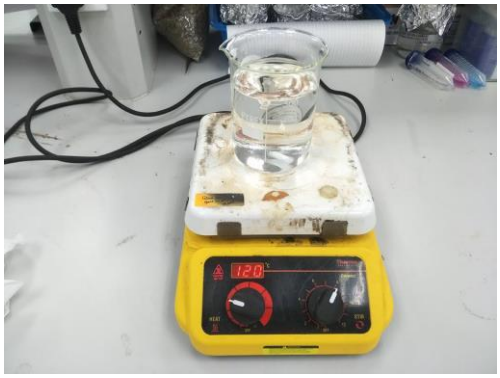

Figure 3.5: The beaker containing DI water was heated up and stirred.

While stirring, 1ml of 0.1% Sulfuric Acid solution (H_2SO_4) is added to the solution to activate the powder completely. After the powder is fully dissolved in the solution, the solution pH is adjusted to 6.6 +/- 0.1 with Potassium hydroxide (KOH) as shown in Figure 3.6. The final solution was poured into 2 separate Erlenmeyer flasks covered with aluminium foil and placed into autoclaved for sterilization process with 121^{o}c and 20min.

Figure 3.6: The solution pH was adjusted to 6.6

Later, the Erlenmeyer flasks is placed in a clean area which will be ready to use after cooled down as shown in Figure 3.7 below. The aluminium foil was kept tight in open space to prevent any kind of contamination until the inoculum process take place.

Figure 3.7: The autoclave medium was place aside for cool down

3.2.3. Sterilization and set up for tubing and pump

All relevant apparatus including beakers, Erlenmeyer flasks, rubber tubing, rubber stopper, autoclaved medium are placed inside biosafety hood as shown in Figure 3.8 for 5minutes UV ray sterilization. After that, the safety hood cover was removed, and the fan was switch on followed by a 3min readying mode which initiate automatically by the safety hood. A layer of 70% ethanol was sprayed on inner surface of the hood, surface of all apparatus and handling glove. By using laboratory tissue, all the surface covered with ethanol was wiped clean and dried. Later, rubber tubing was used to connect pump and the Erlenmeyer containing medium and an air filter was placed in the middle of the tube to filter out any dirt or possible contamination.

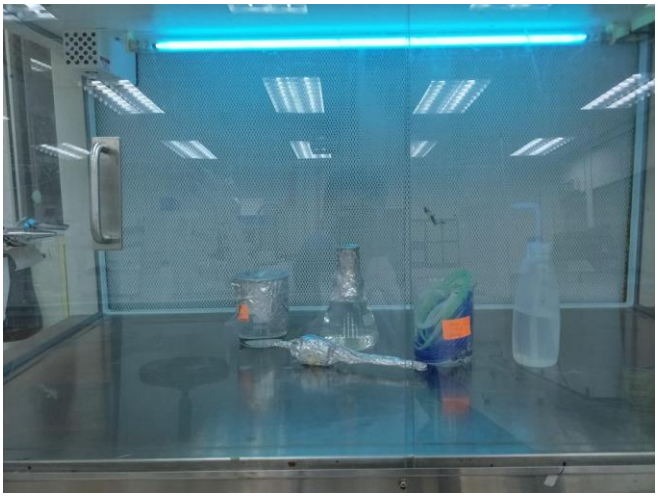

Figure 3.8: Relevant apparatus undergoing UV ray sterilization

3.2.4. Microalgae inoculum

The *Chlorella vulgaris* strain which stored inside chiller was removed and placed inside biosafety hood. Alcohol is sprayed on the centrifuge tube, inner side of the biosafety hood and personnel glove before handling the inoculum process start. Initially, each flask is fill up with 500ml BBM medium and 30ml of the original *Chlorella vulgaris* strain will be pour into the flask to mix with medium that prepared in advance. The flask will then bring to the algae room and connect with tube that continuously supply air aeration from ambient air with normal CO_2 concentration as shown in Figure 3.9 below. Afterwards, latest batch of cultivated microalgae has replaced the strain and was used in inoculum for new batch with same portion.

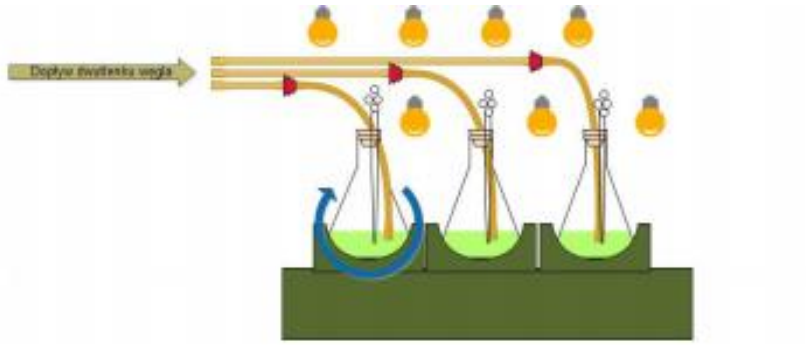

Figure 3.9: Illustration diagram of microalgae cultivation setup

Source: Biłos, Patyna, Płaczek, & Witczak (2016)

3.2.5. Controlled Environment for Cultivation

After the inoculum process, the flasks containing microalgae was left to growth in the algae room as shown in Figure 3.10 which located within Bioprocess lab (FIST). The lighting was provided to the flask containing microalgae by two fluorescent lamps. As shown in Figure 3.11, the actual light intensity was measured to be 750lux using a light meter (EXTECH, Easy View 30). At the same time, the air conditioner temperature was set to be 23^{o}c. but the actual temperature measured using an infrared thermometer (FLUKE, 572-2) as shown in Figure 3.12 was 24.2^{o}c which still within acceptable range. A simple aeration system was set up by using conventional aquarium air pump and rubber tube. Normal air which contained CO_2 was pump into the Erlenmeyer flask containing microalgae at a rate of 2L/min. The light intensity and temperature were checked regularly

to ensure the reading within suitable range which does not cause destructive effect on microalgae. Similarly, the tubing, air filter and pump was also inspected daily to be well functions and without any leakage.

Figure 3.10: Outside view of algae room

Figure 3.11(left) & 3.12(right): Light intensity measurement by light meter & temperature measurement by Infrared thermometer.

