THE OPTIMIZATION OF CO_2 SEQUESTRATION BY MICROALGAE IN POME MEDIUM

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ABSTRACT

Recently, CO_2emissions cause a lot of issues such as Green House gas emissions and drastic climate changes. The cultivation of microalgae in POME medium as nutrient sources are believed can help reduce CO_2 emission to atmosphere and as well as act a POME treatment process. Microalgae has received a lot of attention in recent years due to their fast growth and ability to accumulate high quantity of lipid inside their cells for biodiesel production while acting simultaneous functions for CO_2 sequestration (Demirbas, 2011; Rahaman et al., 2011).

The aim of the study is to optimize the CO_2 sequestration by microalgae cultivation in POME medium. The study dealt with optimization of the level of % v/v of CO_2concentration and illumination intensity (lx) for harvesting microalgae by centrifugation and optimization of biomass growth. The method involved are microalgae cultivation, the analysed of dry biomass and optimization process. The biomass is measured by method of dry cell biomass.

In the optimization of biomass growth, the 2^2 factorial designs are used to investigate the effect of variableCO_2concentration in the sparging air mixture and illumination intensity. The factorial experiments at the area containing the maximum biomass concentration are complemented with the Yates' Method, Linear Regression and Steepest Ascent, respectively. Results showedthat the highest biomass yield (g/L) is 1.129. In the optimization, for Yate's method, the illumination intensity shows a significant effect on microalgae growth instead of CO_2 concentration. While, by using Linear Regression, the data was confirmed that the area investigated does not contain the maximum yield. The application of the Steepest Ascent method based on the linear equation of the factorial experiments indicate that the biomass yield can be increased as CO_2 concentration and illumination intensity is constantly increased.

Overall, main factor of illumination intensity and CO_2 concentration are important for microalgae growth.

ABSTRAK

Sejak kebelakangan ini, pelepasan CO_2 menyebabkan banyak isu-isu seperti pelepasan gas Rumah Hijau dan perubahan iklim yang drastik. Pembiakan microalgae dalam medium POME sebagai sumber nutrien dipercayai boleh membantu mengurangkan pelepasan CO_2 ke persekitaran dan juga berfungsi sebagai proses rawatan POME. Microalgae telah menerima banyak perhatian dalam beberapa tahun kebelakangan ini disebabkan oleh pertumbuhan pesat dan keupayaan untuk mengumpul kuantiti lipid yang tinggi dalam sel untuk menghasilkan biodiesel dan disamping berfungsi sebagai penyerapan CO_2 (Demirbas, 2011; Rahaman et al, 2011).

Tujuan kajian ini adalah untuk mengoptimumkan penyerapan CO_2 dengan pembiakan microalgae dalam media POME. Kajian ini ditangani dengan pengoptimuman tahap kepekatan CO_2 (% v/v) dan keamatan pencahayaan (lx) untuk penuaian microalgaemelalui kaedah bingkai putaran dan mengoptimumkan pertumbuhan biojisim. Proses yang terlibat adalah pembiakan microalgae, penganalisaan bahan kering dan proses pengoptimuman. Biomas ditimbang dengan kaedah biomas sel kering.

Dalam mengoptimumkan pertumbuhan biojisim, 2^2 reka bentuk faktorial digunakan untuk mengkaji kesan kepekatan CO_2dalam campuran udara dan pencahayaan. Eksperimen faktorial di kawasan yang mengandungi kepekatan biomas maksimum masing-masing dilengkapkan dengan Kaedah Yates, persamaan garis lurus dan kaedah puncak tercuram.Keputusan menunjukkan bahawa hasil biomas tertinggi (g/L) adalah 1.129. Dalam pengoptimuman, kaedah Yate, keamatan pencahayaan menunjukkan kesan yang besar kepada pertumbuhan microalgaeberbanding kepekatan CO_2. Walaupun, dengan menggunakan Regresi Linear, data telah mengesahkan bahawa kawasan itu disiasat tidak mengandungi hasil maksimum. Penggunaan kaedah Ascent tercuram berdasarkan persamaan linear satu eksperimen faktorial menunjukkan bahawa hasil biojisim boleh ditingkatkan sekiranya kepekatan CO_2 dan keamatan pencahayaan sentiasa meningkat.

Secara keseluruhannya, pencahayaan dan kepekatan CO_2 adalah penting untuk pertumbuhan microalgae.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

g	Gram
CO_2	Carbon Dioxide
FAME	Fatty Acid Methyl Ester
PPM	Part Per Million
POME	Palm Oil Mill Effluent
CPO	Crude Palm Oil
GHG	Greenhouse Gases
GT	Grand Total
HCO_3^-	Bicarbonate
hr	Hour
L	Litre
m	Metre
ha	Hectare
ml	Millilitre
min	Minute
N	Nitrogen
RuBisco	Ribulose-1,5-bisphosphate carboxylase oxygenase
Kg	Kilogram
Zn	Zinc
P	Phosphorus
Ca	Calcium
K	Potassium
pH	Hydrogen Ion Concentration
PUFAs	Polyunsaturated Fatty Acid
r	Error
$FeCl_3$	Ferric Chloride
s	Second
$Al_2(SO_4)_3$	Aluminum sulfate
TAGs	Triglycerides
µ	Micro
lx	Lux or illuminance
%	Percentage
℃	Celsius Degree
α	alpha
rpm	Revolution Per Minute

