CULTIVATION OF MICROALGAE FOR BIO OIL PRODUCTION AND CARBON DIOXIDE REMOVAL

by

MUHAMMAD HIDAYAT BIN ALIAH

FINAL PROJECT REPORT

Submitted to the Chemical Engineering Programme
in Partial Fulfillment of the Requirements
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Unversiti Teknologi PETRONAS
Bandar Seri Iskandar
31750 Tronoh
Perak Darul Ridzuan

CERTIFICATION OF APPROVAL

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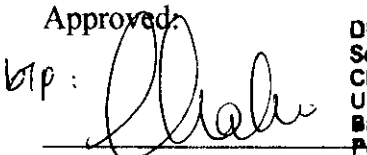

DR. KHALIK MOHAMAD SABIL
Senior Lecturer
Chemical Engineering Department
Universiti Teknologi PETRONAS
Bandar Seri Iskandar, 31750 Tronoh
Perak Darul Ridzuan, MALAYSIA

AP. Dr. Mohd Azmuddin Abdullah
(Chemical Engineering Department)
Project Supervisor

UNIVERSITI TEKNOLOGI PETRONAS
TRONOH, PERAK

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

Muhammad Hidayat b Aliah

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ABSTRACT

The use of fossil fuel is now widely accepted as unsustainable due to depleting resource and the accumulation of greenhouse gases in the environment that have already exceeded the dangerously high threshold of 450ppm CO_2-e. To achieve environmental and economic sustainability, fuel production processes are required not only renewable but also capable of sequestering atmospheric CO_2. Microalgae are a versatile field due to its wide range of application and industries. It has been investigated that microalgae can be used for the production of a number of products ranging from fuel, pharmaceutical, food and health. In the fields of fuel and energy only microalgae can be used to produce biodiesel, bio-oil, bio-syngas and bio-hydrogen. Not limited by that, the production of microalgae can be couple with CO_2 mitigation and wastewater treatment. Development in microalgae cultivation and downstream processes are expected to further enhance cost effectiveness of the biofuel from microalgae. This research entitled **"Cultivation of microalgae for bio-oil production and CO_2 removal"** is done to investigate and to develop a microalgae cultivation system with the aim to improve the CO_2 removal and fixation during the cultivation of microalgae. The main objective of this research is to study on the cultivation technique(s) of microalgae specifically bioreactor, the CO_2 utilization of the system, potential species of microalgae system for both bio-oil production and CO_2 removal system as well as the optimum growth condition for cultivation of microalgae. The scope of study will be narrowed down to the growth rate and survival of microalgae species, utilization of wild algae for the mentioned purposes, the use of microalgae as biomass, the different technique for the production of microalgae and the CO_2 utilization technique using monoculture as well as mix-culture.

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CHAPTER 1

INTRODUCTION

1.1 PROJECT BACKGROUND

The use of fossil fuel for the past 4 centuries has damaged the earth so badly. Fossil fuel is now widely accepted as unsustainable due to depleting resource and the accumulation of greenhouse gasses in the environment that have already exceeded the "dangerously high" threshold of 450ppm CO2-e. To achieve back environmental and economic sustainability, fuel production processes are required that not are only renewable, but also capable of sequestering atmospheric CO2. [2]

The improvement and research in chemical process to reduce the CO2 emission over the year has very large limitation that there is no catalyst that can convert CO2 to O2. The only substance in the world that can do this task is found in every photosynthetic plant called chlorophyll. Photosynthesis process is a nature process that may be the only solution to the polluted earth. Among all plant, Microalgae is the best option for fuel production and CO2 utilization.

Microalgae are a diverse group of prokaryotic and eukaryotic photosynthetic microorganism that grows rapidly due to their simple structure. They can be potentially employed for the production of biofuels in an economically effective and environmentally sustainable manner. The existences of microalgae in nature produce half of the atmospheric O2 and consume greenhouse gasses in the process. [7]

In 1960, Oswald and Golueke [5] presented a conceptual techno-economic analysis, "The Biological Transformation of Solar Energy", proposing the use of

large scale cultivation of microalgae on wastewater nutrient and then anaerobically ferment the algal biomass to methane fuel. The methane is then will be converted into electricity, with the CO2 containing flue gas will be recycled into the culture environment to support the algal production. The concept presented in this paper has attracted a lot of researchers and over the past 40 years, a great deal of research has been carried out on this concept of microalgae fuel production and CO2 utilization. [5]

Microalgae have been investigated to produce a number of biofuels including biodiesel, bio-oil, bio-syngas and bio-hydrogen. Beside energy production, microalgae cultivation system offer wide range of application including food industries, fertilizer, alginates, pigment, pharmaceutical and pollution control (CO2 utilization).

However, major technical challenges have limited the practical application of this technology [5]:

- Maintaining selected algae species in large scale system.
- Lower than anticipated biomass production.
- High cost of harvesting and the overall process.

These limitations however can be overcome by improving and further enhance the cost effectiveness of the biofuels from microalgae system via [7]:

- Couple the fuel production with flue gas CO2 mitigation.
- Utilize the wastewater/seawater for the system.
- Bio-refinery – production of high-value chemicals.
- Development of cultivation and downstream processes (harvesting, drying, thermo chemical, etc).

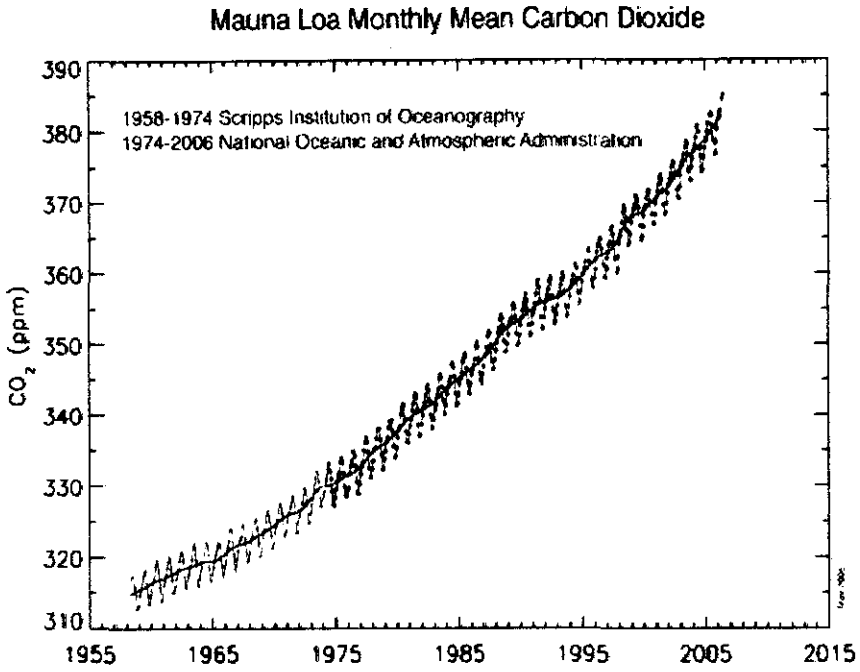


Figure 1.1: CO2 concentration of the world (1960-2005) [14]