3.2.6. Continuous culture

The cultivation was operated in a continuous mode. Since the optimal dilution rate is 0.1 (Kang, 2012), 10% cell suspension of total sample volume from each reservoir were removed daily for tests, and same amount of BBM were added into each reservoir. According to Wong et al. (2017), the steady state is estimated to be observed after 12 days, at this state sample were collected for flotation experiment. The illustration set up diagram for cultivation towards steady growth are shown in Figure 3.13 below. Actual set up does not involve shaker, gas mixing chamber and CO_2 tank due to several limitations. To culture the new batch of *Chlorella vulgaris* for future experimenting, three set of 5ml original *Chlorella vulgaris* is being transferred weekly to three new 100ml Erlenmeyer flasks contained BBM medium.

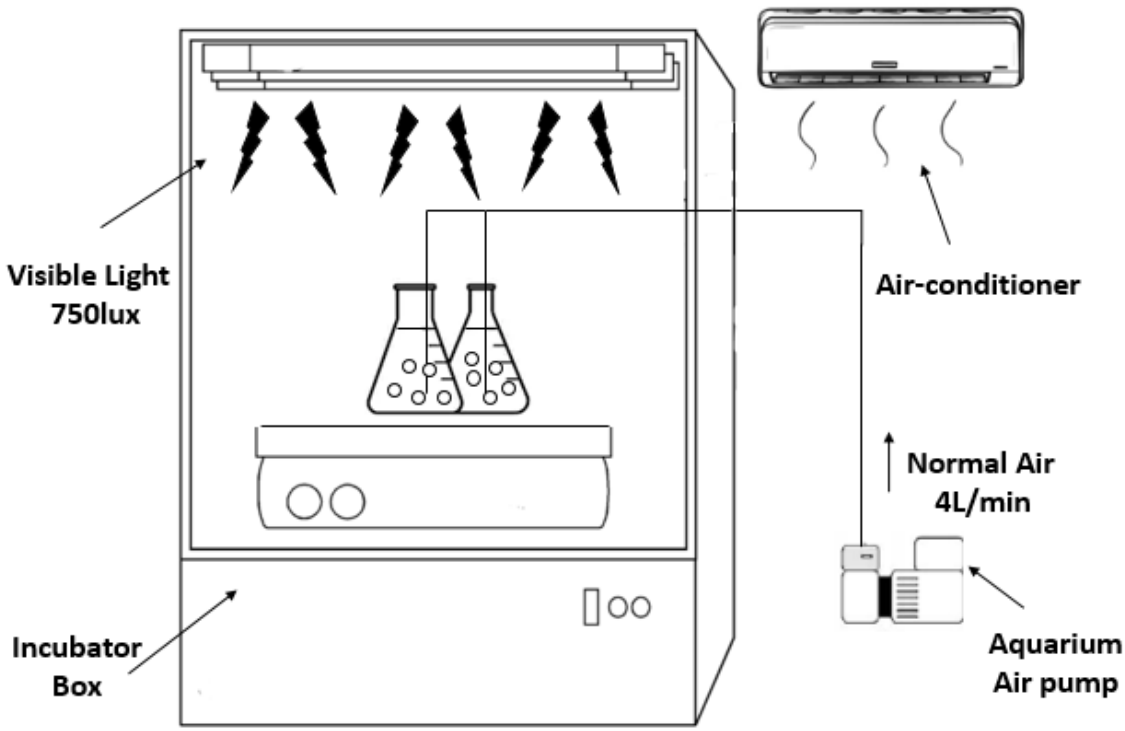

Figure 3.13 Illustration set up for microalgae cultivation

3.2.7. Monitoring of pH

A layer of 70% ethanol was sprayed on both of the glove which will handling the microalgae. The gloves were wipe clean and dried. Flask that containing microalgae was swirled to make sure the suspensions are well mixed. Then, the rubber stopper covering the microalgae culture was removed carefully and optimum amount of suspension which enough to cover a pH meter electro was poured into a beaker slowly. The pH value of the microalgae is being monitored regularly using pH-meter in the certain reading within range 8-10.

3.2.8. Monitoring of Absorbance

Chlorella vulgaris growth was determined by measuring the absorbance using UV-visible spectrophotometer. Microalgae culture was swirled for one minute to ensure the suspension was well mixed. Then, about 2ml of suspension was taken at least 2cm depth from the surface using a sterilized 10ml micropipette. The suspension was then pipetted into a 1ml cuvette and placed inside cell position 1 as shown in Figure 3.14 within the machine. Another cuvette was filled with fresh medium as blank and placed inside the machine. For the determination of an optimum wavelength that can detect the change in concentration of *C.vulgaris*, UV absorbance was scanned with the wavelength ranged from 200nm to 800nm with the same sample. (Wong, et al., 2017) 650nm was the peak absorbance determined and the cell density was measured by this wavelength. All results were summarized in Table 4.1 and Figure 4.1 in the next chapter.

Figure 3.14: Sample and blanked in position to be tested.

3.2.9. Monitoring of Dry Biomass Weight

Microalgae biomass sample were filtered using a glass fibre filter which shown in Figure 3.15 (WHATMAN type 50, 55mm diameter, 100circles). Glass-fibre was inserted with wrinkled side up in filtration apparatus. Vacuum was applied, and disk was washed with three successive 20 mL portions of DI water. Suction was continued to remove all traces of water, vacuum was turned off and washings was discarded. Filter was removed from filtration apparatus and transferred to an inert aluminium dish. The filter was dried in the oven at 105^{o}C for 24 hours. The filter was cold in desiccator to balance temperature and weigh. The weight of filter and aluminium dish (unit in mg) was recorded.

Figure 3.15: Glass fibre filter paper

After that, the filtering apparatus was assembled and filtered as shown in Figure 3.16, and suction was begun. Following, 10ml of well mixed microalgae suspension was retrieved from its culture under aseptic method. Microalgae sample was pipette into the middle of the seated glass-fibre filter. The filter was washed with three successive 10 mL volumes of distilled water, allowed complete drainage between washings, and suction was continued for about 3 min after filtration is complete.

Figure 3.16: Filtration process aided with vacuum machine

The filter was dried with residue at least 1 day at 105°C in an oven as shown in Figure 3.17 below.

Figure 3.17: Filter papers drying inside oven

Later, the filter with residue was left to cool in a desiccator to balanced temperature and weighed as showed in Figure 3.18 below.