1 INTRODUCTION

1.1 Motivation and statement of problem

The palm oil industry has become the backbone of the economy in Malaysia. It continues to face new challenges in the face of globalization. Approximately 16 million tonnes of crude palm oil (CPO) was produced in the year 2012 which increased by 6.7 % from 13.98 million tonnes in the year 2004 (MPOB, 2013). Nevertheless, the expansion of the palm oil industry caused severe negative impacts toward the environment such as deforestation, serious greenhouse gases (GHG) emission and generates the highest pollution load known as palm oil mill effluent (POME) into rivers throughout the country. POME is always regarded as a highly polluting wastewater. Rupani et al., (2010) reported that biomethane emission from POME was estimated at 0.57 million tonnes in year 2009. The emission of biomethane in CO_2-equivalent corresponds to 11.99 million tonnes.

Currently, CO_2 emission is a serious issue of environmental deterioration. It is released into atmosphere mainly as a result of the burning in air of carbon-containing fossil fuels such as oil, natural gas, and coals. The consequence is that atmospheric concentration of CO_2 has increased from 280 ppm in the past to more than 370 ppm presently (Rahaman et al., 2011). With this increase, the concern of serious uncontrolled CO_2 emissions to atmosphere grows, and currently there is a global push to limit the amount of CO_2 emission. One way of reducing CO_2 emission is by CO_2 sequestration.

CO_2 sequestration is a process of carbon capture and storage, where CO_2 is removed from flue gases, such as in power stations, before being stored in underground reservoirs. From an engineering point of view, CO_2 is seen as a promising carbon source for microalgae growth as it is utilized during photosynthesis processes. Microalgae cultivation needs ample CO_2 for photosynthesis. Research indicated that utilizing an atmosphere that contains ample of CO_2 not only helps algae grow, but also regulate the pH value and carbon balance (Rahaman et al., 2011).

Microalgae have received attention more recently as oil-rich organisms with promise as a source of algal biofuels. It can grow in harsh conditions, either marine or wastewater. Although POME is always regarded as a highly polluting wastewater, in order to cope with issues of palm oil effluent and CO_2 emission, POME which is rich with nitrogen source can be used as medium for microalgae cultivation. Reutilization of POME to generate renewable energies in commercial scale has a great potential especially when coupled with CO_2 sequestration.

1.2 Objectives

The study aims to:

- Optimize the CO_2sequestration by microalgae in POME medium.

1.3 Scope of this research

Scopes of the study were identified in order to achieve the research objectives. The scope of research covers:

i) The study of the effects of CO_2concentration and light intensity on algae biomass production at different concentrations (v/v) of CO_2 in air and at different illumination intensities in 1L flask cultures.

ii) The study of CO_2 sparging methods in airlift tubular photobioreactor to achieve adequate and uniform supply of dissolved CO_2 to algae culture growing in it.

1.4 Main contribution of this work

The use of microalgae can be a suitable alternativebecause microalgae are also the most efficient biological producerof oil on the planet and a versatile biomass sourceand may soon be one of the Earth's most important renewable fuel crops, due to the higher photosyntheticefficiency, and highest carbon dioxide fixation capability and oxygen production capability. This is similar with aims of The National Aeronautics and Space Administration (NASA) program of developing an algae fuel by growing the algae in waste water with the aim generating of high quality of liquid fuel and as well as systemizing an effective way eliminate CO_2(Ding, 2011). Reutilization of POME to generate renewable energies in commercial scale has a great potential especially when coupled with CO_2 sequestration and wastewater treatment.

1.5 Organisation of this thesis

The structure of the reminder of the thesis is outlined as follow:

Chapter 2 provides a description of microalgae characteristics and culture conditions affected the microalgae growth. A general description on oil palm processing and palm oil mill effluent (POME) are presented. This chapter also provides general design features of liquid-gas ejector and as well as the fundamental of liquid-gas principles.

Chapter 3 gives a review of process flow on CO_2 sequestration of mixed wastewater microalgae in POME medium. The experiment begins by setting up the CO_2 system. The system divided into two systems which are gas mixing system for flask culture and gas mixing system for tubular photobioreactor. The microalgae are cultivated in POME medium approximately seven days at culture conditions of different CO_2 concentration and light intensities. The biomass yield from microalgae is determined by method of dry biomass analysis.

Chapter 4 provides results of interaction between light intensity and CO_2concentration on microalgae growth. The microalgae growth is analysed by method of Yates', Linear Modelling and Path of Steepest Ascent Method. The main effect and interactive effect between light intensity and CO_2 concentration are analysed by Yates' method. While, Linear Modelling proposed to determine the maximum yield effect obtained from the experiment. As no maximum yield of biomass obtained, Path of Steepest Ascent method is built around to find the direction in which the increase of yield is steepest.