1.2 PROBLEM STATEMENT

Algae are a large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multi-cellular forms. Algae have been used long time ago for a lot of purposes such as medicine and food. Recent finding found that algae in fact is very beneficial in industry especially in handing global warming and energy crisis that now has been the main global problem now days.

Among the usage of algae in industry are as CO_2 removal unit and as the new source for bio-oil (bio-fuel). There are some specific species of algae that has been discovered that is really good for this purpose such as *Gracilaria, Sargassum and Pleurochrysis Carterae* (CRMP647). One of the main challenges faced by a lot of active researchers around the world to fully utilize algae in order to produce bio-oil and to remove CO_2 is to mass produce the algae itself (Microalgae Cultivation). This is due to technical difficulties and limitation in cultivating the microalgae and other downstream process such as drying.

Microalgae cultivation system generally has 2 main objectives which are to produce fuel (energy) and to reduce CO2 concentration via CO2 utilization. Development of a microalgae cultivation system should also take into account this aspect as the current global pollution need the fuel production not only renewable and low CO2 emission but to heal the global warming as it operates.

1.3 OBJECTIVES

The objective of this research is to develop a cultivation system of microalgae for bio-oil production and CO2 removal. In achieving this objective, cultivation technique has to developed and well understood including mix-culture and wild culture. In this research suitable microalgae strain(s) must be choose to be cultivated in culture media that is different in nutrient content. This research will monitor the cell growth rate of the microalgae to compare the growth rate between tested species. The grown microalgae will be extracted and the bio-oil content and bio-fuel content of the microalgae will be analyzed.

1.4 SCOPE OF STUDY

This project focuses on the cultivation of microalgae and CO2 mitigation technique which require:

- Develop cultivation technique for microalgae cultivation.
- Testing different microalgae strains for cell growth rate.
- Finding and develop the practical way(s) to cultivate the algae for bio-oil production and CO2 utilization.
- Determine and control the parameters for microalgae cultivation.
- Extraction of grown microalgae for bio-oil and bio-fuel production.
- Determine the potential of wild microalgae for bio-fuel, bio-oil and CO2 utilization.

CHAPTER 2

LITERATURE REVIEW

2.1 MICROALGAE

Alga/Algae are a large and diverse group of simple, typically autotrophic organism, ranging from unicellular to multicellular form. Microphyte/Microalgae are microscopic and unicellular algae species ranging from a few micrometer (μm) to a few hundreds μm. Microalgae are capable to perform photosynthesis where they produce half of atmospheric oxygen and consume simultaneously green house gas, carbon dioxide [10].

Microalgae are a very simple "plant" that may exist as simple as a unicellular form. Plant has the ability to heal the world pollution by providing a source of energy and clean air through a simple process called;

PHOTOSYNTHESIS

(CHLOROPHYLL)

$$6CO_2 + 6H_2O + \text{ENERGY} \longrightarrow C_6H_{12}O_6 + 6O_2$$

(SUN) (FUEL)

Unlike higher plants, microalgae do not have roots, stems and leaves. Microalgae, capable to perform photosynthesis, are important for life on earth; they produce approximately half of the atmospheric oxygen and use simultaneously the greenhouse gas carbon dioxide to grow photoautotrophically. It has been estimated that about 200,000-800,000 species exist of which about 35,000 species are described.

The example of the specieses are *Scenedesmus obliquus*, *Chlamydomonas rheinhardii*, *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Spirogyra sp.*, *Dunaliella bioculata*, *Dunaliella salina*, *Euglena gracilis*, *Prymnesium parvum*, *Tetraselmis maculata*, *Porphyridium cruentum*, *Spirulina platensis*, *Spirulina maxima*, *Synechoccus sp.*, *Anabaena cylindricall* [11].

Usage of algae [12];

- Food and Agar
- Fertilizer and Nutrients
- Alginates, Stabilizing Substance and Pigments
- Pharmaceutical and Health Product
- Pollution Control and Possible Energy Source

Figure 2.1 (a) and (b): Usage of microalgae

Microalgae, as biomass, are a potential source of renewable energy, and they can be converted into energy such as biofuel and gas. The properties of the microalgae product are almost similar to those of offish and vegetable oils, and therefore, it can be considered as a substitute of fossil oil. There are many advantages of culturing microalgae as a resource of biomass which are:

i. Algae are considered to be a very efficient biological system for harvesting solar energy for the production of organic compounds.

ii. Algae are non-vascular plants, lacking (usually) complex reproductive organs.

iii. Many species of algae can be induced to produce particularly high concentrations of chosen, commercially valuable compounds, such as proteins, carbohydrates, lipids and pigments.

iv. Algae are microorganisms that undergo a simple cell division cycle.

v. The farming of microalgae can be grown using sea or brackish water.

vi. Algal biomass production systems can easily be adapted to various levels of operational or technological skills.

2.2 MICROALGAE INDUSTRIES

A microalgae industry is a very versatile industry that it has very wide application and downstream processes. In energy industries, microalgae can be transformed to source of energy via several processes. Figure below explain microalgae industries and energy converting process.