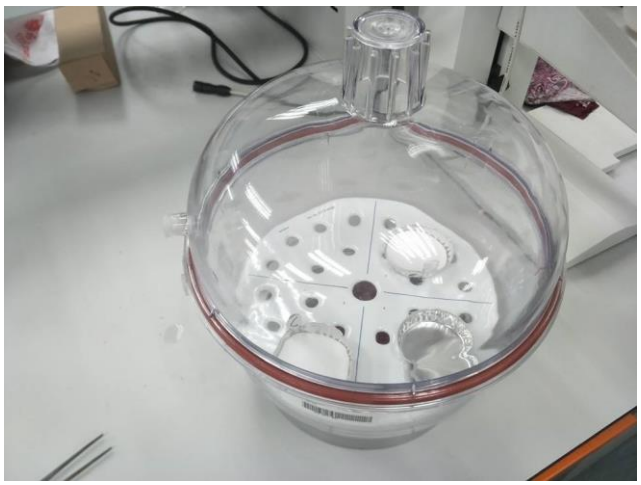

Figure 3.18: Desiccator containing filter paper with aluminium weight dish

As shown in Figure 3.19, the weight of filter and dish and residue was measured by an electronic balance. The result was recorded daily on the graph to see the growth curve and it is ready when a exponential trend like the Figure 3.20 is shown on the graph. All data was summarized and demonstrated in Table 4.2 and Figure 4.2 in the following chapter.

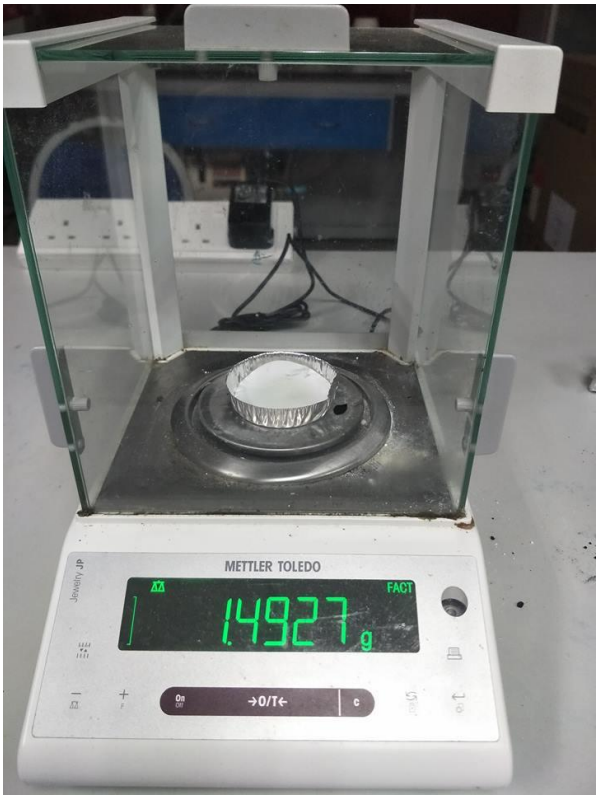

Figure 3.19: Measuring weight for biomass dry weight

3.2.10. Theory

Biomass Dry weight

Overall, measurement of biomass dry weight of a microalgae involves retrieving the aliquot from the culture, drying the sample into a constant weight and eventually expressing as weight of dried cell matter per sample volume. It was a crucial parameter for estimating biomass productivity and percentages of cell concentration. (Zhu, 1997) For 12days in consecutive, a 10mL sample of microalgae suspension from same culture batch were used in determination of Biomass dry weight calculate using equation below:

$$Biomass\ dry\ weight\left(\frac{mg}{L}\right) = \frac{(A-B)\ x\ 1000}{Sample\ volume,ml} \qquad \text{Eq (4.1)}$$

Where;

A= weight of filter + dried residue, mg

B= weight of filter, mg

Specific growth rate

The specific growth rate (μ) in the exponential phase was calculated according to following equation (Wong, et al., 2017):

$$\mu = \ln(x_2/x_1) / (t_2 - t_1) \qquad \text{Eq 4.2}$$

Where x_2 and x_1 are the optical density (OD650) at t_2 and t_1 respectively.

Biomass productivity

Whereby, the Biomass productivity (B) in the exponential phase was calculated by following equation:

$$B = (B1 - B0) / (T1 - T0) \qquad \text{Eq 4.3}$$

B_0 and B_1 are the mean dry biomass concentration at the times T_0 and T_1, respectively.

A growth curve graph as showed by Figure 3.20 below was used to compare with according to trend of acquired results and trend showed by the graph to determine in which state the cultivating microalgae has achieved.

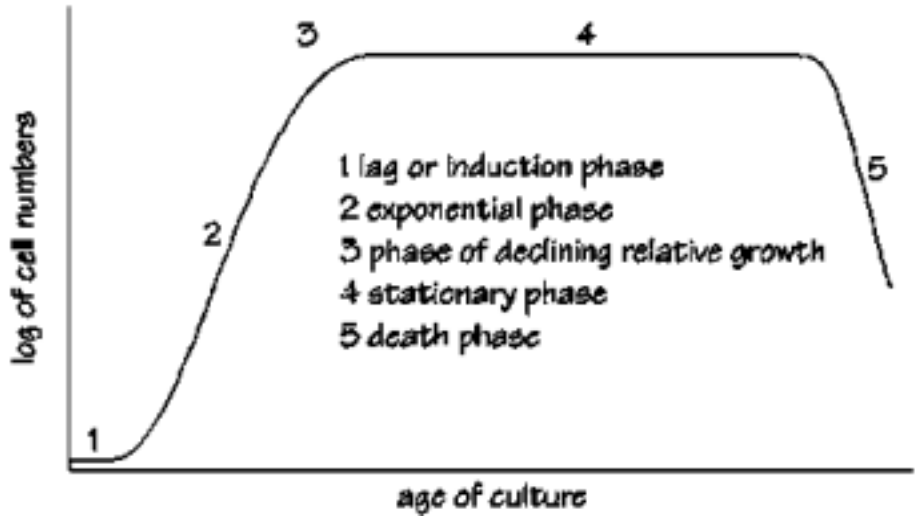

Figure 3.20: Microalgae growth curve graph

Source: Arnas Svaldenis, (2014)

3.2.11. *Jatropha* protein oil emulsion preparation and flotation experiment

During the cultivation of *C.vulgaris* microalgae, the process of *Jatropha* protein extraction was done simultaneously. *Jatropha curcas* came in form of raw seed and protein extraction requires a series of techniques, instrument and glassware which was described in Figure 3.21 including oil extraction, solid discharge removing and centrifuging.

When *Jatropha* protein was ready, a trial and error process was done to identify the optimum ratio between *Jatropha* protein and sunflower oil to produce JPOE which was effective for flotation. Different ratio of *Jatropha* protein and sunflower oil was mixed together from range of 1:1 to 7:1 to identify which one bring the best outcome on bio-flotation.

