CARBON DIOXIDE SEQUESTRATION BY ALGAE IN POME MEDIUM

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NOR SHAHIDA BINTI HAMDAN

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
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JANUARY 2014

SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology).

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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Dedication

I dedicate my dissertation work to my beloved
father and mother, Mr. Hamdan bin Md. Yatim and Mrs. Hasnah binti Man.
My friends, who always gave me inspiration, encouragements and support
towards the success of this study.
Thanks for everything.

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ABSTRACT

Carbon dioxide (CO_2) has been identified as one of the primary greenhouse gases (GHG) in the atmosphere. Carbon dioxide emissions have increased by about 35% since 1990 and cause the global warming. In Malaysia, this problem is due to the rapid growth of palm oil industries. A lot of carbon dioxide is emitted in producing crude palm kernel oil. Besides, palm oil mill effluent (POME) is the waste discharged by the palm oil industries with high biochemical oxygen demand (BOD), which has created serious water pollution in Malaysia, but it contains micronutrient need for microalgae growth. CO_2 emission can be reduced through CO_2 sequestration by photoautotrophic algae through photosynthesis. In this study, microalgae (*Chlorella* sp.) was cultured in POME medium in conical flasks and the interaction effect of light intensity and CO_2 concentration in the sparged mixture of air and CO_2 on algae growth (expressed as cell dry weight and specific growth rate) were identified. Then the results obtained from this experiment were analyzed with 2-level factorial design by using Design Expert 6.0.8 Software. In this experiment, the light intensity was supplied by fluorescent lamps at 2,000-12,000 lux. Meanwhile, the microalgae culture was sparged by 5 L/min of air mixture with CO_2 concentration (2-10% v/v). From this study, it was found that the increasing of light intensity and the increasing of CO_2 concentration gives the highest cell dry weight. At low light intensity, cell dry weight decreased even the CO_2 increased. The interaction between light intensity and CO_2 concentration in cell dry weight production was insignificant. In response to specific growth rate of *Chlorella* sp., at low CO_2 concentration, increasing light intensity did not affect the specific growth rate. On the other hand, when CO_2 concentration was high, increasing light intensity would reduce the specific growth rate of *Chlorella* sp. Both factors (light intensity and CO_2 concentration) were identified as the significant in effecting specific growth rate of *Chlorella* sp. As conclusion, since the CO_2 sequestration was measured based on the cell dry weight produce, light intensity was the significant factor for CO_2 sequestration by *Chlorella* sp. in POME medium. Nevertheless, too high a light intensity would cause photo-inhibition and reduced the growth rate of Chlorella sp. which in turn slows down the process of CO_2 sequestration.

ABSTRAK

Karbon dioksida telah dikenal pasti sebagai salah satu daripada gas-gas rumah hijau utama di atmosfera. Pengeluaran karbon dioksida telah meningkat sebanyak kira-kira 35% sejak tahun 1990 dan menyebabkan pemanasan global. Di Malaysia, masalah ini adalah disebabkan oleh pertumbuhan pesat industri minyak sawit. Banyak karbon dioksida dilepaskan dalam menghasilkan minyak mentah isirong sawit. Selain itu, sisa kumbahan kilang minyak sawit adalah sisa yang dilepaskan oleh industri minyak sawit dengan permintaan oksigen biokimia yang tinggi, yang telah mewujudkan pencemaran air yang serius di Malaysia, tetapi ia mengandungi mikronutrien yang diperlukan untuk pertumbuhan mikroalgae. Pelepasan gas karbon dioksida boleh dikurangkan melalui pemencilan karbon dioksida oleh photoautotrophic algae melalui fotosintesis. Dalam kajian ini, mikroalgae (*Chlorella* sp.) telah dikultur dengan sisa kumbahan kilang minyak kelapa sawit di dalam kelalang kon dan kesan interaksi keamatan cahaya dan kepekatan karbon dioksoda di dalam campuran udara dan gas karbon dioksida kepada pertumbuhan algae (dinyatakan sebagai berat sel kering dan kadar pertumbuhan spesifik) telah dikenal pasti. Kemudian keputusan yang diperolehi dari eksperimen ini telah dianalisis dengan 2-level factorial design menggunakan perisian Design Expert 6.0.8. Dalam eksperimen ini, keamatan cahaya dibekalkan oleh lampu neon di 2,000-12,000 lux. Sementara itu, kultur mikroalgae itu dibekalkan sebanyak 5 L/min campuran udara dengan kepekatan karbon dioksida (2-10% v/v). Daripada kajian ini, didapati bahawa peningkatan keamatan cahaya dan peningkatan kepekatan karbon dioksida memberikan berat sel kering yang tertinggi. Pada keamatan cahaya yang rendah, berat sel kering menurun walaupun karbon dioksida meningkat. Interaksi antara keamatan cahaya dan kepekatan karbon dioksida dalam penghasilan berat sel kering tidak penting. Sebagai tindak balas kepada kadar pertumbuhan spesifik *Chlorella* sp., Pada kepekatan karbon dioksida yang rendah, peningkatan keamatan cahaya tidak menjejaskan kadar pertumbuhan spesifik. Sebaliknya, apabila kepekatan karbon dioksida adalah tinggi, peningkatan keamatan cahaya akan mengurangkan kadar pertumbuhan spesifik *Chlorella* sp. Kedua-dua faktor (keamatan cahaya dan kepekatan karbon dioksida) telah dikenal pasti sebagai faktor yang penting pada kadar pertumbuhan spesifik *Chlorella* sp. Kesimpulannya, kerana pemencilan karbon dioksida telah diukur berdasarkan berat sel kering, keamatan cahaya merupakan faktor penting bagi pemencilan karbon dioksida oleh *Chlorella* sp. dalam medium POME. Walau-bagaimanapun, keamatan cahaya yang terlalu tinggi akan menyebabkan perencatan fotosintesis dan mengurangkan kadar pertumbuhan *Chlorella* sp. yang seterusnya melambatkan proses pemencilan karbon dioksida.

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LIST OF ABBREVIATIONS

μ	Specific growth rate
BBM	Bold's Basal Medium
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DOE	Design of Experiment
GHG	Greenhouse Gas
lux	SI unit of illumination intensity
pH	Hydrogen Ion Concentration
POME	Palm Oil Mill Effluent
rpm	rotation per minute
RSM	Response Surface Method
RuBisCO	Ribulose-1,5-biphosphate carboxylase oxygenase
sp.	Species

1 INTRODUCTION

1.1 Motivation and statement of problem

Carbon dioxide (CO_2) has been identified as one of the primary greenhouse gases (GHG) in the atmosphere (Choo and Subramaniam, 2012). Carbon dioxide emissions have increased by about 35% since 1990 (Kaladharan *et al*., 2009). Koorosh and Adal (2012) stated that the increasing of carbon dioxide emissions causes the global warming which leads to acid rain and air pollution. Lam and Lee (2011) said that in Malaysia this problem is due to the rapid growth of palm oil industries. Malaysia has become the second largest palm oil producer in the world and its palm oil industry served as a backbone to Malaysia economy. However, in order to produce crude palm kernel oil a lot of carbon dioxide is emitted (Choo and Subramaniam, 2012). In addition, it also contributes to others negative impacts to environment such as deforestation and habitat destruction when a new oil palm plantation is started or a new palm oil mill is built.