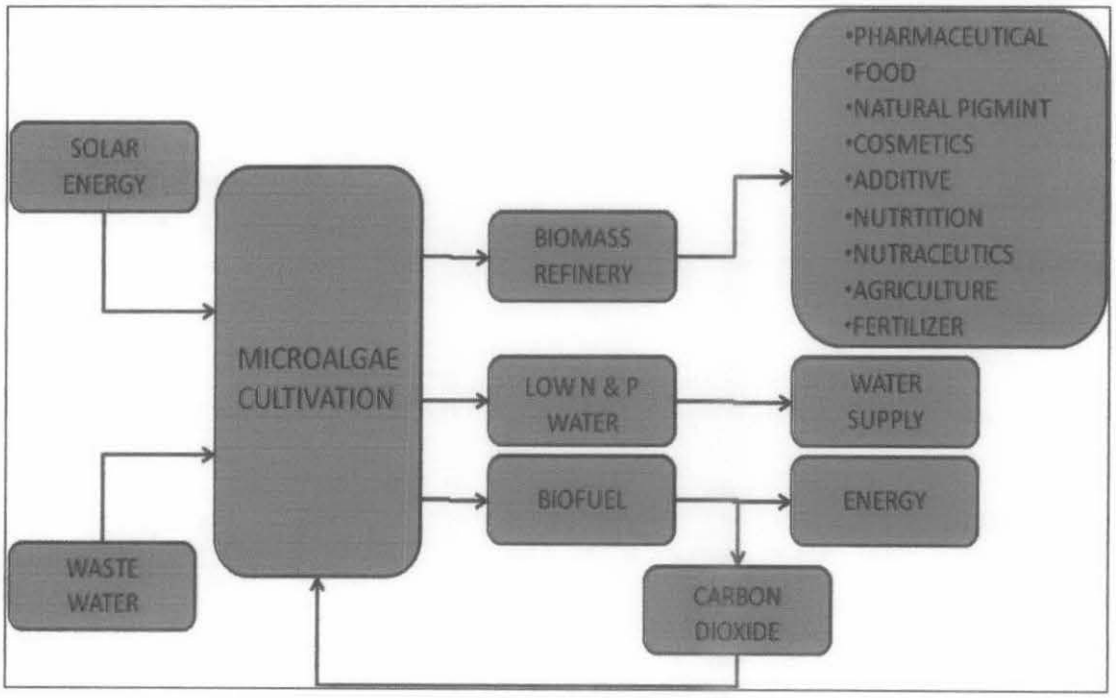


Figure 2.2: Microalgae industries

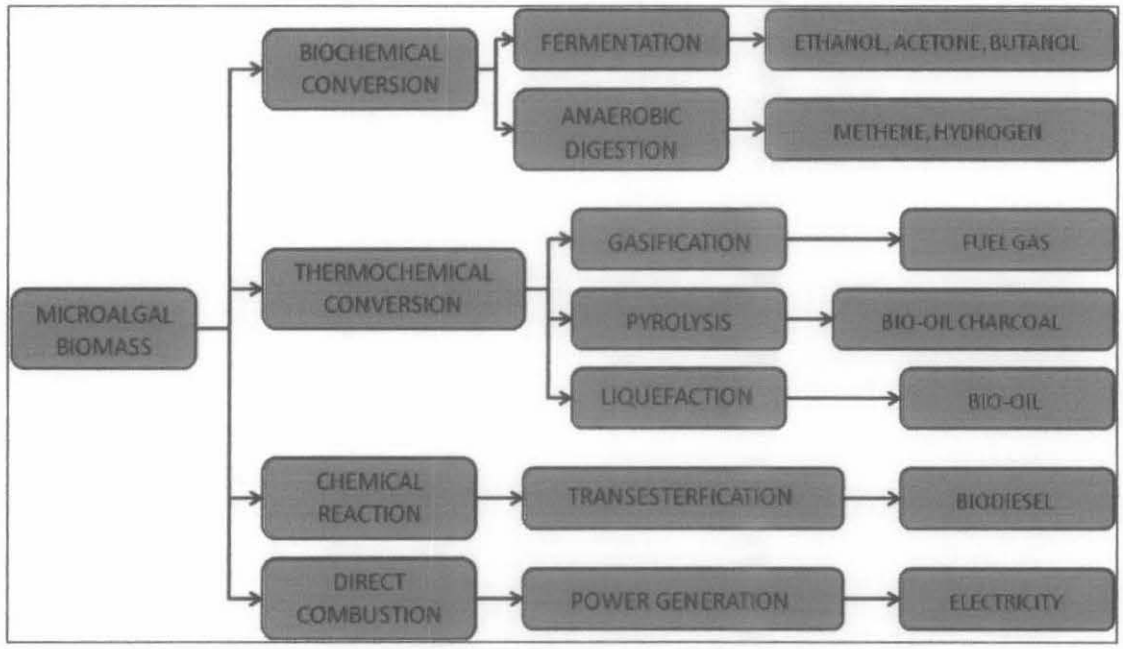


Figure 2.3: Energy Converting Processes from Microalgae

2.3 MICROALGAE CULTIVATION SYSTEM

There are 3 established ways to cultivate microalgae which are;

1. Open Pool
2. Closed Loop System
3. Photo bioreactor/Bioreactor/PBR

For this Final Year Project, simple bioreactor will is chosen be developed rather than the other 2 method of cultivation of microalgae. A bioreactor is a device or system that supports a biologically active environment. Bioreactor typically is a cylindrical vessel in which a chemical process is carried out which the process involve organism or biochemically active substance derived from such organisms. The process may be aerobic or anaerobic. Like other reactors, bioreactors also operate in a specific mode whether batch, semi-batch of continuous mode.

A photo bioreactor is a bioreactor which incorporates some type of light source to provide photonic energy input into the reactor. Because algae tend to produce a lot of compounds and other organism, a closed photo bioreactor needed to be developed for maintaining monoculture of algae. The bioreactor is chosen because of its advantages compared to other method which are;

- Prevent or minimize contamination.
- Offer better control over bio-cultural conditions (p_H, light, carbon dioxide, temperature).
- Prevent water evaporation.
- Lower carbon dioxide losses due to out-gassing.
- Permit higher cell concentrations.

Designing the bioreactor will take consideration of lots of internal and external factors to optimize the production and quality of product of the reactor. Some of the factors that needed to be considered are;

- Species of the algae.
- Operating mode.
- Operating condition.
- Extraction method.
- Culture media and nutrient content.