After *C.vulgaris* microalgae reached its exponential growth phase and the JPOE was ready, the project was proceeded to the final stage. The performance of JPOE working on bio-flotation removal was evaluated under different operating parameter including dosage, pH and mixing time. Figure 3.22 has shown the overall process for flotation evaluation experiment.

As shown below, Figure 3.21 has listed out all process of *Jatropha* protein extraction in detail. Relevant photos were attached in *Appendix C.*

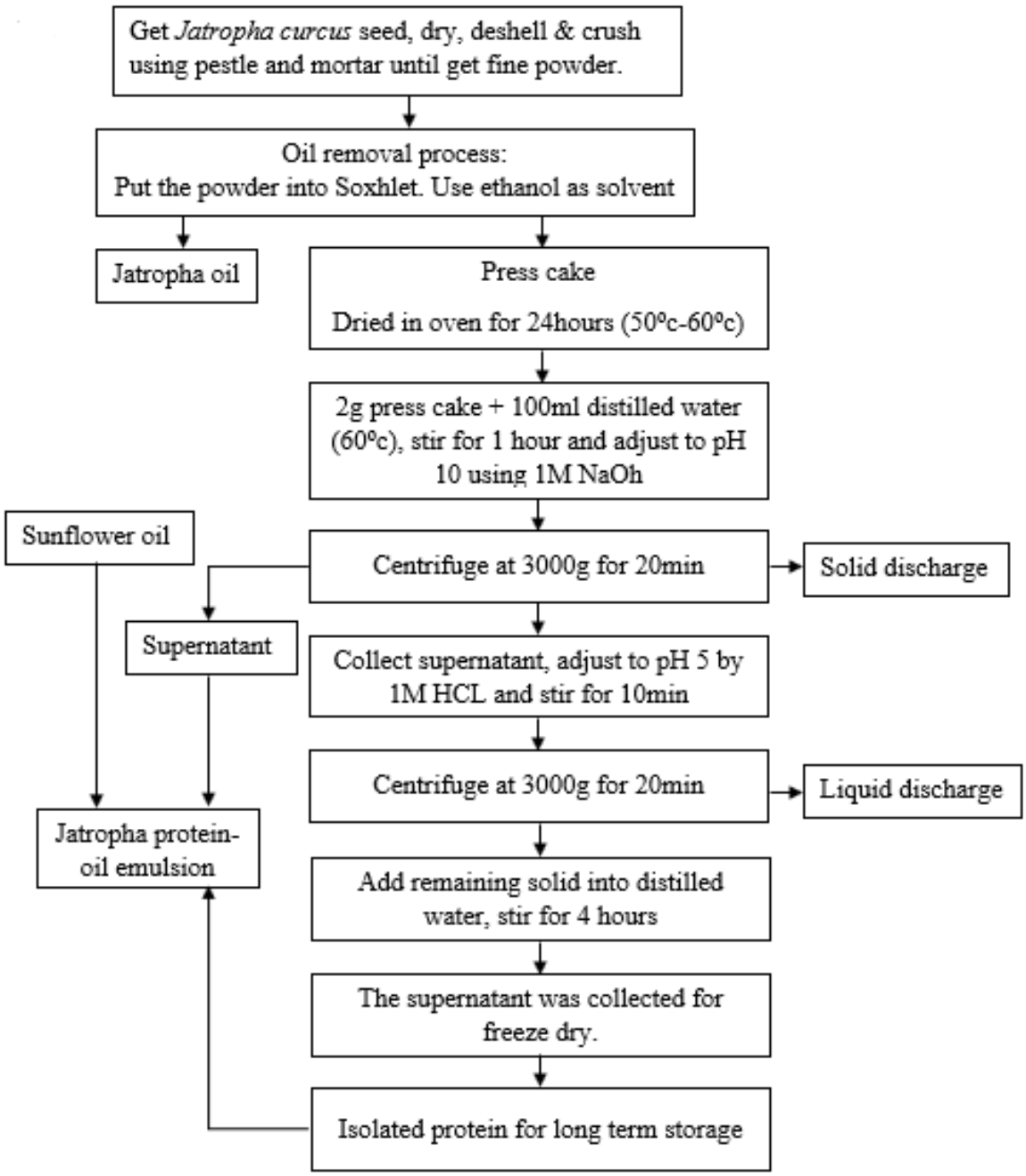

Figure 3.21: Overall JPOE production process

The flowchart in Figure 3.22 state all stages of flotation experiment process using *Jatropha* protein oil emulsion and *C.vulgaris* microalgae. Relevant photos of this process were attached in *Appendix C.*