CO_2 emission can be reduced through CO_2 sequestration, which is the process of carbon capture from atmosphere (Kenneth, 2010). A recent study found that photoautotrophic algae have the potential to sequestrate CO_2 through carbon fixation during photosynthesis (Kaladharan *et al*., 2009). Peter *et al*. (2006) summarized the advantages of using microalgae as its rapid growth, its ability to produce more biofuel than oil plants, its ability to sequester CO_2, the low toxicity of biodegradable fuel without sulfur, it is not being involved in competition with food crops, and also it is not being involved in natural habitat destruction. In addition, microalgae are rich in micronutrients. Table 1.1 lists the micronutrients that containing in microalgae.

Table 1.1: Micronutrient contain in microalgae (Peter *et al*., 2006).

Micronutrient	**Content (%)**
Protein	25-40
Carbohydrate	5-30
Lipid/oils	10-30

Microalgae need certain condition to grow. They needs light, nutrients and a warm condition. It can be grown in wastewater treatment ponds, animal waste and other liquid waste. Nitrogen, phosphorus, potassium and magnesium are the nutrients needed for microalgae growth. According to Choong (2012), palm oil mill effluent (POME) contains significant amounts of these nutrients and can be used as the medium for microalgae culture.

POME is the waste discharged by the palm oil industries which has created serious water pollution in Malaysia (Habib *et al.*, 1997). According to Choong (2012) POME consists of palm fruit water soluble components and suspended materials such as palm fibre and oil residue. Since POME is acidic and contains residual oil that difficult to separate, it cannot be discharged without first being treated. It needs a lot of oxygen to decompose completely. This phenomenon is known as high biochemical oxygen demand (BOD). Sometimes, BOD of POME is up to 100 times higher than domestic sewage. Table 1.2 shows the characteristics of POME and discharged limit.

Table 1.2: Characteristics of POME and discharged limit (Official Portal Malaysian Palm Oil Board, December 2012)

Parameter	**POME (range)**	**POME (mean)**	**Discharge standard (1.1.1984 and thereafter)**
Temperature (^{o}C)	80-90	85	45
pH	3.4-5.2	42	5.0-9.0
Oil and grease	130-18,000	6,000	50
BOD	10, 250-43,750	25,000	100
COD	15,000-100,000	51,000	-
Total solid	11,500-79,000	40,000	-
Suspended solid	5,000-54,000	18,000	400
Total volatile solid	9,000-72,000	34,000	-
Total nitrogen	180-1,400	750	200
Ammoniacal nitrogen	4-80	35	150

During photosynthesis, microalgae will absorb CO_2 and release oxygen. So that, microalgae will sequestrate the CO_2 emission during the production of crude palm kernel oil. Meanwhile, oxygen that is released by microalgae during photosynthesis will be used for POME decomposition. This correlation will reduce the pollution caused by palm oil industries.

Therefore, the aim of this research is to sequestrate the CO_2 that release during the production of crude palm kernel oil by culturing microalgae in POME medium which have high BOD and chemical oxygen demand (COD). The usage of microalgae will provide oxygen for POME decomposition.

1.2 Objectives

The following are the objectives of this research:

- To study the factors of light intensity and % (v/v) CO_2 sequestration that are influencing the CO_2 sequestration of algae in POME medium at shake flask scale.

1.3 Scope of this research

The following are the scope of this research:

i) To study the effect of light intensity (2,000-12,000 lux) and % (v/v) CO_2 (2-10%) on the growth of *Chlorella* sp. in POME medium.

ii) To study the interaction between these two factors using Design Expert 6.0.8 software.

1.4 Main contribution of this work

By doing this research, after considering the parameter of light intensity and CO_2 concentration, the interaction factors affecting the CO_2 sequestration can be achieved by using Design Expert 6.0.8 Software. Besides, this process also can provide the oxygen for POME decomposition. The cost of CO_2 sequestration can be reduced since it does not need any other costly method for CO_2 sequestration like CO_2 injection into soil which is practically used in Canada. Other CO_2 sequestration methods need high cost to set up the technology and transportation. The CO_2 sequestration by microalgae is biologically method, which also known as win-win strategies. The microalgae can get nutrients source that containing in POME which will cause water pollution if release directly to environment without treatment.

2 LITERATURE REVIEW

2.1 Carbon Dioxide (CO_2) Sequestration

This As a means of reducing the emission of carbon dioxide (CO_2) and other greenhouse gases (GHGs) such as methane (CH_4) and nitrous oxide (N_2O) in atmosphere, various carbon sequestration methods have been investigated. Many sequestration strategies have focused on expensive carbon capture technologies. Oelkers and Cole (2008) proposed three major types of carbon storage: geological storage, ocean storage, and mineral carbonation. Geological storage is the process of injection of CO_2 into porous rock formations (Holloway, 2001). Meanwhile, ocean storage means the injection of captured CO_2 into the ocean, which usually greater than 1000 meters in depths to make it isolated from the atmosphere (Adams and Caldeira, 2008). Mineral carbonation was aims to create stable carbonate minerals, such as magnesite ($MgCO_3$) and calcite ($CaCO_3$),by reacting CO_2 with silicate minerals containing magnesium and calcium (Oelkers *et al*. 2008). However, these processes need high cost to set up the technology and transportation to sequestration site.

In Canada, CO_2 injection into soil reservoir has been applied. This method has improved the economics because of the value added component associated with incremental hydrocarbon recovery. But, the problem is in the selecting of the right underground reservoir for storing CO_2 and it has the maximum level of CO_2 can be stored in the reservoir (Koorosh and Adal, 2012).

Recent study by Kaladharan *et al*. (2009) found that microalgae have the potential to sequestrate the CO_2 from atmosphere. Microalgae are the primary producers and it plays an important role in carbon sequestration. Some microalgae species are grown to optimize CO_2 sequestration. CO_2 is used as the carbon source in an autotrophic culture (Kenneth, 2010). Microalgae will sequester CO_2 from the atmosphere to form biomass (Kenneth, 2010). Since the molecular formula of microalgae biomass is $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$, approximately half of the dry weight of algal biomass is carbon

(Chisti, 2007). Based on mass balance, the sequestration value can be quantified; for every pound of algal biomass created, 1.83 pounds of CO2 are sequestered (Chisti, 2007). Biomass is produced according to the following reversible reaction (Chaumont, 1993):

$$6.14\,CO_2 + 3.65\,H_2O + NH_3 \xleftrightarrow{\text{Light}} C_{6.14}H_{10.3}O_{2.24}N + 6.85\,O_2 \quad (2.1)$$

2.2 Microalgae

Microalgae are single cell organisms which represent both bacteria and eukaryotes (Lam & Lee, 2011). Microalgae species are divided into four categories depending on their pigmentation, life cycle and basic cellular structure: diatoms (*Bacillariophyceae*), green algae (*Chlorophyceae*), blue-green algae (*Cyanophyceae*) and golden algae (*Chrysophyceae*) (Khan *et al.*, 2009). William and Laurens (2010) have identified the microalgae main components which are carbohydrates, proteins, nucleic acids and lipids (typically phospholipids and glycolipids).