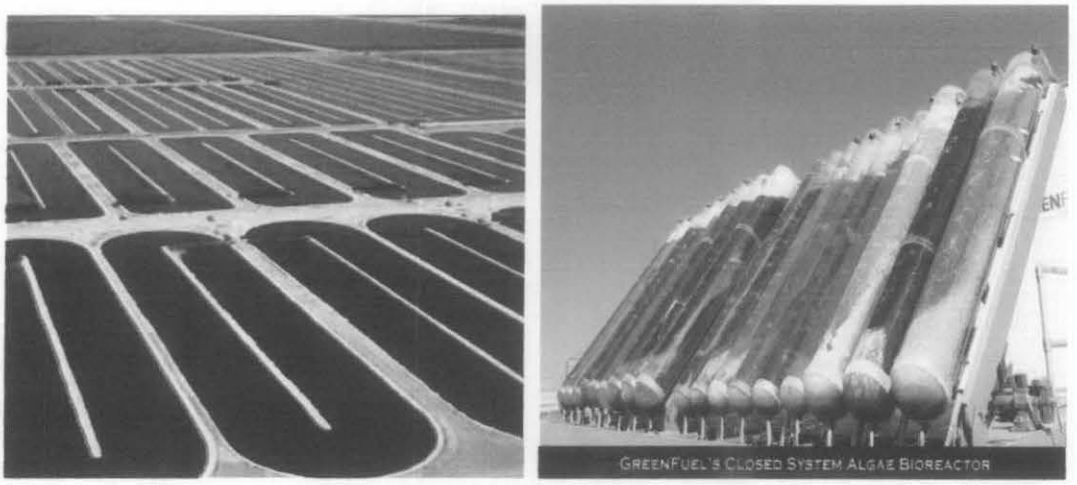


Figure 2.4 (a) and (b): Microalgae cultivation system

2.3.1 Microalgae Strain for CO_2 Fixation

Microalgae can fix the CO2 from different sources, which can be categorized as [1],

1. CO2 from atmosphere
2. CO2 from flue/flaring gas (industrial exhaust gas)
3. Fix CO2 in the form of soluble carbonates (NaHCO3, Na2CO3)

Traditionally, microalgae are cultivated in closed system or open pool, which are aerated or exposed to air to allow the microalgae to capture carbon dioxide from atmosphere for cell growth. As the atmospheric concentration of Carbon Dioxide is only 0.03-0.06%, it is expected that mass transfer limitation could slow down the whole process thus limiting the efficiency of the system. Industrial exhaust gas may contain up to 15% of Carbon Dioxide, providing a rich CO_2 feed for the microalgae cultivation system and a potentially providing are more efficient CO_2 fixation route.

A number of species has been identified to be able to utilize carbonates such as Na_2CO_3 and $NaHCO_3$. Some of the species typically have high extracellular carboanhydrase activities, which responsible for the conversion of carbonate to free CO_2 to facilitate the Carbon Dioxide assimilation. Direct uptake of bicarbonate by an active transport system has been found in several species. Some other species in other hand has shown tolerance of high level of CO_2 concentration.

In example,

1. *Chlorococcum littorale* is a marine alga that shows tolerance of up to 40% concentration of CO_2.
2. *Scenedesmus obliquus* and *Chlorella kessleri* also show good tolerance to high CO_2 concentration (grew well on 18% CO_2 supply).
3. *Scenedesmus obliquus* and *Spirulina sp.* Can be cultivated at 30°C.
4. *Chlorococcum littorale* showed high CO_2 fixation exceeding 1 g CO_2 $Liter^{-1}$ Day^{-1}.

The tolerance of microalgae to high temperature is important in reducing cooling cost of exhaust gas. Several *Chlorella sp.* has been identified that can grew at up to 42°C of water containing more than 40% of CO_2 concentration. Table show the species studied for cultivation of microalgae for CO_2 fixation.

Species	CO_2 %	T°C	P g l^{-1} day^{-1}	P_{CO2} g l^{-1} day^{-1}
Chlorococcum littorale	40	30	-	1.0
Chlorella kessleri	18	30	0.087	0.163
Chlorella sp. UK001	15	35	-	>1.0
Chlorella vulgaris	15	25	-	0.624
Chlorella sp.	40	42	-	1.0
Dunaliella	3	27	0.17	0.313
Haemotococcus pluvalis	16-34	20	0.076	0.143
Scenedesmus obliquus	18	30	0.14	0.26
Botryococcus braunii	-	25-30	1.1	>1.0

Table 2.1: Microalgae strain studied for CO_2 bio-mitigation

2.3.2 The Combined CO_2 bio-mitigation and biofuel production strategy

The merit of CO_2 bi-mitigation lies primarily in the fact that the biomass produced in the process of CO_2 fixation can be converted efficiently into biofuel for energy production. A study shows that the biofuel is 2.3 times more expensive than fossil fuel. Development in biofuel and microalgae field can lower the price of biofuel. [1]

Recent commercial scale microalgal farming facility to produce *Haematococcus pluvalis* for biodiesel production using 2 stages closed to open system operation shows that daily production of 1.9 kg dry biomass was achieve with 25 000 l Photobioreactor, correspond to biomass productivity of 0.076 g l^{-1} day^{-1} at biomass concentration of 0.3 g/l. Annual production of microalgae oil equivalent to 420 GJ ha^{-1} is obtained. Maximum production rate achieve for *Haematococcus pluvalis* was equivalent to 1014 GJ ha^{-1}. It is estimated that the production of 3200 GJ ha^{-1} $year^{-1}$ can be achieve using a fast growing *Chlorella sp.* This is a rate possible to replace the reliance to fossil fuel which current use equivalent of 300 EJ per year and eliminate fossil fuel CO_2 emission about 6.5 Gigatons of carbon per year using only 7.3% of surplus arable land.[1]

2.3.3 Combination of wastewater treatment with CO_2 fixation and biofuel production.

Combination of wastewater treatment and microalgae CO2 fixation provides additional economic potential incentive due to the savings from chemicals nutrient and the environmental benefit. It provide a pathway to remove nitrogen, phosphorus, and metal from wastewater and produce microalgae biomass which can be further exploited as biofuel production without using freshwater. The potential of CO2 fixation combines with wastewater treatment has been heavily investigates by lots of researchers with several strains tested for this purpose. [1]

1. B. braunii was shown to be able to remove nitrogen and phosphorus from secondarily treated wastewater.
2. C. vulgaris was cultivated in wastewater discharge from steel-making plant with the aim to develop an economically feasible system to remove ammonia from wastewater and CO2 from flue gas simultaneously.
3. Scenedesmus obliquus in was successfully cultivated on artificial wastewater under the winter and summer tropical condition.