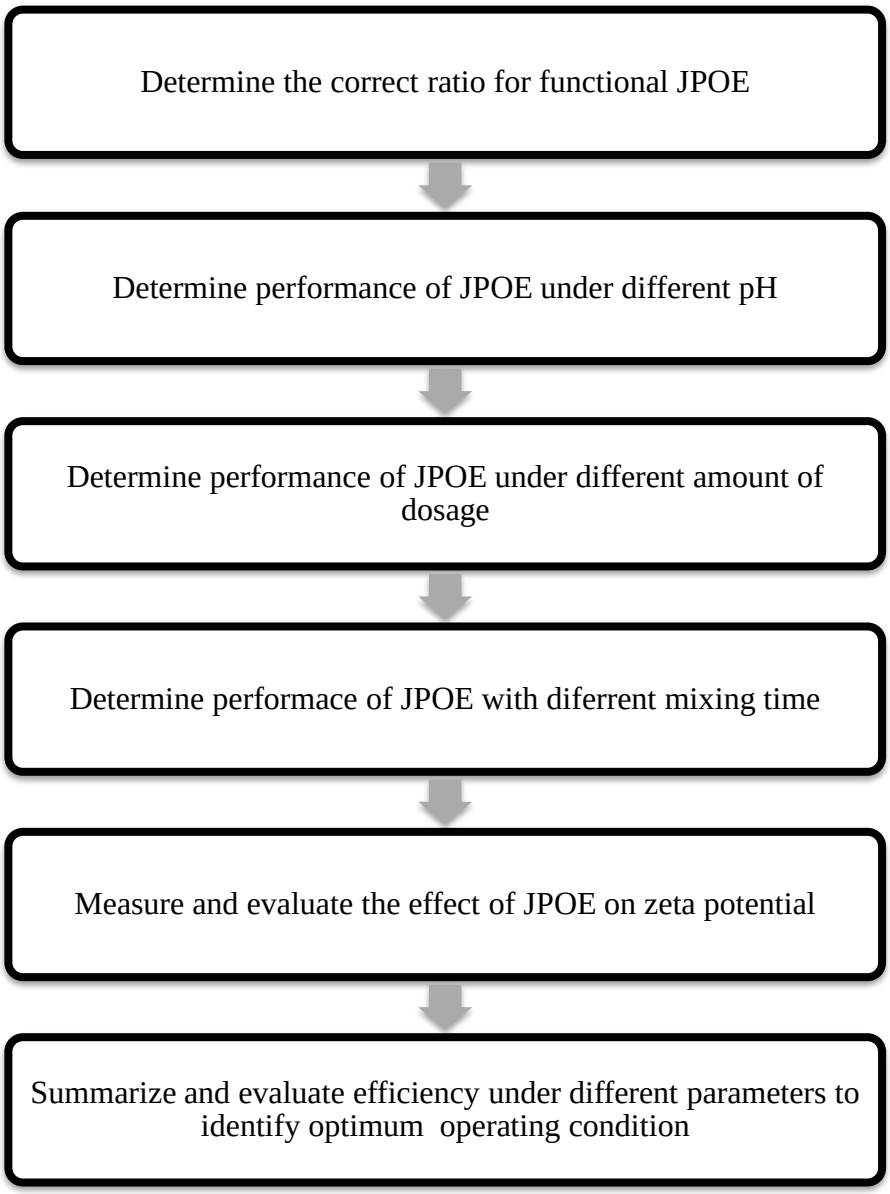

Figure 3.22: Overall flotation experiment process

CHAPTER 4

RESULT AND DISCUSSION

4.1. GROWTH PERFORMANCE

To follow microalgae growth, directly or indirectly, is always difficult because both the cell compound to quantify and the moment of sample collection, affect cell density estimation. These problems increase with dilute cultures and with small size cells, like the ones from *Chlorella vulgaris* (2–10μm). Evaluation by absorbance and by biomass dry weight is here presented and discussed. The effect of culture conditions on cell growth rate, in particular the aeration, light intensity, carbon source, pH, temperature is studied, as well as the eventual role of the specific surface area at the air/water interface.

Absorbance (650nm)

According to (Gómez, 2015), a new culture of microalgae needs about 12days to achieve continuous exponential phase of growth as shown in Figure 4.4 which is the desirable operating mode for any microalgae production plant in the industry. At the same time, Gomez (2015) study concluded that the absorbance of a microalgae culture which followed a linear trend as a function of time is mutual related to microalgae cell density during the exponential phase of growth.

Similarly, a document from Michigan State University stated that, amount of light absorbed is directly proportional to the concentration of absorbing compounds in that sample, so a spectrophotometer can also be used to determine concentrations of compounds in solution. Finally, because particles in suspension will scatter light (thus preventing it from reaching the light detector), spectrophotometers may also be used to estimate the number of cells in suspension.

Therefore, measuring the absorbance of a microalgae culture for the first 12days was very important to identify its growth performance. Absorbance reading for a new batch of culture cultivated during this study from Day 1 to 12 is measured by a spectrophotometer and fresh medium as blank with wavelength of 650nm. The data was

summarized in Table 4.1 and the relationship between absorbance over cultivation times (day) was demonstrated in Figure 4.1 respectively.

Table 4.1: Absorbance reading for 12days of continuous cultivation

Cultivation time (Day)	Absorbance (650nm)
1	0.050
2	0.070
3	0.100
4	0.137
5	0.157
6	0.179
7	0.204
8	0.243
9	0.319
10	0.377
11	0.437
12	0.501

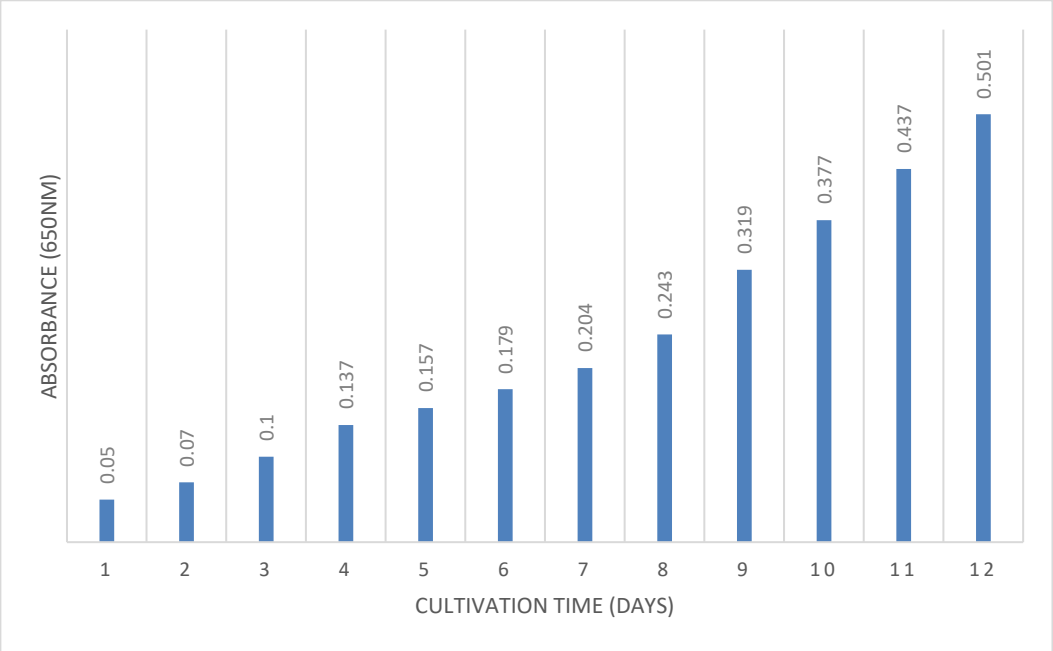

Figure 4.1: The graph of absorbance versus cultivation time

Based on the graph, the absorbance reading of *C.vulgaris* microalgae has shown a gradually increment trend from day 1 to day 12 within the range from 0.05 to 0.501. The initial absorbance reading under wavelength 650 is 0.05 on day1 and day 12 recorded

the highest reading which is 0.501. The total increment within this period is 0.451. This growth performance shown that the BBM medium was suitable and fully capable to support the reproduction of *C.vulgaris* microalgae cell throughout the whole exponential growth phase. Similar to Wong, et al. (2017) study which compare the growth performance between 13 type of growth medium, *C.vulgaris* which growth by BBM result in the highest ranking (3.389) among the selected growth media within a cultivation of 12days.