The advantages of using microalgae as CO_2 sequester: (1) rapid growth rate; (2) as a source of biofuels; (3) low toxicity of biodegradable fuel without sulfur; (4) not being involved in natural habitat destruction; (5) rich in micronutrients; (6) synthesize and accumulate large quantities of neutral lipids/oil; (7) oil yield exceed the yield of the best oilseed crops; (8) can be cultivated in saline/brackish water/coastal seawater or non-arable land; (9) does not being involved in competition with food crops; (10) utilize nitrogen and phosphorus from a variety of wastewater sources (11) produce value-added by-products (e.g. biopolymers, proteins, polysaccharides, pigments, animal feed and fertilizer); (12) do not need herbicide and pesticide; and (13) higher biomass productivity (Peter *et al*., 2006; Khan *et al*., 2009; Tredici, 2010).

Microalgae are said to be rapid growth rate because microalgae can double their biomass in less than 24 hours (Tredici, 2010). Moreover, once in every 3-4 hr

microalgae are able to divide its cell under favorable growing conditions (Williams and Laurens, 2010). Khan *et al*. (2009) said that microalgae have ability to uptake the large amount of nutrients from water sources because of their simple cellular structure and large surface to volume ratio. This promotes their growth rate. In addition, when grown in open pond culture system, the algae can be grown in wastewater treatment systems using the waste water stream effluent as a water and nutrient source.

Chlorella sp. (Figure 2.1) was used in this study. There are several advantages of using *Chlorella* sp. This is because *Chlorella* sp. can produce high biomass under high CO_2 concentration due to its ability to fix up to 74% of the original CO_2 with only 2 seconds of CO_2 residence time (Sebastian *et al*., 2013). Sebastian *et al*. (2013) also stated that under ambient air concentration of CO_2 (0.037%) *Chlorella* sp. will growth rapidly. Besides, *Chlorella* sp. can reduce the BOD value. Violeta *et al*. (2011), in their studies, found that BOD was reduced more than 87.1% during the growth of *Chlorella* sp. For application, *Chlorella* sp. usually used in cultivation of marine organism for human food, and for zooplanktons culture in marine fish hatchery (Igor, 2001).

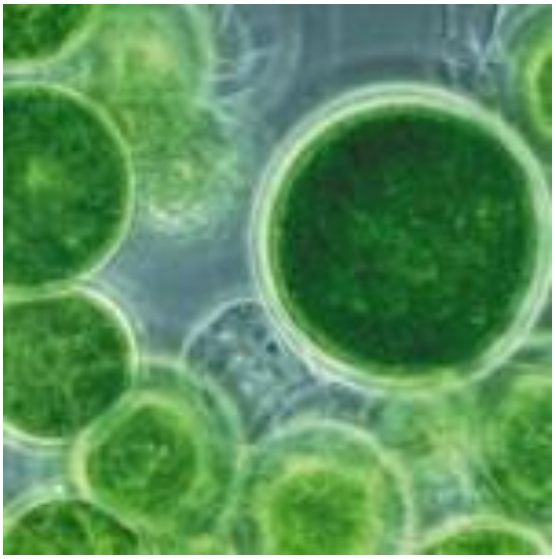

Figure 2.1: *Chlorella* sp.

2.3 Microalgae Growth Condition

2.3.1 Carbon dioxide

Carbon dioxide (CO_2) is the carbon source for the process of photosynthesis by microalgae (Ryu*et al*., 2012). During photosynthesis, CO_2 is dissolved in water and becomes HCO_3^-, which was absorbed by microalgae (Ciferrum, 1983). Hence, CO_2 are significant key factors for microalgae cultivation and balances the CO_2 ecosystem as well. However, each microalga shows different behaviour towards CO_2 consumption. Some microalgae required higher CO_2 concentration to avoid binding of Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) to Oxygen rather than CO_2 (Rahaman *et al*., 2011), meanwhile some microalgae growth restricted by increasing injection rate of CO_2 into the culture medium.

With regards to this research, in order to maximizing CO_2 uptake by microalgae, CO_2 were supplied at different volumetric flowrate so that the effect of CO_2 towards microalgae growth can be observed throughout the study.The recommended range for CO_2 concentration for *Chlorella* sp. culture is 2-10% (Mariana *et al*., 2013).

2.3.2 pH

pH range for microalgae cultivation is 7–9, with the optimum range being 8.2–8.7 (Coutteau, 1996). The pH value must be maintained because too low or too high pH value will cause disruptions of microalgae cells and leads to the death of culture, the increase in pH occurs over the time in very dense cultures condition (Sabrettin,2012).

2.3.3 Temperature

Most common cultured temperature of microalgae is between 20 to 30˚C (Chisti, 2007). But, it is depending on the culture medium composition, the species and strain cultured

(Woertz*et al*., 2004). Exceeding the optimum temperature 2-4˚C will result in the total loss of culture (Mata *et al*., 2010).

2.3.4 Aeration

Mixing is necessary to provide turbulent flow (Chisti, 2007). This turbulent flow is provide to prevent sedimentation of the algae, to ensure that all cells are equally exposed to light and nutrients, to avoid thermal stratification and facilitate gas transfer rate between the culture medium and the air (Mata *et al*., 2010). Besides, aeration is functioning to strip out accumulated oxygen because when the level of oxygen dissolved greater than air saturation value, it will inhibit the photosynthesis (Chisti, 2007).

2.3.5 Nutrient

Growth medium must contain the inorganic elements that constitute the algal cell. The essential elements are nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg) (Peter *et al*., 2006). Furthermore, Chisti (2007) also recommended adding the iron (Fe) and silicon (Si) as the nutrient for microalgae growth.

2.3.6 Light

Algae are affected by the light intensities to which they are exposed. Exposure to too little light will decrease biomass productivity because it has prohibited logarithmic growth. Exposure to too much light inhibits growth and kills the organism. This is known as photo-inhibition (Esra *et al*., 2007). Photo-inhibition means that there is no further growth in an algal culture as a result of increasing light intensities. Photo-inhibition results when maximum growth rate is achieved given an unchanging suite of nutrient conditions. Up to the light saturation value, algae will grow exponentially with increasing irradiance. Above this light saturation value, a further increase in irradiance actually reduces the biomass growth rate. Most algae become photo-inhibited at irradiance levels slightly greater than the light level at which their growth rate peaks

(Karcher, 2010). Previous study by Igor (2001) found that the suitable range of light level for *Chlorella* sp. culture is 2,000-12,000 lux.