Chapter 5 draws together a summary of the thesis and outlines the future work which might be derived from the model developed in this work.

2 LITERATURE REVIEW

2.1 Overview

This paper presents the experimental studies of optimization of the CO_2 sequestration by microalgae cultivation in POME medium. The studies involved the determination of the effects of CO_2 concentration and light intensity on algae biomass and the study of CO_2 sparging in airlift tubular photobioareactor to achieve adequate and uniform supply of dissolved CO_2 toalgae culture growing in it. The analysis of the product was done by using cell dry weight method and as well as mathematical analysis which includes of Yates' Method, Linear Modelling and Path of Steepest Ascent method.

2.2 Introduction

Microalgae are prokaryotic and eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular or multicellular structure. Microalgae are devided into four categories: diatoms (Bacillariophyceae), green algae (Chlorophyceae), blue-green algae (Cyanophyceace) and golden algae (Chrysoohyceae), depending on their pigmentation, life cycle and basic cellular structure (Man and Keat, 2011). Demirbas A. and Demirbas M.F., (2011) reported that most of algal species are obligate phototrophs. Microalgae present in all existing earth ecosystem, not just aquatic but terrestrial, representing a big variety of species living in a wide range of environmental condition. Besides, they can also be cultivated under extreme agro-climatic conditions (Doan and Obbard, 2012). Microalgae contain fatty acid as membrane components, storage products, metabolite and source of energy (Demirbas, 2011).

Table 2.1: Chemical composition of microalgae on a dry matter basis

Species of sample	Proteins	Carbohydrates	Lipids	Nucleic acid
Scenedesmus obliquus	50-56	10-17	12-14	3-6
Scenedesmusquadricauda	47	-	1.9	-
Scenedesmus dimorphus	8-18	21-52	16-40	-
Chlamydomonas rheinhardii	48	17	21	-
Chlorella vulgaris	51-58	12-17	14-22	4-5
Chlorella pyrenoidosa	57	26	2	-
Spirogyra sp.	6-20	22-64	11-21	-
Dunaliella salina	49	4	8	-
Euglena gracilis	57	32	6	-
Prymnesium pavum	39-61	14-18	14-20	-
Tetraselmis maculate	52	15	3	-
Porphyridium cruentum	28-39	40-57	9-14	-
Spirulina platensis	46-63	8-14	4-9	2-5
Spirulina maxima	60-71	13-16	6-7	3-4.5
Synechoccus sp	63	15	11	5
Anabaena sylindrica	43-56	25-30	4-7	-

By Demirbas A. and Demirbas M.F (2011)

2.3 Advantages of Microalgae

Poor sewage treatment has been blamed as being one of the causes of corals slowly dying in the sea the east coast of peninsular Malaysia, as algae was found to have smothered some reefs (Ding, 2011). In contrast, in economic terms, microalgae can be seen as microorganisms capable of producing highly valuable compounds (e.g., natural pigments and polyunsaturated fatty acids (PUFAs)) starting from inexpensive resources (Carvalho, 2001). The polyunsaturated fatty acids (PUFAs) are classified into two main groups: omega-3 of which the parent essential fatty acid is alpha-linolenic acid and omega-6 of which the parent essential fatty acid is linoleic acid (Edwards et al., 1998). PUFAs are capable of producing rich-biomass and oil. As a proven, microalgae have received attention more recently as oil-rich organisms with promise as a source of algal biofuels (Edwards et al., 1998). Man and Keat, (2012) found that Botryococcus braunii, Chlorella protothecoides and Chlorella vulgaris, have been identified as the most promising strains for biofuel production. Consequently, biofuel production is expected to offer new opportunities to diversify income and fuel supply source, to develop long

term replacement of fossil fuel and reduce the Greenhouse emissions, booting the carbonization of transportation fuels and increasing the security of energy supply.

Since the available quantities of waste oils and animal fats are not enough to match the today's demand for biofuel due to limited land areas needed for cultivation (Table 2.2), thus the transition to second generation biofuels, such as microalgae can also contribute to a reduction in land requirements. Compared to other biofuel feedstock, biofuel produced by microalgae is clean burning, non-toxic and carbon neutral to environment (Rahaman et al., 2011). This clean biofuel can control the problem of global warming resulted from greenhouse emission. Apart being used for bioenergy generation (biodiesel, biomethane, biohydrogen), microalgae also used for CO_2 mitigation.

Table 2.2: Comparison of some biodiesel

Crop	Oil yield (L/ha)	Land area needed (M/ha)[a]	Percent of existing US cropping area[a]
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil palm	5950	45	24
Microalgae (70% oil of sample, by wt)	136,900	2	1.1
Microalgae (70% oil of sample, by wt)	58,700	4.5	2.5

[a] For meeting 50% of all transport fuel needs of the United Satetes by Demirbas, (2011)

2.4 Oil Palm Processing

Several unit operations are involved in oil palm processing. The simplified process flow diagram of oil palm processing is shown in Figure 2.1. The process involves several stages in which huge amounts of water and steam are required for washing and sterilizing respectively. The stages include are sterilization of fresh fruit

bunches, stripping, digestion and processing of fruits, clarification and kernel oil recovery (Man et al., 2011; Ling, 2007; Ta et al., 2010) This has resulted in huge amounts of wastewater, known as palm oil mill effluent or POME.