2.3.4 Microalgal Nutrition

Microalgae cultivation is the core of the microalgae CO2 mitigation strategy and extensive research has been carried out in this field. Among the important aspect in microalgae cultivation is microalgae nutrient. It is important to develop well balance media for optimum microalgae cultivation and CO2 fixation. Growth medium must provide sufficient nutrient to support microalgae growth. Important chemicals for microalgae growth are;

1. Carbon
2. Nitrogen
3. Phosphorus
4. Sulfur
5. Metal (Iron, Magnesium and Silicon in some cases)

2.4 POTENTIAL OF WILD ALGAE AND MIXCULTURE

2.4.1 The Need of Wild Microalgae

Since the Industrial Revolution, the CO_2 concentration in the atmosphere has been steadily increasing. This is because of increasing consumption of fossil fuels accompanied by activities of human life. Associated with this, the issue of global warming is now causing great anxiety. Under such circumstances, researchers have so far tried a variety of approaches to the recovery and fixation of CO_2. Possible methods for effective use of CO_2 by fixing it using biological techniques have been studied. Special attention has been paid to microalgae, because they are fast in CO_2 fixation and can be industrially mass-cultured at high density.

Researches has so far carried out confirmatory tests with respect to the effect of various exhaust gas components, such as CO_2, SO_x, NO_x, and heavy metal contained in dust, on the microalgae culture, and also the tests on culture conditions, including atmospheric temperature and solar radiation. The search for wild strains of microalgae, its evaluation method, outdoor year-round tests, fundamental tests for use of microalgae as fuel, and feasibility studies should be done so that microalgae CO_2 mitigation can be successfully carried out.

2.4.2 Wild Microalgae CO_2 Mitigation System

2.4.2.1 Screening of Wild Strain and Its Evaluation Method

Sampling of wild strain can be done either on fresh or seawater. Survival screening method can be done using small pond (0.8 mwidth x 2.5 m length x 0.3 m depth). For the isolated strains, the absorption coefficient ε of algal cell suspension is measured as well as the other algal properties by the oxygen-reaction monitor method. The productivity of these strains was evaluated by the method as follows. Raceway-type cultivator model radiated from the upper part is used as shown in Fig. below. The light intensity in the algal suspension is assumed that as expressed by the law of Lambert-Beer and

also that the rate of photosynthetic reaction depends on the light intensity of the each point of the depth. [14]

$$I = I_o \cdot exp(-\varepsilon \cdot C \cdot x)$$

Here, the O2 production rate is used as the measure of the rate of photosynthetic reaction. The O2 production rate of microalgae in raceway cultivator is expressed by:

$$q = Kp \cdot C \int_0^H \frac{I}{(I+\phi)} - \frac{p}{Kp} dx$$

Where C = concentration of algal biomass (kg/m3),
I = light intensity (lux),
Kp = productivity constant (mL/[kg .h]),
ǿ =light dependencyconstant (lux),
ρ = respiratory rate (mL/[kg .h]),
x = optical pass length

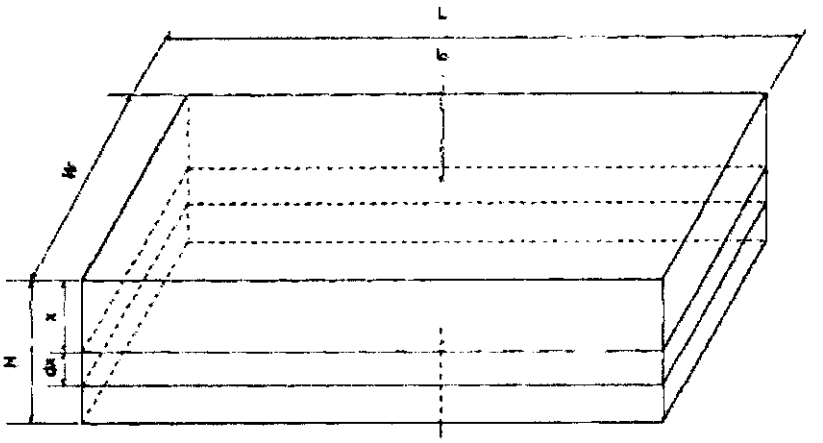


Figure 2.5: Wild strain screening pond

2.4.2.2 Outdoor Cultivation Test

Using the raceway-type cultivator, 17-mo long-term outdoor cultivation tests were carried out. Actual flue gas of thermal power plant and actual sea water were used in the test. [14]

Test Conditions

The test conditions are shown as follows.

1. Algal Strains

NANNP-2 *(Nannochloropsis salina)*

PHAEO-2 *(Phaeodactylum triwrnutum)*

Tetra. sp. *(Tetraselmis sp.,* TM-S3)

2. Medium

Standard medium of f/2 sea water based on SERI

3. Raceway-type cultivator

Size = 0.8 m width x 2.5 m length x 0.25 m depth

Liquid velocity = 0.1-0.2 m/s

4. Test place

Shin-Sendai Thermal Power Station, Tohoku Electric Power Co. Inc: (Sendai City, Japan)

5. Fuel

Heavy oil

6. Flue gas composition

CO_2 = 14.1%; 02 = 1.3%, SOx = 185 ppm, NOx = 125 ppm

2.4.2.3 Results

In Fig. below, the data of duration period of algal cultivation are shown. In case of NANNP-2 and PHAEO-2, the duration period of algal cultivation is at most 2 month, and the long and stable cultivation was difficult. On the other hand, *Tetra, sp.* (TM-S3) collected from the sea near the experimental place was cultivated through I yr stably without a break. The algal productivity of *Tetra sp.* is shown Fig. 4. The algal productivity is high the period from May to September owing to high solar radiation and average water temperature, and the maximum algal productivity in this period was 40 g/(m2.d) .

In the period from March to September when solar radiation is larger than 10 MJ/(m2.d), the maximum algal productivity showed the same tendency as the average water temperature, and it can be seen that the algal productivity is highly dependent on the average water temperature when solar radiation is higher than some critical value. [14]

Table 1
Physical Properties of Microalgae

Properties		Microalgae	NANNP-2	PHAEO-2	TM-S3	TM-T1	TM-S4	TM-S8	TM-S9
Productivity constant	K_p	mL/(kg·h)	2.10E + 05	1.14E + 05	3.29E + 05	1.96E + 05	2.72E + 05	3.07 + 05	3.85E + 05
Light dependency constant	ϕ	lx	6420	1560	9430	1990	3580	4830	6260
Respiratory rate	β	mL/(kg·h)	5710	8610	4310	5690	9480	5320	3860
Adsorption coefficient	ϵ	m^{-1} $(kg/m^3)^{-1}$	112	115	165	126	120	115	152

NANNP-2: *Nannochloropsis salina*.
PHAEO-2: *Phaeodactylum tricornutum*.
TM: *Tetraselmis* sp.