2.4 Palm Oil Mill Effluent (POME)

POME is a thick brownish colloidal suspension containing mixture of water (95–96%), oil (0.6–0.7%) and total solids (4–5%), including 2–4% of suspended solids (Wu *et al*., 2007). The suspended solid is originating from the mixture of sterilizer condensate, separator sludge and hydrocyclone wastewater in a ratio of 9:15:1, respectively (Wu *et al*., 2010). Since no chemical are added during extraction process, it is classified as non-toxic effluent (Khalid and Wan Mustafa, 1992). POME is discharged with high temperature (80-90^{o}C), acidic (pH around 4.5), high biochemical oxygen demand (BOD), high chemical oxygen demand (COD), contain oil, grease and suspended solids (Khalid and Wan Mustafa, 1992; Md Din *et al*., 2006; Wu *et al*., 2007). Therefore, the palm oil mill industry in Malaysia has been identified as one of the industries that have contributed to the water pollution throughout the country. As stated by Lam and Lee (2011), based on 17.56 million tons of the total crude palm oil production in year 2009, it was estimated about 8 million m^3 (11,600 million gallon) of POME was generated from Malaysian palm oil mills. Table 2.1 shows the typical characteristics of POME. From Table 2.1, the total nitrogen contents are still high compared to the discharge standard for wastewater, which is 200 mg/L (Lam & Lee, 2011). If the POME is discharged untreated, it can certainly cause considerable environmental problems. Meanwhile, nitrogen source is one of the nutrients needed to promote microalgae growth. The basic nitrate concentration required to grow microalgae effectively is in the range of 200–400 mg/L (Li *et al*., 2008). Other minerals that are required for microalgae growth, such as Fe, Zn, P, Mg, Ca and K, are also present in POME (Habib *et al*., 1998).Thus, POME provide an alternative option as a chemical remediation to grow microalgae for biomass production.

Table 2.1: Characteristics of sterilization condensate, separator sludge and hydrocyclone wastewater (Whiting, 1978).

Parameters[a]	Sterilizer condensate	Separator sludge	Hydrocyclone wastewater
pH	5.0	4.5	-
BOD[b]	23000	29000	5000
COD	47000	64000	15000
TSS	5000	23000	7000
Total dissolved solid	34000	22000	100
Total nitrogen	600	1200	100
Ammoniacal nitrogen	20	40	-
Oil and grease	4000	7000	300

a All parameters are in units of mg/l except pH.

b The sample for BOD analysis is incubated at 30 ^{o}C for 3 days.

2.5 Bold's Basal Medium (BBM)

Several media such as CFTRI media, OFERR media, Revised media, Bangladesh medium No (3), Zarrouk's media and Bold's Basal Media (BBM) has been identified as microalgae culture medium. According to Sankar and Ramasubramanian (2012), *Chlorella* sp. grown best in Bold's Basal Media compared to others. Microalgae growth is expressed as concentration of microalgae biomass. From Table 2.2, we can conclude that Bold's Basal Medium give higher biomass production. This is due to nutrients contains in the medium. In this study, Bold's Basal Medium was used to culture the stock microalgae and preparing inoculums. Table 2.2 below shows the experimental result conducted by Sankar and Ramasubramanian (2012).

Table 2.2: Chlorophyll b content in (mg/L) *C.vulgaris* cultured in six different types of culture media (Sankar and Ramasubramanian, 2012)

Culture Days (Days)	**BBM (mg/L)**	**Bangladesh M3 (mg/L)**	**Revised M6 (mg/L)**	**OFERR M (mg/L)**	**CFTRI M (mg/L)**	**Zarrouk's M (mg/L)**
5	0.35	0.33	0.23	0.20	0.11	0.06
10	0.47	0.37	0.31	0.26	0.21	0.18
15	0.50	0.47	0.38	0.34	0.26	0.20
20	0.56	0.51	0.40	0.37	0.32	0.30

Each value is expressed as mean ± SEM (n=9) X Statistically significant at $P<0.05$.

2.6 Mathematical Analysis

2.6.1 Factorial Design

The experimental variables, factors and interaction effects on the responses can be investigated by using a factorial design (Zatilfarihiah *et al*., 2009). 2-level factorial design (2^n) is commonly used in mathematical analysis. The maximum result based on statistical principles with the less number of experiments can be identified from this method (Mullai *et al*., 2010). From this method, the number of experiment need to be conducted can be calculated. The factorial experiments make use the Yates' Method (Yates, 1937) to analyze the main effects and the interactive effects. Main effect can be categorized as independent variables where all the effects based on the experimental error. Meanwhile, failure of one factor to show the same result on the response at different level of other factor was actually interaction. Thus, the interactive effect was for two or more variables that dependent between them (Ding, 2011).

2.6.1 Yates' Method

This mathematical method was used in the factorial experiment. Yates' method is used to analyze on the main effects and interactive effects. Based on Yates' method calculation, it will show the yield response surface whether it is curved or uncurved, whether flat, increasing or decreasing based on one or more of experimental variables and the direction. In order to detect the effect of difference on the yields, the experimental variables were under two levels equidistant from the centre point (Ding, 2011). Yet, advance analysis using Design of Experiment (DOE) software such as Design Expert Software was developed in order to simplify the analysis.

DOE software was developed with the purpose of providing many powerful statistical tools such as two-level factorial screening designs, general factorial studies, response surface methods (RSM), mixture design techniques and combinations of process factors, mixture components, and categorical factors.

3 MATERIALS AND METHODS

3.1 Flowchart of Research Methodology

The brief description of process flow for CO_2 sequestration by algae in POME medium is shown in Figure 3.1.

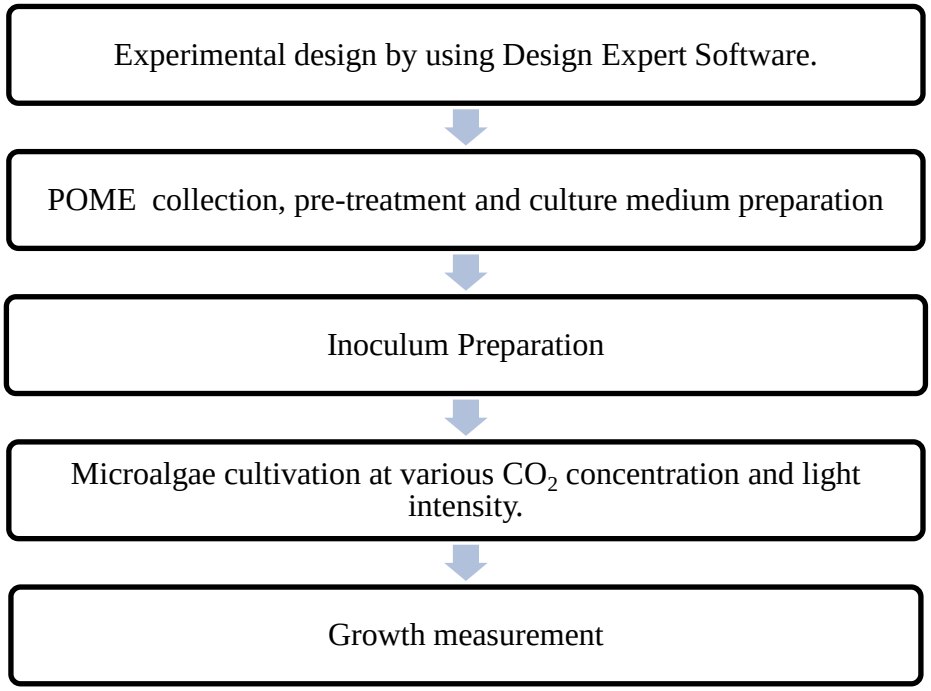

Figure 3.1: Process flowchart on CO_2 sequestration process.