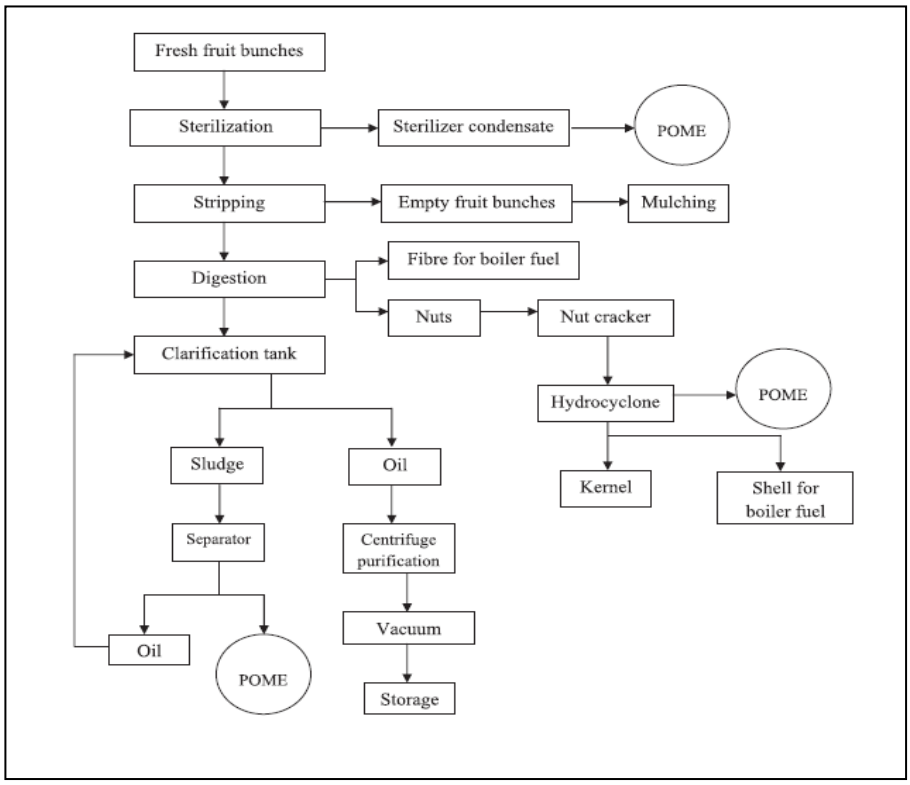

Figure 2.1: Process flow diagrams of palm oil mills by Man and Keat (2011)

2.5 Palm Oil Mill Effluent (POME)

POME is a non-toxic liquid waste with unpleasant smell and has high enough of COD and BOD values to cause serious pollution and environmental problem to rivers (Man and Keat*.*, 2011). Raw POME is a colloidal suspension containing 95-96% water, 0.6-0.7% oil and 4-5% total solid including 2-4% suspended solids (Man et al., 2012).

Culturing of microalgae in wastewaters offers an inexpensive alternative to conventional form of tertiary wastewater treatments and at the same time to utilize the nitrogen and phosphorus compound in wastewaters to generate microalgae biomass for biofuel production. The wastewater in the secondary and tertiary treatments plants contain significant amounts of nitrate and ortho-phosphate which are not removed during primary treatment (Man et al, 2012). Nitrogen source (usually appears in nitrate form) plays an important role in promoting microalgae growth. In order to grow microalgae effectively, the basic nitrate concentration required in the range of 200-400 mg/L (Man et al., 2012). Other mineral such as Fe, Zn, P, Mg, Ca and K that are required for microalgae growth are also present in POME (Man and Keat, 2012).

Besides, Khalid and Wan Mustafa, (1992) found that POME has big role as carbon sources because it contains organic acids in complex forms and has low ash contents.

2.6 Influence of Culture Conditions

2.6.1 Carbon Dioxide, CO_2

CO_2 is the sole source of carbon and it is supplied continuously for the process of photosynthesis and biomass production (Ryu et al., 2001). CO_2 dissolves in water and becomes $HCO_3^{-,}$ and then the HCO_3^- is absorbed by algae cells for photosynthesis (Ciferrum, 1983). It has been proven from previous research that optimum consumption of CO_2 and high lipid hydrocarbon content of microalgae provides the best circumstances for the production of liquid biofuel (Rahaman et al., 2011). Hence, CO_2 are significant key factors for microalgae cultivation and promote an efficient oil production which currently oil is in demand due to the natural resources scarcity and balances the CO_2 ecosystem as well.