Table 2.2: Physical properties of tested microalgae strain

CHAPTER 3: METHODOLOGY / PROJECT WORK

For this project, a small scale cultivation system is developed to study the growth rate and growth conditions of microalgae species. The cultivation system is based on Algal Culture for Peneid Hatchery system [9] and the CO_2 removal system is based on Carbon Dioxide Removal from Air by Microalgae Cultured in a Membrane-Photo bioreactor [4]. The system developed for this FYP is a batch system that is theoretically low cost with significant efficiency, few parameters controllers and easy to maintain.

General procedure involve in the algal culture are [9]:

- Culture Media Preparation – Nutrient and chemical for the algal growth
- Isolation of Suitable Strain, Strains maintenance and Initial preparation
- Algae Production in Batches of ascending volume
- CO2 Removal System is Utilize During The Cultivation of Microalgae
- Extraction (harvesting) of dried microalgae.
- Utilization of wild microalgae.

Figure 3.1 illustrate the general idea of utilizing microalgae for the purpose of bio-fuel (energy).

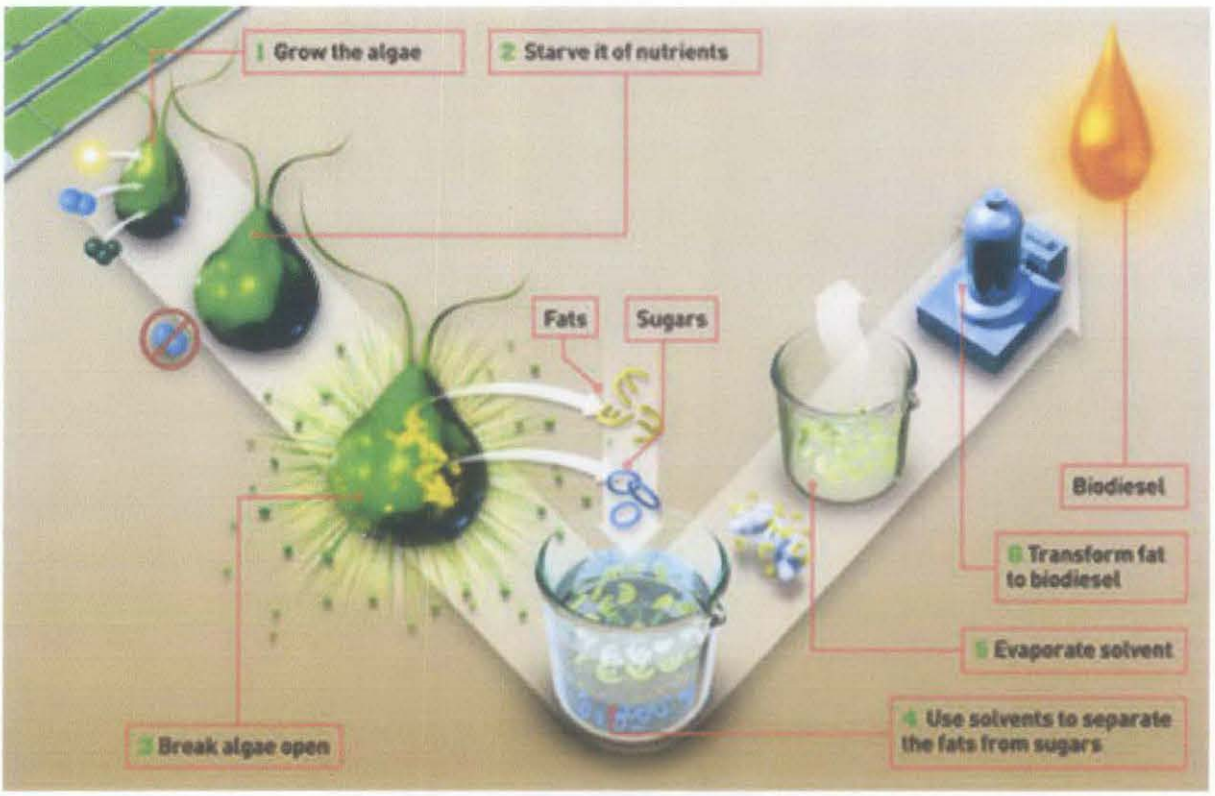


Figure 3.1: Cultivation of Microalgae for Biomass Production [11]

3.1 CULTURE FACILITIES

Early stage of cultivation needed enclosed room facilities so that the culture operation is free from contamination of unwanted species that could enter the culture via air-draught. Two sections in the enclosed space can be useful so that element of cultivation which is strain maintenance and algae production has own specific space. The temperature of the production room is maintained at 20-24°C while strain maintenance room is controlled at 20-22°C. The cultivation room should place the culture media which are 20ml test tube, 250ml flask, 2 liter flask, 30 liter tank and 300 liter tank.

The culture room also equipped with day-light fluorescent lightings to emit light intensity of 3000-5000lux that is required for algal culture. The strain room is equipped with mini laboratory for all strains maintenance and cultivation works.

Among facilities that available at the lab are electronic balance, compound microscope, working bench, sink, magnetic stirrer and hot plate, Bunsen burner, haemacytometer, automatic pipettes, test tube racks and hanging shelf for strains.

Figure 3.2: Culture Facility for Microalgae Cultivation

3.2 INITIAL PREPARATION

3.2.1 Culture Container (size)

The culture container may range from glassware, fiberglass container and tank and concrete tank. Sufficient number of 20 ml test tubes and 250ml conical flasks are requires for early stage of strain development. Other larger culture containers are 2 liter conical flask, 30 and 300 liter tank, and 1.5 and 5 ton culture container. This large volume culture container can be constructed using fiberglass or concrete with inner wall are coated with good finishing or white pigmented fiberglass resin.