3.2 Experimental Design

Factorial experiment was design by using Design Expert Software. The parameter range identified was 2,000-12,000 lux for light intensity and 2-10% for CO_2 concentration. A 2^2 full factorial design with 3 centre points was chosen. The experimental runs were generated by Design Expert version 6.0.8 and shown in Table 3.1

Table 3.1: Factorial Experimental Design

No of Experimental Run	Light Intensity (lux)	CO_2 Concentration (%)
1	12,000	10
2	7,000	6
3	2,000	10
4	2,000	2
5	12,000	2
6	7,000	6
7	7,000	6

3.3 POME Collection and pre-treatment

Culture medium of POME from polishing pond was obtained from Dominion Square Palm Oil Mill Sdn. Bhd.,Gambang, Pahang, Malaysia. POME was treated first by removing the solid particle by centrifugation (Eppendorf Refrigerated Centrifuge model 5810R) at 10,000 rpm for 10 minutes. Then, POME was autoclaved (Hiraclave, Hirayama, model PH PM088) at 121^{o}C for 20 minutes to prevent contamination.

3.4 Bold's Basal Medium (BBM)

3.4.1 Preparation of stock solution for Bold's Basal Medium (BBM)

Medium was prepared in aqueous solution. Three stock solutions were prepared separately by using ultrapure water to prevent contamination. All chemicals used are

indicated in Table 3.2. To fully dissolve all chemicals, preparation should be done at 50-60^{o}C under stirring condition.

Table 3.2: List of chemicals used to prepare BBM stock solution (Daphnia Research group, 2007).

Stock Solution	**Chemical Name**	**Formula**	**Weight (g)**	**Ultrapure Water (mL)**
A	Dipotassium Phosphate	K_2HPO_4	1.875	250
	Potassium dihydrogen phosphate	KH_2PO_4	4.375	
	Magnesium sulphate heptahydrate	$MgSO_4.7H_2O$	1.875	
	Sodium Nitrate	$NaNO_3$	6.250	
	Calcium chloride dehydrate	$CaCl_2.2H_2O$	0.625	
	Sodium Chloride	NaCl	0.625	
B	Ethylenediaminetetraacetic acid tetrasodium salt	EDTA - Na_4	5.000	100
	Potassium hydroxide	KOH	3.100	
	Ferrous sulphate heptahydrate	$FeSO_4.7H_2O$	0.498	
	Sulphuric acid conc. (wt per mL = 1.84g)	H_2SO_4	0.1mL	
	Boric acid	H_3BO_3	1.142	
C	Zinc sulphate heptahydrate	$ZnSO_4.7H_2O$	0.353	25
	Manganese chloride tetrahydrate	$MnCl_2.4H_2O$	0.058	
	Copper (II) sulphate pentahydrate	$CuSO_4.5H_2O$	0.063	
	Cobalt (II) nitrate hexahydrate	$Co(NO_3)_2.6H_2O$	0.020	
	Sodium molybdate (VI) dehydrate	$Na_2MoO_4.2H_2O$	0.048	

3.4.2 The preparation 1L of BBM medium

To prepare 1 L of BBM, 10 mL of stock solution A, 1 mL of stock solution B and 0.1 mL of stock solution C were added into the 1 L Schott bottle that containing 900 mL of ultrapure water. Then, top up with ultrapure water until the volume become 1 L. Next, the mixture was stirred for 30 minutes to ensure the BBM medium mix properly. Lastly, the pH value was checked and must be in the range of 6.7 ± 0.3.

3.5 Inoculums Preparation

All the culture materials, including 2 L conical flasks, air filters, rubber stoppers, silicon rubber tubing and BBM medium were autoclaved at 121^{o}C for 20 minutes to prevent contamination. Final culture volume was 2 L which contain 10% of inoculation volume (Ding, 2011). 200 mL of microalgae were poured into 2 L conical flask containing 1800 mL of BBM medium. All operations were conducted in the biohazard safety cabinet to prevent contamination. The microalgae were aerated with filtered air by using aquarium air pump and cultivated under 1,300 lux of illumination intensity for 7 days (Ding, 2011). 300 mL of inoculums was prepared for conical flask cultivation.

3.6 Microalgae Cultivation

All the culture materials, including 1 L conical flasks, air filters rubber stoppers, silicon rubber tubing and POME medium were autoclaved at 121^{o}C for 20 minutes. Final culture volume was 1 L which contains 30% of inoculation volume (Ding, 2011). 300 mL of inoculums were poured into conical flask containing 700 mL of POME medium. All operations were conducted in the biohazard safety cabinet. The microalgae were then aerated with filtered 5 L/min air mix with %(v/v) CO_2 by gas mixing system, and cultivated under appropriate light intensity for 7 days (Ding, 2011; Figure 3.2).

Figure 3.2: Microalgae culture in POME medium

3.7 Growth Measurement

Productivity of biomass was measured by cell dry weight. 40 mL of culture samples taken for 7 days at every 24 hours were centrifuged at 10,000 rpm for 15 minutes (Ding, 2011). The cell pellet was rinsed twice with distilled water and was dried overnight at 70°C oven (Weena *et al*., 2005).

Cell dry weight of *Chlorella* sp. was calculated using Equation 3.1.

$$\text{Cell Dry Weight (g)} = \text{Weighing boat With cell pellet (g)} - \text{Weighing boat without cell pellet (g)} \quad (3.1)$$

Specific growth rate was calculated by using Equation 3.2 (Mariana *et al*., 2013)

$$Specific\ Growth\ Rate, \mu\ (day^{-1}) = \frac{(\ln N_2 - lnN_1)}{t_2 - t_1} \qquad (3.2)$$

Where N_1 is concentration of cell at the beginning of exponential growth phase, while N_2 is concentration of cell at the end of exponential growth phase. t_1 is the time at the beginning of exponential growth phase and t_2 is the time at the end of exponential growth phase.

4. RESULT AND DISCUSSION

4.1 Introduction

To identify the variables that influencing the CO_2 sequestration by algae (*Chlorella* sp.), the sparged flask method were used. Two variables which affect the CO_2 sequestration were selected; they were light intensity and CO_2 concentration. An experimental design of 7 runs containing 4 factorial experiments and 3 central points were generated according to two-level factorial design. The light intensity range was chosen at 2,000-12,000 lux. Meanwhile, the range of CO_2 concentration in gas mixture was chosen at 2-10% v/v proportion in air. The aeration rate was 5 L/min.

4.2 Result of Factorial Design Experiment

The individual and interactive effects of CO_2 concentration and light intensity were studied by culturing the microalgae at different conditions. The responses were measured in term of cell dry weight and specific growth rate. The values of parameter for *Chlorella* sp. culture condition and its corresponding results are shown in Table 4.1.

Table 4.1**:** Culture conditions of *Chlorella* sp., the cell dry weight obtained and specific growth rate of *Chlorella* sp.