Absorbance measurement was said to be able to determine the concentration of photosynthetic pigments in a water sample which was related to phytoplankton biomass. This method was used to determine chlorophyll a,b and c in Gomez (2015) study. Therefore, higher absorbance reading means higher pigment and chlorophyll in a microalgae suspension. The colourful chlorophyll within the microalgae will be able to absorb the light transmitted by spectrophotometer and the amount of absorbed light was proportional to the cell density or cell number in suspension. (Hu, 2014)

The rapid increment of absorbance can be seen as the reproduction ability of the microalgae. The trend shown in Figure 4.1 can be explained by the excessive nutrient contents in the growth media. Reproducing capability of *C.vulgaris* can be affected by any addition or deficient of macronutrients (nitrogen, phosphorus and carbon) and micronutrients (magnesium, sulphur and iron).

Continuous aeration of air which contain nitrogen was supplied to the microalgae culture throughout the project with an aquarium pump with output of 4L/min. Specifically, nitrogenous compounds was proved to be important factor affecting protein, Chl-a, Chl-b production of *C.vulgaris*. (M. & J., 2005) In Wong et al. study, when nitrogen is limited, significant decline in the cell division rate and low optical density/absorbance value was found in medium with no nitrogen.

When the cell is starved by limited nitrogen supply, it leads to decrease. Relatively, the higher the nutrient contents, the higher the cell division rate, the higher the absorbance reading. Therefore, under condition with enough nutrients, the absorbance of microalgae culture will be directly proportional with the cultivation time.

Biomass dry weight

The weight of aluminium weighing dish and glass fibre filter disk were weighed before and after filtration with filtration residue. Equation 4.1 was used in the calculation of the biomass dry weight of the microalgae in the sample. The detailed calculation for biomass dry weight was shown in appendix. All data was tabulated in Table 4.2 and a graph showing the biomass dry weight over cultivation time was sketched as Figure 4.2.

Table 4.2: Biomass Dry Weight for 12days of continuous cultivation

Day	Initial Weight(g)	Final weight (g)	Difference(mg)	Biomass Dry Weight (mg/L)
1	1.4894	1.4901	0.70	70.0
2	1.4902	1.4915	1.30	130.0
3	1.4977	1.4993	1.60	160.0
4	1.4907	1.4927	2.00	200.0
5	1.5301	1.5323	2.20	220.0
6	1.4854	1.4886	3.20	320.0
7	1.4892	1.4934	4.20	420.0
8	1.4872	1.4920	4.80	480.0
9	1.4844	1.4900	5.60	560.0
10	1.4888	1.4950	6.20	620.0
11	1.4870	1.4936	6.60	660.0
12	1.4911	1.4982	7.10	710.0

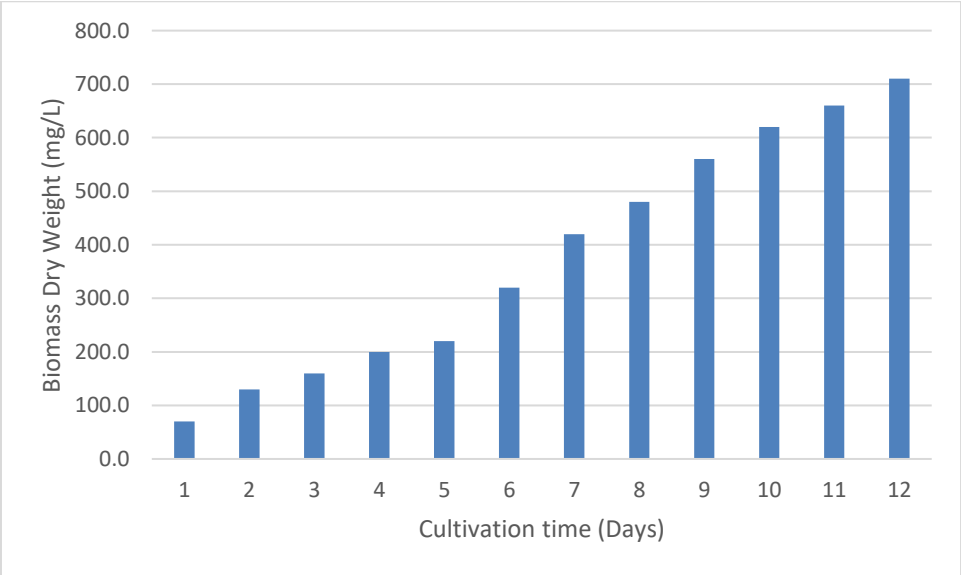

Figure 4.2: The graph of biomass dry weight versus cultivation time

Based on the graph, biomass dry weight has continuously increasing along with the cultivation time from day1 to day12. This result was similar to the absorbance reading and provided a more specific and convincing evidence for the growth of microalgae in terms of dry cell weight in certain amount of sample. The measurement starts with first day of inoculum where the biomass dry weight was the lowest (70mg/L) and end with day 12 which have the highest reading (710mg/L). The different in term of biomass dry weight in this 12 day was 640mg/L which indicated that the microalgae cell has reproduced 10times of its original number of cells within this period.

The glass fibre filter paper after undergo filtration process from day1 to day12 was shown in Figure 4.3. Filter paper in the figure obviously shown different in colour and concentration of filtration residue increasing slowly day by day. This was because the concentration of microalgae was increasing, and the more concentrated microalgae has more chlorophyll with brighter green colour.

Figure 4.3: Filter papers with residue from day 1 to day 12

As proved by the absorbance reading and biomass dry weight results, the C.vulgaris microalgae was undergoing exponential growth phase as shown in Figure 3.20 in previous chapter. At this stage, the microalgae will continue reproduce in a rapid speed with optimum condition provided similar to large scale production in the industry. Therefore, the fresh microalgae were being used in flotation experiment to mimic the

situation in actual large-scale microalgae production which able to have a more convincing study result.

Biomass productivity and overall specific growth rate

To further discuss the growth performance of *C.vulgaris* in this 12 days of exponential growth, the specific growth rate as absorbance, specific growth rate as biomass dry weight, and biomass productivity of the microalgae was calculated. The results were comparing to a similar project with same species of microalgae, same cultivation time, and same growth media in Table 4.3.

Table 4.3: Comparison between current project and referred project data.

	Actual data	**Referred Data (Wong, et al., 2017)**
Absorbance reading (Day 12)	0.501	3.389
Overall Specific Growth Rate (as absorbance)	0.209	0.278
Biomass dry weight (mg/L) (day 12)	710	1420
Biomass productivity (mg L^{-1} day^{-1})	58.182	114.208
Overall specific growth rate (d^{-1}) (as dry biomass)	0.211	0.279

From the table, it shown there was difference in all numbers even though the species and cultivation time was completely same. This has shown that there were other factors which affected the growth performance of microalgae particularly *C.vulgaris*.