3.2.2 Equipment Sterilization

It is important that the equipment that is used in strain maintenance or inoculums production must be sterilize to avoid possible contamination that will lead to failure of algal culture. Sterilization process that is being used area as follow:

1. All test tube and flask used that is being used for strain maintenance (inoculums production) are:
    a. Immersed in dilute mulric acid (20-30% diluted HCl or HSO_4)
    b. Washed with soap solution
    c. Rinse with tap water
    d. Final Rinsing using distilled water
2. Dried, plugged by cotton with top wrapping of aluminum foil or screw capped
3. Sterilization is done by oven drying at 125°C or by autoclaving at 1kg/cm2 pressure at 125°C
4. Larger container 30 liter – 5 ton, wash with soap solution, rinse, disinfection using dilute solution of Chlorox 940ppm) and dried to maintain high level of cleanliness.

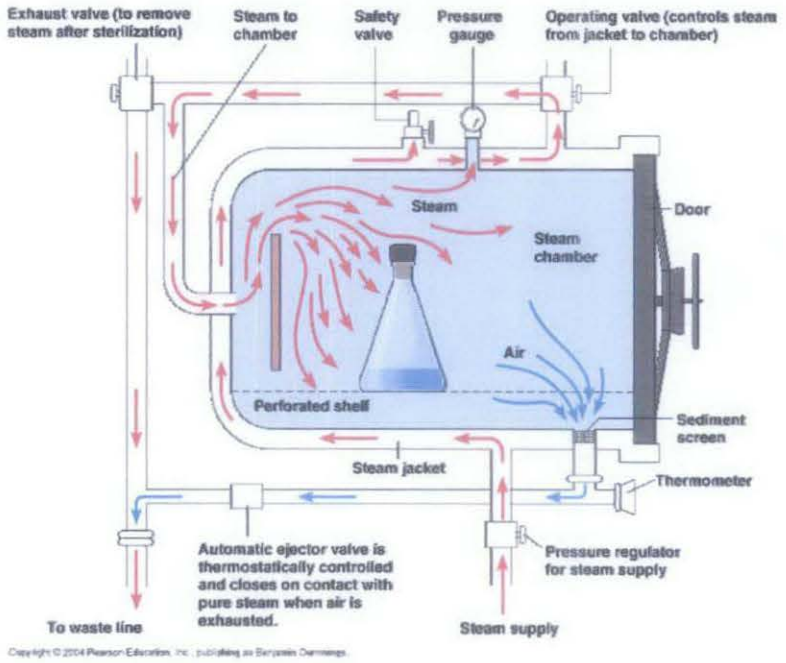


Figure 3.3: Autoclave

3.3 CULTURE MEDIA PREPARATION

Seawater is filtered before being pumped and used as culture media. Fresh water is obtained from public water supply. Air supply is provided using vortex blower. CO_2 supply is from gas-cylinder with flow regulator. Use of sodium bicarbonate also can be use to supply the CO_2. CO_2 is provided from below using any a sort of membrane in a culture container and it passes through the culture media as small bubbles to increase the CO2 utilization and fixation [4].

For equipment sterilization, electric drying oven is used for the sterilization of all glassware while an autoclave is mainly used for the sterilization of fresh and seawater and some nutrient media used in culture works.

Chemicals that are needed for algal culture are listed below [9]:

- Sodium Nitrate ($NaNO_3$)
- Potassium Nitrate (KNO_3)
- Di-Sodium EDTA ($C_{10}N_2Na_2O_8 \cdot 2H_2O$)
- Boric Acid (H_3BO_3)
- Sodium Dihydrogen Phosphate ($NaH_2PO_4 \cdot 4H_2O$)
- Ferric Chloride ($FeCl_3 \cdot 6H_2O$)
- Manganese Chloride ($MnCl_2 \cdot 4H_2O$)
- Concentrated Hydrochloric Acid (HCl)
- Sodium Silicate (Na_2SiO_3)
- Zinc Chloride ($ZnCl_2$)
- Cobalt Chloride ($CoCl_2 \cdot 5H_2O$)
- Ammonium Molybdate ($(NH_4)6Mo_7O_4 \cdot 4H_2O$)
- Copper Sulphate ($CuSO_4 \cdot 5H_2O$)
- Thiamine Chlorhydrate; Vitamin B1
- Cyanocobalamine; Vitamin B12
- Clewat-32 or EDTA metal complex
- Ammonium Sulphate ($(NH_4)_2SO_4$)
- Urea (NH_2CONH_2)
- Calcium Superphosphate ($Ca_5(PO_4)_3OH$)

There is a lot of recommended culture media for microalgae production. The more commonly used are Conway (AQUACOP, 1983) and TMRL (Liao and Huang, (1973).

Stock Solution	Constituent	Amount
Main Mineral Solution	$NaNO_3$ or KNO_3	100.00g or 116g
	Disodium EDTA	45.00g
	H_3BO_3	33.60g
	$NaH_2PO_4 \cdot 4H_2O$	20.00g
	$FeCl_3 \cdot 6H_2O$	1.30g
	$MnCl_2 \cdot 4H_2O$	0.36g
	Trace Metal Solution	1.0ml
	Distilled Water	1000.0ml
Trace Metal Solution	ZnCl	2.10g
	$CoCl_2 \cdot 6H_2O$	2.00g
	$(NH4)6Mo_7O_2 \cdot 4H_2O$	0.90g
	$CuSO_4 \cdot 5H_2O$	2.00g
	Distilled Water	100ml
Vitamin Solution	Thiamine Chlorhydrate, B1	200ml
	Cynocobalamin, B12	10ml
	Distilled Water	100ml
Silicate Solution	Sodium Silicate (Na_2SiO_3)	20ml
	Distilled Water	1000ml
Nitrate Solution	Potassium Nitrate	100ml
	Distilled Water	1000ml

Table 3.1: Composition of Conway Media

Constituents	Amount	Distilled Water
KNO_3	100g	1000ml
$Na_2HPO_4 \cdot 12H_2O$	10g	1000ml
$FeCl_3 \cdot 6H_2O$	3g	1000ml
$Na_2SiO_3 \cdot 9H_2O$	1g	1000ml

Table 3.2: TMRL Enrichment Medium

Note: Use 1ml of either Conway Media or TMRL enrichment per each liter of culture media

Preparation of stock solution is by mixing the necessary chemicals for each solution separately using 2 l conical flask over a hot plate with a magnetic stirrer. No hot plate for vitamin mixing. Sterilize each solution (except for vitamin) at 125°C plus 1kg/cm2 pressure for 30 minute. Store the solution in a cool dry place.