Each microalgae shows different behavior towards CO_2 consumption. Some microalgae required higher CO_2 concentration to avoid binding of RuBisCO to Oxygen rather than CO_2 (Rahaman et al., 2011), whereas some microalgae growth restricted by increasing injection rate of CO_2 into the culture medium. In contrast, according to Man et al., (2012), higher concentration of CO_2 supplement could stimulate microalgae to grow faster compared to atmosphere air because it promotes photosynthetic efficiency of microalgae to reproduce within a shorter time and thus more quantity of microalgae biomass could attained. Actually, higher density of microalgae helps it to achieve higher tolerance towards CO_2 and faster growth rate.

However, most of the researchers agreed that microalgae strains are less tolerant toward high concentration of CO_2 and may possible inhibit their growth (Rahman et al., 2010). This due to high concentration of CO_2 induced low pH in the culture medium and reduces the activity of the extracellular carbonic anhydrase of microalgae which is responsible in carbon concentration mechanism.

2.6.2 Temperature

Microalgae can tolerate a range of temperature and their response to temperature variations can affect their (1) nutritional requirements, (2) rates and nature of metabolism and (3) cell composition (Richmond, 1999). Most commoly cultured species of microalgae tolerate temperatures between 15 and 26˚C, depending on the culture medium composition, the species and strain cultured (Woertz et al., 2004; Mata et al., 2010) but exceeding the optimum temperature 2-4˚C will result the total loss of culture (Mata et al., 2010).

Hence, to obtain higher oil yield, researchers agreed that the best temperature for microalgae growth was within ranges 15 and 26˚C. However, temperature is also influenced by other environment parameters, such as light intensity (Woertz et al., 2004). To maintain at these optimum temperature, other parameters which indirectly may affect the temperature conditions should always be ensured in control.

2.6.3 Aeration

Mixing is necessary to prevent sedimentation of microalgae, ensure that all cells are equally exposed to light and nutrients, avoid thermal stratification and facilitate gas transfer rate between the culture medium and the air (Briassoulis et al., 2010 and Mata et al., 2010). Low mixing rates hamper gaseous mass transfer and might even permit biomass settling. Conversely, high mixing rates can cause shear damage to cells, besides requiring a large energy input (Kumar et al., n.d). Generally, there are three types of the common mixing methods; pumping, mechanical stirring and gas injection.

Mechanical stirring has been reported to provide good mixing efficiency and good transfer rate but like to produce significant hydronamic stress. Converse to gas injection, produces lower hydronamic stress which is good conditions for microalgae grow, while providing good gas transfer and reasonable mixing efficiency. To provide good mixing, the effects of gas injection with different flow rates are investigated in this study.

2.6.4 Light Requirements

Recommended range of light intensity for growing micro-algae in hatcheries is 2500–8000 lux (Oellermann, 2001). Solar intensity greatly varies during the day and during the year. Microalgae activity increases with light intensity up to 200–400 μE m^{2-} s^{1-} (10,800 – 21,600 lux m^{2-} s^{1-}), where the photosynthetic apparatus becomes saturated, to decrease at higher light intensities (Ding, 2011). Photo-inhibition has therefore been observed during the central hours of a sunny day when irradiance can reach up to 4000μ$Em^{2-}S^{1-}$ (Rebolloso et al., 1999). It is more likely to occur at low microalgae concentration, such as during start-up (Göksan et al., 2003), because the light intensity to which the microalgae are actually exposed is not reduced by mutual shading (Ding, 2011).

Light saturation is characterized by a light saturation constant, as shown in Figure 2.2, which is the intensity of light at which the specific biomass growth rate is half its maximum value, μ_{max}. Light saturation constants for microalgae tend to be much lower than the maximum sunlight level that occurs at midday. For example, the light saturation constants for the microalgae *Phaeodactylum tricornutum* and *Porphyridium cruentum* are 185 μE m^{2-} s^{1-} (9,990 lux m^{2-} s^{1-}) and 200 μE m^{2-} s^{1-} (10,800 lux m^{2-} s^{1-}) (Molina et al., 2000), respectively. In comparison with these values, the typical midday outdoor light intensity in equatorial regions is about 2000 μE m^{2-} s^{1-} (108,000 lux m^{2-} s^{1-}). Because of light saturation, the biomass growth rate is much lower than would be possible if light saturation value could be increased substantially.

Above a certain value of light intensity, a further increase in light level actually reduces the biomass growth rate. This phenomenon is known as photo-inhibition. Microalgae become photo-inhibited at light intensities only slightly greater than the light level at which the specific growth rate peaks. Photo-inhibition results from generally reversible damage to the photosynthetic apparatus, as a consequence of excessive light (Camacho et al., 2003). Elimination of photo-inhibition or its postponement to higher light intensities can greatly increase the average daily growth rate of algal biomass (Chisti, 2007).