The biomass productivity and accumulation of specific compounds such as oil in microalgae mainly depend upon the environment conditions and media compositions. Environmental conditions that influence algal productivity and oil yield include duration and quality of sunlight, temperature, relative humidity, evaporation, precipitation, topography of lands, nutrients, carbon sources and water qualities.

Quality and quantity of biomass and oil yield of microalgae also depends on the kind of species and strains from local environments. Growth medium gave direct influence on specific product accumulation in microalgae for example fatty acid composition within the biomass. Also, pH conditions and media components highly related to oils and nutraceutical important pigment accumulations in microalgae as well.

Specific microalgae will require specific growth medium to achieve high biomass productivity and promising lipid accumulation. (Santhoshkumar, 2015) Among all growth medium for *C.vulgaris*, BBM was already proved scientifically to be the best especially in terms of protein concentration and chlorophyll pigments in dry cell biomass. (Wong, et al., 2017) Therefore, even though there is different in productivity and growth rate between these two projects, both has shown promising productivity and rapid specific growth rate.

Flotation efficiency and zeta potential analysis

The flotation experiment was done using *C.vulgaris* microalgae with JPOE afterwards. Figure 4.4 below shown the visible effect of JPOE successfully flocculated microalgae suspension to the surface of its culture. Then, the flotation dewatering efficiency by JPOE was evaluated under several working parameters including mixing time, pH and dosage to identify its optimum condition with effectiveness.

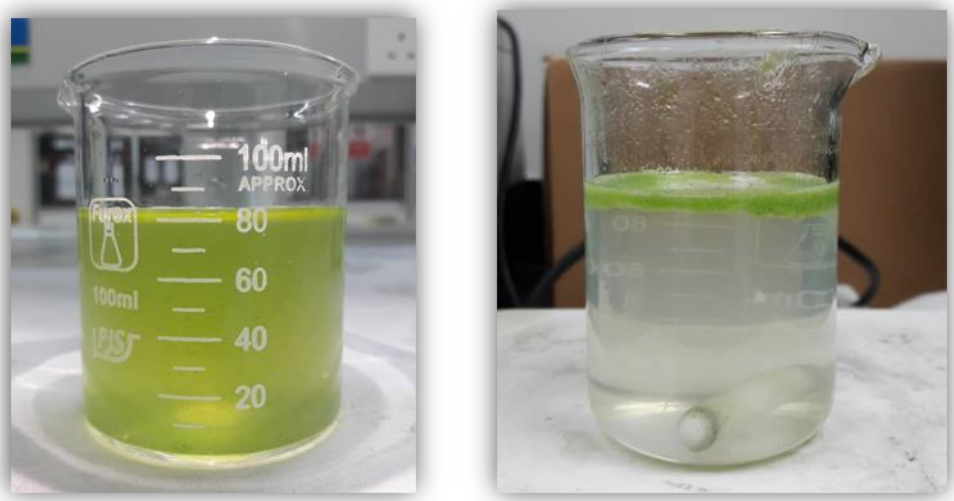

Figure 4.4: Beaker containing microalgae before and after flotation experiment

At the same time, the effect of JPOE affecting the changes in microalgae zeta potential was measured and shown in Figure 4.5. The JPOE able to neutralize the surface charge of *C.vulgaris* cell from -18mV to approximately 0 which enable it to float on the surface of its culture medium. Finally, the JPOE was determined best to work under pH 2, dosage of 20ml/L and mixing time of 4 minute which able to achieve 81% removal of microalgae.

A zeta potential analyser was used to measure the change of zeta potential under the influence of JPOE and different pH. The result for Zeta potential under pH 2 to 8 was recorded in Figure 4.5 below. pH 2 has the lower zeta potential which indicate the microalgae cell under this condition has less negative surface charge and more floatable.

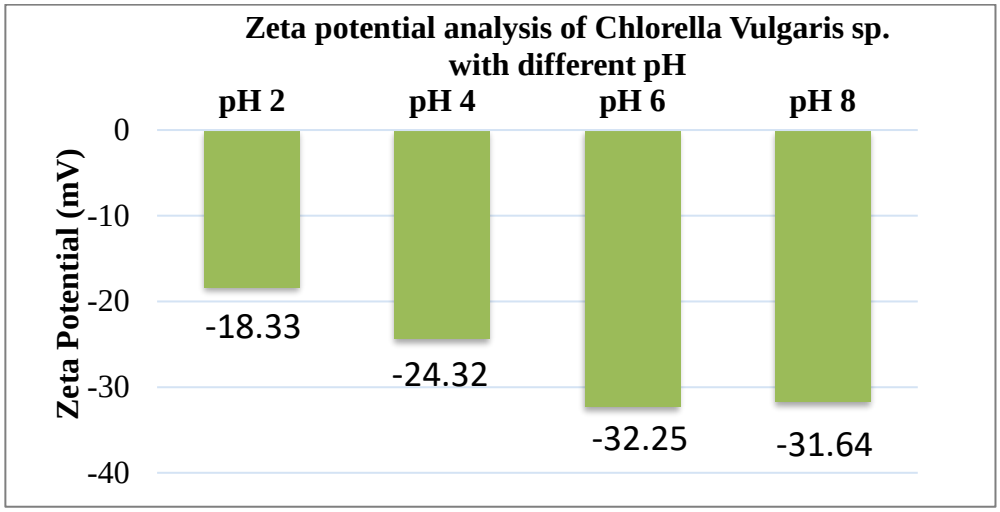

Figure 4.5: Zeta potential measurement of microalgae at different pH

The removal efficiency was acquired by comparing the condition before and after JPOE was added in terms of absorbance, total suspended solid, and zeta potential. Key data was summarized and tabulated in Table 4.4 below. This data was obtained with operating parameter, dosage 20ml/L, mixing time 4min, and pH 2.

Table 4.4: Efficiency analysis of JPOE under optimum condition

Efficiency Analysis	Before	After
UV-VIS Spectrophotometer	0.657A	0.121A
Total Suspended Solid	220 mg/L	42 mg/L
Zeta potential	-18.33mV	≈0mV

4.2. TIME MANGEMENT AND COST ANALYSIS

TIME LINE

The progress of this project proceeds according to the time line as shown by Table 4.5 below from week 1 to week 14.

Table 4.5: Project progress from week 1 to week 14.