No. of experimental run	**Factor 1**	**Factor 2**	**Response 1**	**Response 2**
	A: Light intensity (lux)	**B: Concentration of CO_2 (%)**	**Cell Dry Weight (g)**	**Specific growth rate, μ (day^{-1})**
1	12,000	10	**0.043**	1.147
2	7,000	6	0.025	1.210
3	2,000	10	0.019	**2.649**
4	2,000	2	0.029	1.172
5	12,000	2	0.038	1.143
6	7,000	6	0.026	1.262
7	7,000	6	0.022	1.222

Data collected for cell dry weight and specific growth rate for each run was analysed using Design Expert 6.0.8 software. From Table 4.1, it shows that the highest cell dry weight was obtained at highest light intensity (12,000 lux) and highest CO_2 concentration (10%). Meanwhile, highest specific growth rate was obtained at the lowest light intensity (2,000 lux) and highest CO_2 concentration (10%). There were no correlation between cell dry weight and specific growth rate because cell dry weight was obtained to identify the cell produce at the end of experiment. Meanwhile, specific growth was identified to determine how fast the cell growth per time at exponential phase.

4.3 Effect of Factors on Cell Dry Weight

By applying two-level factorial design analysis on the experimental data, the factorial model was used to explain the cell dry weight (CDW) of microalgae produced. The factorial model was as proposed in Equation 4.1.

$$CDW\ (g) = 0.032 + 0.00825\ A - 0.00125\ B + 0.00375\ AB \qquad \text{Eq. (4.1)}$$

where A and B represent the light intensity (lux) and CO_2 concentration (%), respectively.

Equation 4.1 appropriately describes the effect of light intensity and CO_2 concentration to cell dry weight obtained in this study. This equation indicates that the light intensity was positively significant factors, whereas CO_2 concentration was negatively significant factor (Sayyad *et al*., 2006). The analysis of variance (ANOVA) for the model is shown in Table 4.2.

Table 4.2**:** Analysis of variance for the model of cell dry weight response.

Source		Sum of square	DF	Mean square	F value	Prob>F
Model		$3.348x10^{-4}$	3	$1.116 x10^{-4}$	25.75	0.0376*
A		$2.732 x10^{-4}$	1	$2.723 x10^{-4}$	62.83	0.0155*
B		$6.25 x10^{-6}$	1	$6.25x10^{-6}$	1.44	0.3527
AB		$5.625 x10^{-5}$	1	$5.625x10^{-5}$	12.98	0.0691
Curvature		$1.074 x10^{-4}$	1	$1.074x10^{-4}$	24.79	0.0380*
R^2	0.9748					
Adjusted R^2	0.9369					

*Significant ($p<0.05$)

From Table 4.2, it shows that the model was significant since the model F-value is 27.75. The high value of regression coefficient (R^2 = 0.9748) could explain that the model was an accurate representation of the data (Zatilfarihah *et al*., 2009). Table 4.2 also showed that light intensity (A) was significant term for cell dry weight production, which means this term had great impacts on cell dry weight produce.

Figure 4.1 shows the interaction graph for factor of cell dry weight produced by *Chlorella* sp. From the graph, at 2,000 lux of light intensity, cell dry weight decrease with the increasing of CO_2 concentration. Meanwhile, at 12,000 lux of light intensity, cell dry weight increased slightly when CO_2 concentration increases. It shows that light intensity and CO_2 concentration are interacting in response to cell dry weight produced. Nevertheless, this interaction is not significant up to 95% confident level (Table 4.2). This can be clearly seen in the Figure 4.1, where the error bars were overlapped to each other as the CO_2 concentrations were changed for both the light intensity.

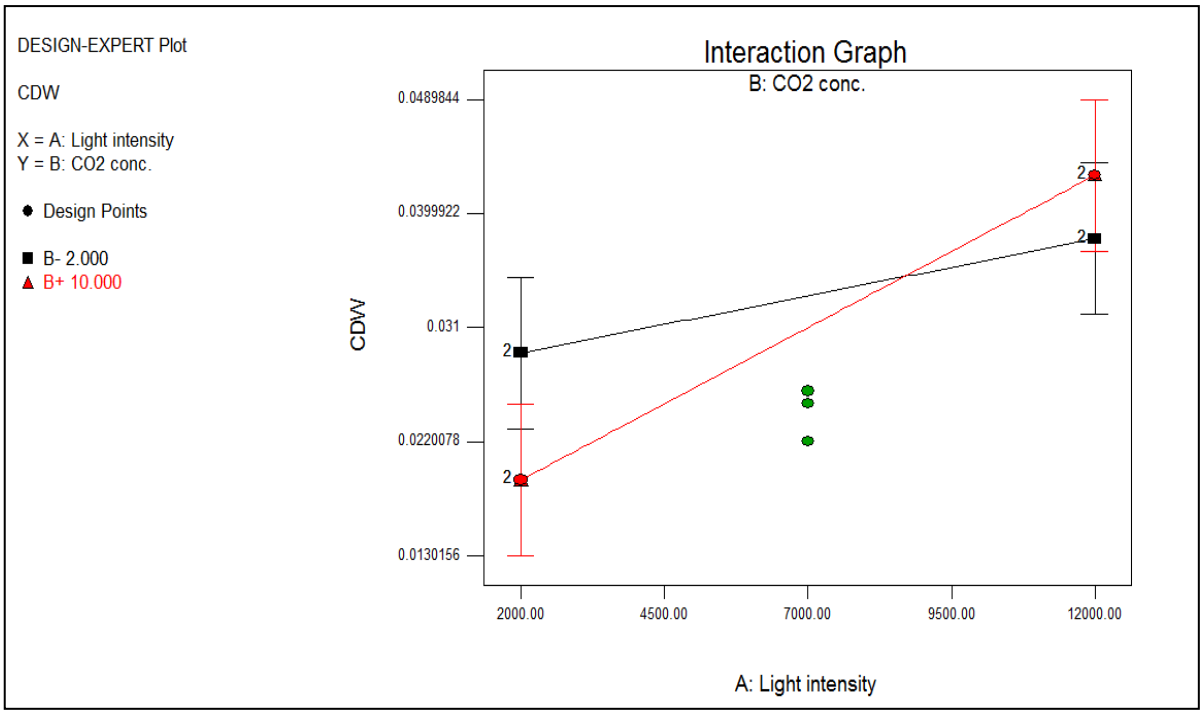

Figure 4.1: Interaction graph of light intensity and CO_2 concentration on cell dry weight produce by *Chlorella* sp.

Table 4.2 also illustrates that the curvature for the model is significant. Nevertheless, Figure 4.1 depicts that the significant curvature was not helpful as it was a negative curvature. The aim of current study is to sequester CO_2 by consumption of microalgae during growth. Thus, it is expected that the higher the cell dry weight obtained the greater the amount of CO_2 will be sequester. As a result, negative curvature only demonstrates that the range of study is not correct and it is still far from the optimum point.