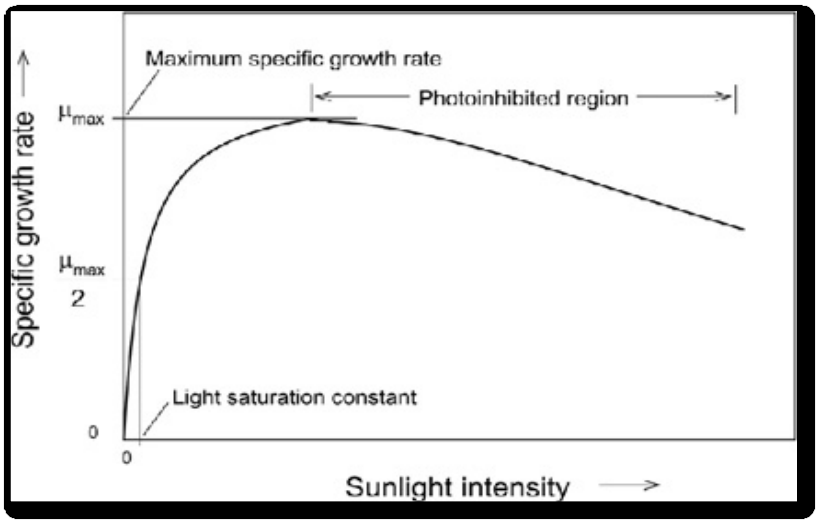

Figure 2.2: Effect of light intensity on specific growth rate of microalgae.
By Chisti (2007)

2.7 Liquid – Gas Ejector

The ejector as shown Figure 2.3 has great market potential because it offers some remarkable advantages which include: it can alleviate environmental problems by using low grade thermal energy sources such as solar energy, geothermal energy and waste heat to drive the system instead of high grade electric energy, hence it can reduce CO_2 emissions resulting from the combustion of fossil fuels (He et al., 2009); it is also relatively simple in design and can provide reliable operation at low capital cost, hence these advantage have led to ejectors being used in processes requiring the drawing of a vacuum (McGovern et al., 2012); and most importantly ejectors are generate very small bubbles, large hold-ups and high mass transfer rates (Rahman et al., 2010). In comparison with others diffusers (Cheng et al., 2006; Ferreira et al., 2002), corbanation column (Put et al., 2011), venturi (Hong et al., 2012; Thalasso et al., 1995), gas sparger (Ryu, 2009), plain bubbling and hollow-fiber modules (Carvalho, 2001), ejector is most preferable for CO_2 injection to water due to higher gas transfer rates.

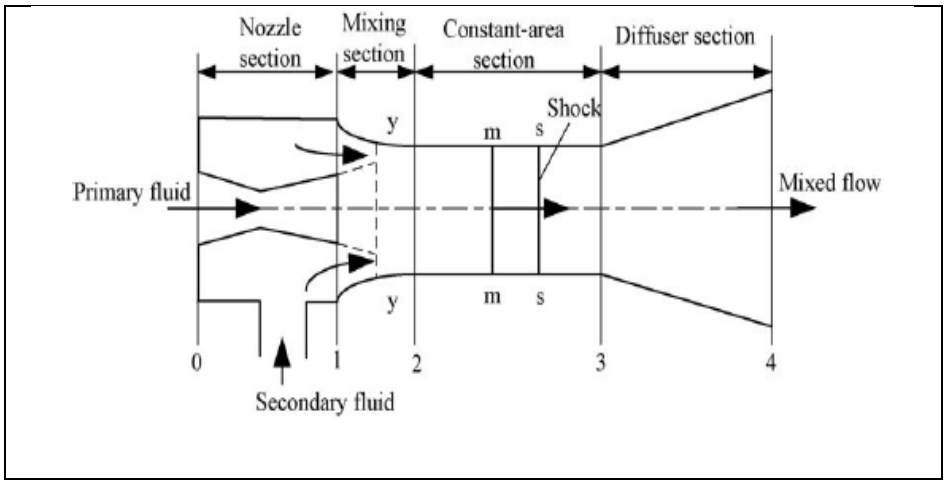

Figure 2.3: Principle Structure of Ejector by He et al. (2009)

2.7.1 The Fundamental Principle of Gas-Liquid Ejector

Basically, there are several sections of ejector; nozzle section, mixing section and diffuser section (He et al., 2009). Each section plays important roles for an efficient liquid-gas ejection. The process begins at nozzle section, a primary fluid is accelerated to supersonic speed by the convergent-divergent primary nozzle, which forms low pressure region at the nozzle exit plane and produces the entrainment effect to entrain the secondary fluid (He et al., 2009; Chunnanond and Aphomratna, 2004). Then the mixing at mixing section begins after the secondary flow chokes. In this case, a sudden reaction in the mixture velocity and rise in the pressure take place and the fluid mixture easily undergoes phase change and condensation shock may occur (He et al., 2009; (Chunnanond and Aphomratna, 2004). At this stage culture medium and CO_2 gas completely dissolved and hence reducing an effect of large bubbles size. This mixing causes the primary flow to be retarded whilst secondary flow is accelerated (Chunnanond and Aphomratna, 2004).