ACTIVITY	WEEK													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
FYP 2 briefing from Dr Che Ku														
Discussion on the project about each other workflow and task														
Revise on all the methodology • Materials • Chemicals • Apparatus & Machine														
Prepare documents for lab/workshop usage, training attendance, risk assessment														
Application to access algae lab														
Start the experiment following the planned methodology														
Poster preparation														
Group extended abstract writing														
Thesis writing														
Submission of first draft														
Mock presentation of project														
Poster design with supervisor and group member														
Improvise individual thesis, poster and extended abstract														
Acquire presentation approval from supervisor														
Poster printing, final rehearsal for presentation with members														
Presentation of the project														
Laboratory clean up														
Submission of the finalized individual thesis, extended abstract, peer evaluation form and general handling form														

BUDGET AND COST ANALYSIS

Major materials and chemicals used in the project was listed below in Table 4.6 along with quantities, price per unit and total price calculated.

Table 4.6: List of Material and apparatus for Project Set up

No.	**Items**	**Quantities**	**Price per unit (RM)**	**Total Price (RM)**
1.	BBM powder (10L)	1	450	450
2.	Air pump	1	20	20
3.	Rubber Tubing (2m)	1	5	5
4.	Plug Extension (1m)	1	10	10
5.	Fluorescent light	2	Lab provided	-
6.	Ethanol (70%)	1	Lab provided	-
7.	Erlenmeyer flask	4	Lab provided	-
8.	Parafilm	1	Lab provided	-
9.	*Jatropha* seed (1kg)	1	50	50
10.	Sunflower oil (1L)	1	11	11
11.	Ethanol (99.9%)	1	Lab provided	-
TOTAL COST (RM)				546

CHAPTER 5

CONCLUSION AND RECOMMENDATION

In this part, the observations and statistics from all three objective will be summarized as an overall result of a single project. The final product was *Jatropha* protein-oil emulsion and bio-flotation method under its function.

5.1. GENERAL SUMMARY

All three main objective of this project was fulfilled. The cultivation of *C.vulgaris* in lab scale was done smoothly and has been able to continuously supply fresh microalgae for flotation experiment.

A biomass productivity of 58.182 mg $L^{-1}day^{-1}$ was recorded within 12 days of cultivation. Overall specific growth rate as absorbance and as biomass dry weight was recorded as 0.209/d and 0.211/d respectively.

The cultivation continued to go on after the 12 days and a series of inoculation has been done. Total of 5 different batch microalgae has been cultivated initially started with *C.vulgaris* strain from UMT and Bold's Basal Medium.

At the same time, the protein extraction process of *Jatorpha* seed has been executed in the same lab. Protein extracted was mixed with sunflower oil to produce protein oil emulsion and the effective ratio was identified as 1:1. Afterwards, the bio-flotation was proved to be effective and continue to test with different operating parameters.

5.2. SUMMARY OF FINDING

Finally, efficiency of the JPOE under different operating parameters was evaluated. JPOE under optimum condition of pH 2, mixing time 4 minutes and 20ml/L dosage able to achieve more that 81% removal efficiency.

5.3. CONCLUSION

In conclusion, the bio-flotation by JPOE on *C.vulgaris* microalgae was successfully developed. The novelty and achievements of this bio-flotation including:

- This bio-flotation has high efficiency which able to achieve >81% removal within 5minutes
- This method is also environment friendly because minimal usage of chemicals was used and produce small footprint for chemical recovery
- Energy saving was one of the achievements as minimum mechanical process was needed using this method
- JPOE can made by used *Jatropha* press cake and help in reducing *Jatropha* press cake waste from the industry after oil extraction
- This method only required very low cost. Approximately only RM0.16 material cost to harvest a litre of microalgae. The high performance and organic characteristic of this new method was believed able to reduce the overall production cost of microalgae in the future

5.4. RECOMMENDATION

As recommendation, JPOE required additional experimental investigation for being able to work in:

- Acquire effective flotation under original pH of the microalgae culture
- The pH adjustment step can be eliminated and further reduce the usage of chemicals
- The JPOE may try to use different type of oil like palm oil, coconut oil, or *Jatropha* oil
- This bio-flotation method should also be tested on more type of both marine and freshwater microalgae
- Should improvise potential of JPOE working under continuous system

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APPENDIX A

BIOMASS DRY WEIGHT CALCULATIONS

Biomass dry weight of *Chlorella vulgaris* was calculated by following formula:

$$Biomass\ dry\ weight\left(\frac{mg}{L}\right) = \frac{(A-B)\ x\ 1000}{Sample\ volume, ml}$$

A= weight of filter + dried residue, mg

B= weight of filter, mg

Below is sample calculation for biomass dry weight on cultivation time Day 1. Same calculation was done for the next 11days in consecutive.

Biomass dry weight (Day1)

A = 1.4901g x 1000 = 1490.1mg

B = 1.4894g x 1000 = 1489.4mg

Sample volume = 10ml

Biomass dry weight mg/L = (1490.1-1489.4) mg x 1000 / 10ml

= 70mg/L

APPENDIX B

SPECIFIC GROWTH RATE AND BIOMASS PRODUCTIVITY CALCULATIONS

The specific growth rate (μ) in the exponential phase was calculated according to following equation (Wong, et al., 2017):

$$\mu = \ln(x_2/x_1) / (t_2 - t_1)$$

Where x_2 and x_1 are the optical density (OD650) at t_2 and t_1 respectively.

Specific growth rate as absorbance (Day12)

$x_1 = 0.05$; $x_2 = 0.501$

$t_1 = 1$; $t_2 = 12$

Specific growth rate, μ = ln (0.501/0.05) / (12-1)
= 0.209

Specific growth rate as biomass dry weight (Day12)

$x_1 = 320$mg/L ; $x_2 = 70$mg/L

$t_1 = 1$; $t_2 = 12$

Specific growth rate, μ = ln (710/70) mg/L / (12-1)
= 0.211

Biomass productivity (B) was calculated by following equation:

$$B = (B_1 - B_O) / (T_1 - T_O)$$

where B0 and B1 are the mean dry biomass concentration at the times T0 and T1, respectively.

Biomass productivity (mg L^{-1} day^{-1})

B_1 = 710mg/L ; B_o = 70mg/L

t_1 = 12 ; t_o = 1

Biomass productivity (mg L^{-1} day^{-1}) = (710-70) mg/L / (12-1)

= 58.182 mg L^{-1} day^{-1}

APPENDIX C

PHOTOS OF JPOE PRODUCTION AND FLOATION EXPERIMENT

Raw *Jatropha curcas* seed and Jatopha Powder after crushed

***Jatropha* protein-oil emulsion and microalgae after flotation in centrifuge tubes**