Igor (2001) found that highest cell dry weight of *Chlorella* sp. was at 12,000 lux of light intensity. Meanwhile, Sebastian *et al.* (2013) found that when CO_2 concentration increases, cell dry weight also increases. However, at 2,000 lux light intensity and 10% of CO_2 concentration, this study shows the lowest cell dry weight produced. This is due to the low light intensity provided was not enough because the dark brown colour from tannic acid in POME reduced the light passing through the culture and affect the photosynthesis reaction (Hadiyanto and Nur, 2012). In addition, at highest CO_2 concentration, the cell dry weight produced decreased at low light intensity, meanwhile the cell dry weight increased at high CO_2 concentration and at high light intensity

(Granados and Korner, 2002). A plant cannot use more CO_2 if there is insufficient excitation of electrons due to low light intensity. At low light intensity, the rate of photosynthesis is low even there is plenty carbon dioxide. Increasing the light intensity will accelerate the speed of photosynthesis. Meanwhile, Claveland (2006) stated that under low light intensity, photosynthesis is enhanced by increasing light, but is unaffected by increases the carbon dioxide. When light intensity is high, increases the carbon dioxide accelerate photosynthesis. Since photosynthesis can be measured by an increase in cell dry weight, the increase in rate of photosynthesis shows that the increasing of cell dry weight (Photosynthesis, n.d).

4.4 Effect of Factors on Specific Growth Rate

In similar way, two-level factorial design analysis was performed to evaluate the effect of light intensity and CO_2 concentration on specific growth rate of *Chlorella* sp. The factorial model was constructed as in Equation 4.2.

$$Specific\ growth\ rate\ (day^{-1}) = 1.53 - 0.38\ A + 0.37\ B - \ 0.37\ AB \qquad \text{Eq. (4.2)}$$

where A and B represent the light intensity (lux) and CO_2 concentration (%), respectively.

The effect of light intensity and CO_2 concentration to specific growth rate was described by Equation 4.2. From this equation, it shows that the light intensity was negatively significant factors, whereas CO_2 concentration was positively significant factor. The analysis of variance (ANOVA) of the model was shown in Table 4.3.

Table 4.3: Analysis of variance for model of specific growth rate response.

Source	Sum of square	DF	Mean square	F value	Prob>F
Model	1.68	3	0.56	753.94	0.0013*
A	0.59	1	0.59	790.45	0.0013*
B	0.55	1	0.55	739.67	0.0013*
AB	0.54	1	0.54	731.70	0.0014*
Curvature	0.15	1	0.15	203.18	0.0049*
R^2	0.9991				
Adjusted R^2	0.9978				

*Significant ($p<0.05$)

Table 4.3 shows that the model for specific growth rate was significant since the model F-value is 753.94. The high value of regression coefficient (R^2 = 0.9991) means that calculated model is able to explain 99% of the result (Sayyad *et al*., 2006). Table 4.3 also showed that light intensity (A), CO_2 concentration (B) and interaction factor (AB) were significant terms for specific growth rate, which means these terms had great impacts on specific growth rate of *Chlorella* sp. The curvature was also significant, but it is a negative curvature as in previous case. Thus, the range of light intensity and CO_2 concentration were not acceptable.

Figure 4.2 shows the interaction response to the factor of specific growth rate for *Chlorella* sp. The graph shows that at low CO_2 concentration (2% v/v), increasing light intensity did not affect specific growth rate. On the other hand, when CO_2 concentration were high (10% v/v), increasing light intensity would reduce the specific growth rate. Based on Figure 4.2, it can be explained that the high light intensity inhibit the growth of *Chlorella* sp. Esra *et al*. (2007) said that higher light intensity can caused photo-inhibition. The consequences of photo-inhibition in photosynthesis are a reduction of the maximum quantum yields for CO_2 uptake (Long and Humphries, 1994). Based on Figure 4.2, it also shows that high CO_2 concentration at low light intensity (2000 lux) will increase the growth rate of *Chlorella* sp. Sabrettin (2012) stated that specific growth rate is depend on CO_2 level where the higher the CO_2 concentration supply, the higher is the specific growth rate. The stimulatory effect of CO_2 on growth parameters

of *Chlorella* sp. could be related to the increased availability of carbon as a result of the higher dissolve inorganic carbon (DIC) concentration (Mariana et al., 2013). Besides, CO_2 abundance influences several key enzymes in carbon metabolism, such as carbonic anhydrase and Rubisco. Thus, increasing CO_2 concentration can enhance the carboxylating activity and repress the oxygenating activity of Rubisco, resulting in increased specific growth rate (Mariana et al., 2013).

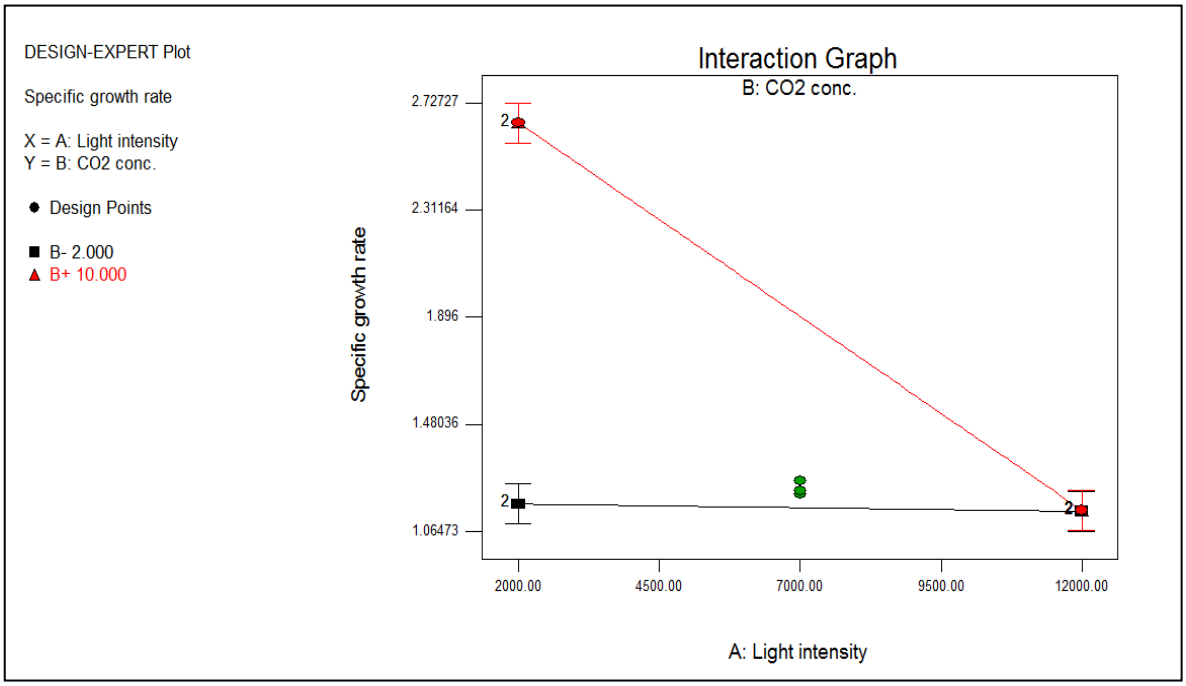

Figure 4.2: Interaction graph of light intensity and CO_2 sequestration on specific growth rate of *Chlorella* sp.