By the end of the mixing chamber, two streams are completely mixed and the static pressure was assumed to remain constant until it reaches the throat section. Finally, the ejection processes end up at diffuser section. At this section, the mixture of primary and secondary flows passes through the diffuser, and converts kinetic energy into pressure energy. At the diffuser exit, the velocity is reduced to zero and the pressure is lifted high enough to cause discharge (He et al., 2009). The higher pressure at the diffuser exit is believed to help disperse CO_2 throughout cells culture in tubular photobioreactor.

2.8 Summary

Pome oil mill effluent (POME) has many advantages especially as nutrient source for microalgae cultivation. Culturing of microalgae in wastewaters offers an expensive alternative to conventional form of tertiary wastewater treatment and at the same to utilize the nitrogen and phosphorus compound in wastewater to generate microalgae biomass for biofuel production. The microalgae growth affected by several conditions; light requirements, CO_2, temperature and aeration. All these factors are very important for microalgae but light intensity and CO_2 are mostly affected the microalgae growth. However, the growth is restricted if excessive CO_2 and light intensity supply to microalgae culture. The recommended CO_2 percentage consumed by microalgae is ranging from 0 to 5 %. Whereas, the recommended range of light intensity is between 2500 to 8000 lx. The used of CO_2 for microalgae cultivation is actually an alternative way to sequester the CO_2 emission to environment. Microalgae cell wall is very rigid and tough. A suitable dispersion method must be chosen so that the loss of CO_2 to environment can be avoided. In this study, the liquid-gas ejector is found to be better than the others recommended CO_2sparger.

3 MATERIALS AND METHODS

3.1 Overview

This chapter is discussed on research methodology which includes several steps as described in Figure 3.1. The method of analysis to fulfil the scope and objectives are also discussed. The research is done to optimize the carbon dioxide sequestration by mixed microalgae in POME medium in shake-flask cultivation of 1000 mL working volume. The parameters of CO_2 volumetric flow rate and illumination intensity were adjusted to gain the maximum yield of microalgae biomass. The general steps of the research including the set-up of CO_2 system, experimental and mathematical methods which were:

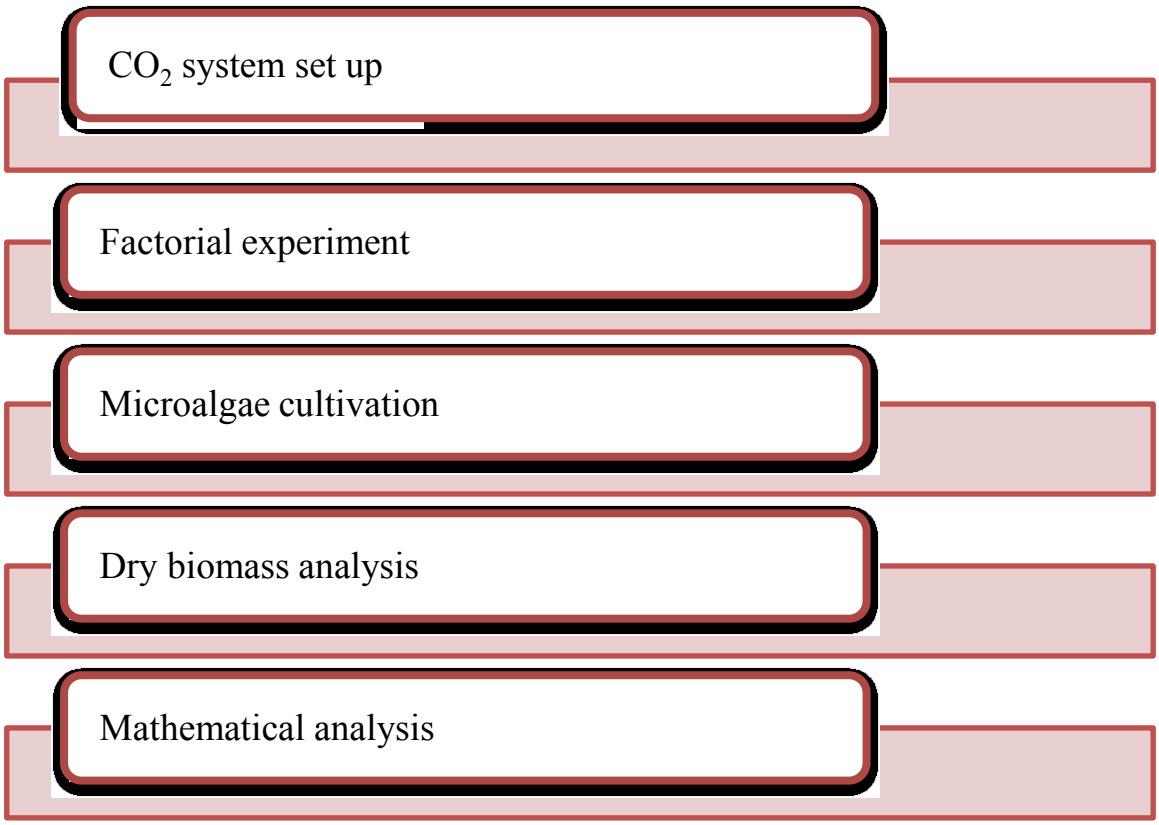

Figure 3.1: Process flow chart on CO_2 sequestration of mixed microalgae in POME medium

3.2 Algae Strain and Medium

Cultures of mixed microalgae were collected from the final pond of the Seri Senggora Palm Oil Mill in Pahang, Malaysia. It was maintained in 1L liquid cultures in 2L Erlenmeyer flask closed with cotton wool.