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Present study was aimed to determine the factors affecting the CO_2 sequestration. In this study, microalgae (*Chlorella* sp.) were cultured in POME medium to sequestrate CO_2. The effectiveness of sequestration was measured in term of cell dry weight and specific growth rate by using Design Expert 6.0.8 Software based on two-level factorial analysis.

As a conclusion, the objective of this study was successfully achieved. Light intensity was identified as the significant factor for cell dry weight production. Even at high CO_2 concentration, cell dry weight decreased if the light intensity was decreased. The interaction between light intensity and CO_2 concentration in cell dry weight production was insignificant. Meanwhile, interaction between factors on response to specific growth rate of *Chlorella* sp. was also identified. Specific growth rate of *Chlorella* sp. was not affected at low CO_2 concentration even though light intensity was increased. At high CO_2 concentration, specific growth rate is higher at low light intensity due to photo-inhibition that was occurred at high light intensity. Thus, light intensity, CO_2 concentration and interaction factors were significant in affecting specific growth rate of *Chlorella* sp. Since the CO_2 sequestration was measured based on cell dry weight production, light intensity was the significant factor for CO_2 sequestration by *Chlorella* sp. in POME medium. Nevertheless, too high a light intensity would cause photo-inhibition and reduced the growth rate of Chlorella sp. which in turn slows down the process of CO_2 sequestration.

5.2 Recommendation

Generally, this study can be further improved. High evaporation rate was occurred during this experiment. This may cause the culture become too concentrated due to the reducing of culture volume. To overcome this problem, Karcher (2010) had found the method by filtering the supply compressed air and pass through a sterilized DI water bath and then mixed with CO_2 in line.

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APPENDICES

Run 1

Light intensity 12000 lux

%CO2 10 %

Time (Day)	Weighing boat (g)	Weighing boat + algae (g)			Cell Dry Weight (g)	Concentration (mg/L)
		1	2	3		
0	10.152	10.156	10.154	10.154	0.002	50
1	10.069	10.075	10.074	10.073	0.004	100
2	9.963	9.974	9.973	9.972	0.009	225
3	9.923	9.941	9.938	9.937	0.014	350
4	10.011	10.037	10.036	10.032	0.021	525
5	9.705	9.739	9.735	9.733	0.028	700
6	10.297	10.327	10.326	10.339	0.042	1050
7	10.146	10.176	10.174	10.189	0.043	1075

Run 2

Light intensity 7000 lux

%CO2 6 %

Time (Day)	Weighing boat (g)	weighing boat + algae (g)			Cell Dry Weight (g)	Concentration (mg/L)
		1	2	3		
0	9.928	9.933	9.931	9.931	0.003	75
1	9.954	9.961	9.960	9.960	0.006	150
2	9.977	9.989	9.985	9.985	0.008	200
3	9.990	10.003	10.002	10.001	0.011	275
4	10.186	10.202	10.201	10.198	0.012	300
5	9.705	9.730	9.764	9.726	0.021	525
6	9.922	9.950	9.948	9.944	0.022	550
7	10.300	10.328	10.327	10.325	0.025	625

Run 3

Light intensity 2000 lux

%CO2 10 %

Time (Day)	Weighing boat (g)	weighing boat + algae (g)			Cell Dry Weight (g)	Concentration (mg/L)
		3	2	3		
0	9.961	9.966	9.964	9.962	0.001	25
1	10.027	10.033	10.033	10.030	0.003	75
2	10.031	10.041	10.040	10.036	0.005	125
3	9.970	9.980	9.977	9.977	0.007	175
4	10.054	10.063	10.063	10.063	0.009	225
5	9.697	9.712	9.718	9.708	0.011	275
6	9.752	9.768	9.767	9.767	0.015	375
7	9.956	9.976	9.975	9.975	0.019	475

Run 4

Light intensity 2000 lux

%CO2 2 %

Time (Day)	Weighing boat (g)	Weighing boat + algae (g)			Cell Dry Weight (g)	Concentration (mg/L)
		1	2	3		
0	1.309	1.319	1.318	1.316	0.007	175
1	1.311	1.332	1.322	1.322	0.011	275
2	1.295	1.312	1.311	1.31	0.015	375
3	1.315	1.332	1.331	1.331	0.016	400
4	1.319	1.336	1.336	1.336	0.017	425
5	1.310	1.329	1.328	1.328	0.018	450
6	9.692	9.715	1.711	9.711	0.019	475
7	10.011	10.043	10.041	10.040	0.029	725

Run 5

Light intensity 12000 lux

%CO2 2 %

Time (Day)	Weighing boat (g)	Weighing boat + algae (g)			Cell Dry Weight (g)	Concentration (mg/L)
		1	2	3		
0	1.300	1.308	1.307	1.307	0.007	175
1	1.319	1.327	1.327	1.327	0.008	200
2	1.289	1.306	1.305	1.305	0.016	400
3	1.313	1.342	1.340	1.340	0.027	675
4	1.317	1.346	1.345	1.345	0.028	700
5	1.310	1.342	1.339	1.339	0.029	725
6	9.696	9.726	9.726	9.726	0.030	750
7	9.823	9.864	9.861	9.861	0.038	950

Run 6

Light intensity 7000 lux

%CO2 6 %

Time (Day)	Weighing boat (g)	Weighing boat + algae (g)			Cell Dry Weight (g)	Concentration (mg/L)
		1	2	3		
0	9.962	9.965	9.964	9.964	0.002	50
1	10.010	10.013	10.013	10.013	0.003	75
2	10.011	10.016	10.015	10.015	0.004	100
3	10.065	10.077	10.076	10.074	0.009	225
4	9.924	9.936	9.935	9.934	0.010	250
5	9.819	9.842	9.836	9.834	0.015	375
6	9.958	9.979	9.978	9.977	0.019	475
7	9.699	9.727	9.725	9.725	0.026	650

Run 7	
Light intensity	7000 lux
%CO2	6 %

Time (Day)	Weighing boat (g)	Weighing boat + algae (g)			Cell Dry Weight (g)	Concentration (mg/L)
		1	2	3		
0	9.956	9.960	9.959	9.957	0.001	25
1	9.955	9.962	9.962	9.957	0.002	50
2	10.005	10.017	10.015	10.009	0.004	100
3	9.704	9.714	9.713	9.712	0.008	200
4	9.706	9.722	9.721	9.720	0.014	350
5	10.061	10.083	10.081	10.078	0.017	425
6	10.004	10.024	10.023	10.023	0.019	475
7	9.952	9.975	9.974	9.974	0.022	550

specific growth rate

run	t1	t2	t2-t1	x1	x2	x2-x1	spesific growth rate (day^{-1})
1	1	7	6	100	1075	975	1.147
2	2	7	5	200	625	425	1.210
3	5	7	2	275	475	200	2.649
4	2	7	5	375	725	350	1.172
5	1	7	6	200	950	950	1.143
6	2	7	5	100	650	550	1.262
7	2	7	5	100	550	450	1.222

(a)

(b)

Figure 7. 1: Figure (a) and (b) shows the gas mixing system.

Figure 7. 2: POME medium collection site

Figure 7. 3: Palm oil mill effluent (POME)