The POME medium used was polishing pond medium as shown in Figure 3.2. The 10 L medium also was taken from Sri Senggora Oil Mills and stored in freezer of 4˚C. Before used, the sample medium was cooled to room temperature, 27˚C. The raw POME was a light brown coloured suspension, and consist mainly 95% of water.

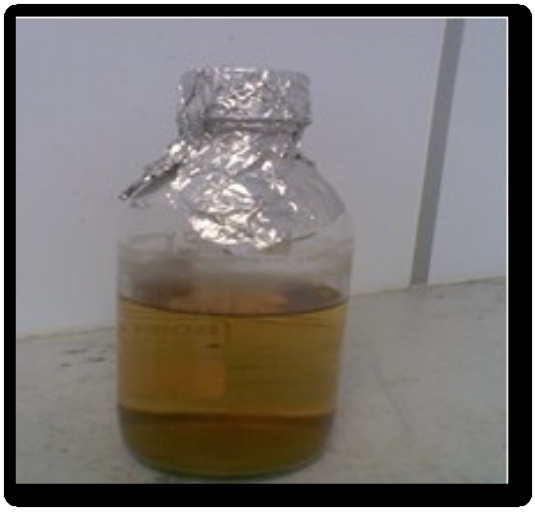

Figure 3.2: POME medium

3.3 CO_2 System Set-up

3.3.1 Gas Mixing System for Flask Culture

The schematic diagram of the gas mixing system for flask culture is shown in Figure 3.3. The system was constructed around one month. The process includes; fabrication, assembly and gas testing. The system mainly contained the following components: pressure gauges, ball valves, air compressor and flow meter mounted vertically. The piping was fabricated from stainless steel tubing obtained from Gas laboratory. A mixture of air with CO_2 was generated by adequate continuous mixing of air with pure CO_2, with flow rates duly monitored by flow meters and pressure of system was set to 1 bar. The mixtures of air-CO_2 were then supplied to the cultures in 1L flask.

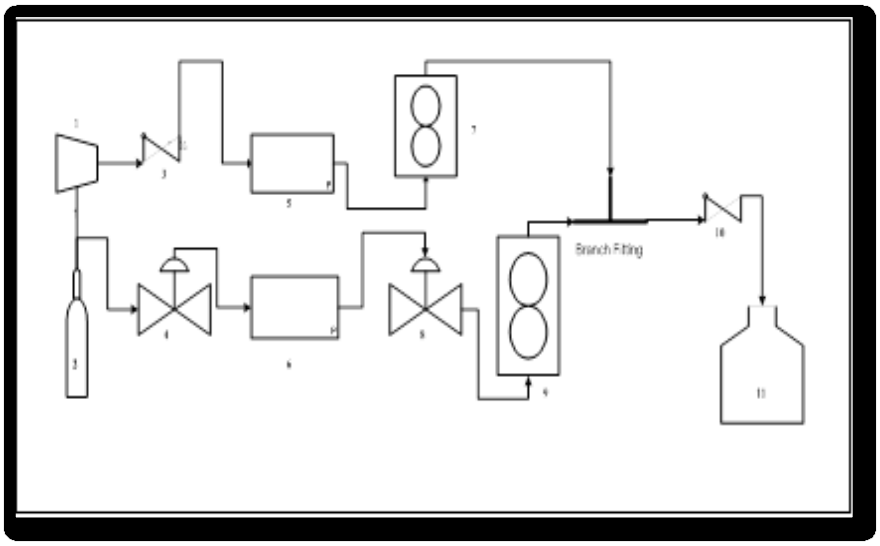

Figure 3.3: Schematic diagram of gas mixing system for flask culture. (1) Air compressor: (2) CO_2 cylinder tank: (3&10) Check valve: (4&8) Ball valve: (5&6) Pressure gauge: (7&9) Rotameter: (11) Flask

3.3.2 Gas Mixing System for Airlift Tubular Photobioreactor

In the study of the effect of varying the light intensity and varying the concentration of CO_2 in photobioreactor, the CO_2 was dispersed into photobioreactor via liquid-gas ejector (Figure 3.4). In the liquid-gas ejector, the culture was pumped through a nozzle and ejected into an inverted slender cone outlet. The nozzle and the inlet of the small-end of the inverted slender cone are enclosed in a housing which experiences low pressure resulting from the passage of culture from the nozzle through the slender cone, and CO_2 released into this low pressure chamber is drawn into the jet of culture and becomes entrained as very small bubbles in the CO_2-culture mixture expanding into the bigger end of the cone and out again into the main bulk of the culture at a downstream location along the horizontal light-receiver of the photobioreactor.