3.4 STRAIN ISOLATION AND MAINTANANCE

3.4.1 Isolation of pure algal strain by agar plating technique

Whenever a certain alga species is required, isolation work has to be done first. A number of isolation technique has been develop by various researchers such as serial dilute culture, agar plating, micromanipulation, glass hook capillary suction and cover glass attachment method. For this research, agar plating technique is used to maintain selected species. Steps in agar plating technique are:

1. Prepare 0.9% agar medium. Weigh out 9gm of agar powder and place it into 2-liter conical flask and add 1000ml sea water.
2. Heat the flask until twice boiling. Nutrient and chemicals are added before autoclaving except foe vitamins.
3. Cover the flask using aluminum foil.
4. Autoclave at 125°C for 30 minutes at $1kg/cm^2$
5. Dry-sterilize the petri dishes for 30 minutes at 150°C and let it cool.
6. Agar plates are prepared aseptically by working near a Bunsen flame and pouring the warm autoclave agar into the sterilize petri dishes: cover up the petri dishes and leave them to cool and set.
7. After 2 hours, the microalgae sample (strains) can be streaked on to the agar surface using sterilize wire loop(previously heated red-hot and cooled).
8. Place the petri dishes upside down on an illuminated glass rack.

The culture method on agar plating is good for isolation of algal species. After isolation, the pure strains can be maintained by inoculating on to a fresh agar plate for 1-4 months.

3.5 CULTIVATION OF SELECTED SPECIES

3.5.1 Production Cultures

The main purpose of production cultures is to get a large volume of algae in the shortest period of time. The production culture starts in 25 ml test tube and the subsequence culture involve respectively in 250 ml and 2 liter Erlenmeyer flasks. For bigger scale cultivation, the culture may use 30 and 300 liter fiberglass tanks and outdoor culture of 1 – 3 tons tanks. The system chosen for this research is a batch culture method with continuous CO_2 supply and monitoring for 2 liter stage.

3.5.2 Culture Method in 25 ml Test Tube

The purpose of this stage is to produce inocula of pure strain algae for larger volume (250 Erlenmeyer flask). The inoculation of test tube culture is done once a week. Preparation of test tube is as in section 3.2.5. The test tubes are filled with 20 ml of enriched culture medium as specified in table below.

Species	Chaetoceros	Diatoms	Other Algae
Conway solution	1 ml	1 ml	1 ml
Vitamin Silicate	0.1 ml	0.1 ml	0.1 ml
Silicate Solution	1 ml	1 ml	-
Nitrate Solution	1 ml	-	-
Seawater 0.2 micron	1000 ml	1000 ml	1000 ml

Table 3.3: Rate of adding various nutrient solutions for culture media

The nutrient media differ in composition for diatoms (i.e. *Chaetoceros spp.* and *Skeletonema costatum*) and algae species (i.e. *Tetraselmis chui* and *Isochrysis galbana*). Every test tube is marked for a specific species need an inoculums volume of 2 ml of the respected species.

3.5.3 Culture Method in 250 ml Erlenmeyer Flasks

The main purpose of this stage is to produce inocula of pure strain algae for larger volume 2-L Erlenmeyer flask. Sterile 250 ml Erlenmeyer flask are use in this culture. The culture media used in this stage are the same shown in table from 3.2.9 section. The flasks are kept on the illuminated shelf for 4 to 11 days to increase microalgae density. Aeration is not supply during this stage. [9]

Figure 3.4 (a) & (b): Culture of microalgae in 25ml and 250ml volume

3.5.4 Research Set up

2 sets of culture conditions for 3 different microalgae strains are prepared.

Culture A	Culture B
0.22 micron filtered Seawater	0.22 micron filtered Seawater
Conway media (1ml / 1000ml seawater)	Conway media (0.5ml / 1000ml seawater)
Same condition for other culture condition (Artificial lightning, Temperature, etc.)	

Table 3.4: Experimental Set Up

Microalgae strains tested and its growth is monitored and compared under 2 different culture conditions. Strains tested are;

1. *Chlorella sp.*
2. *Tetraselmis sp.*
3. *Nannochloropsis sp.*

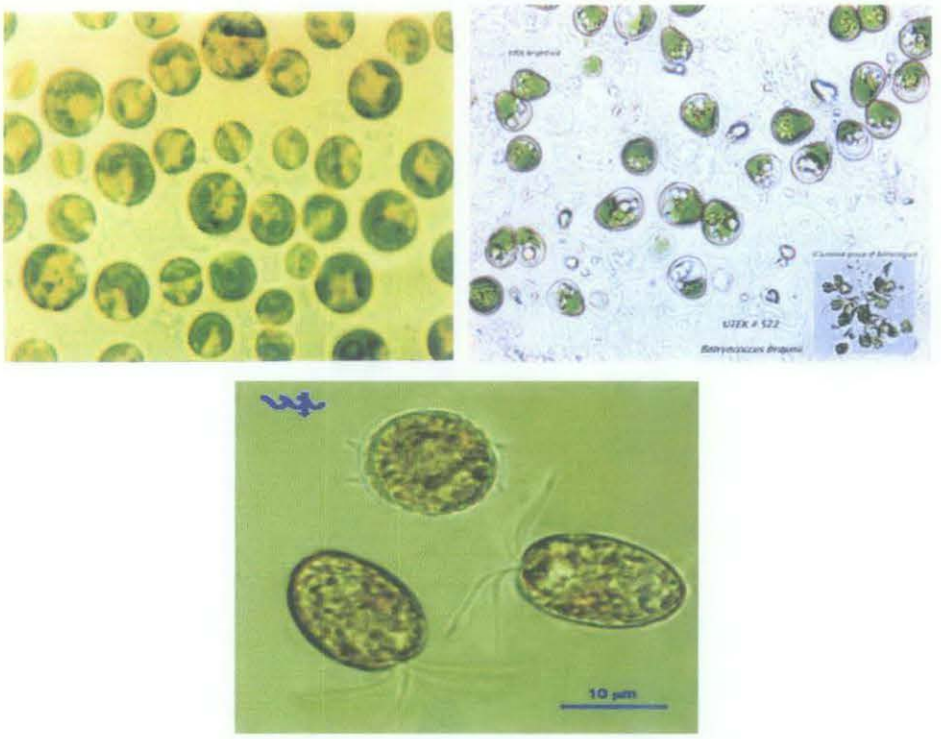
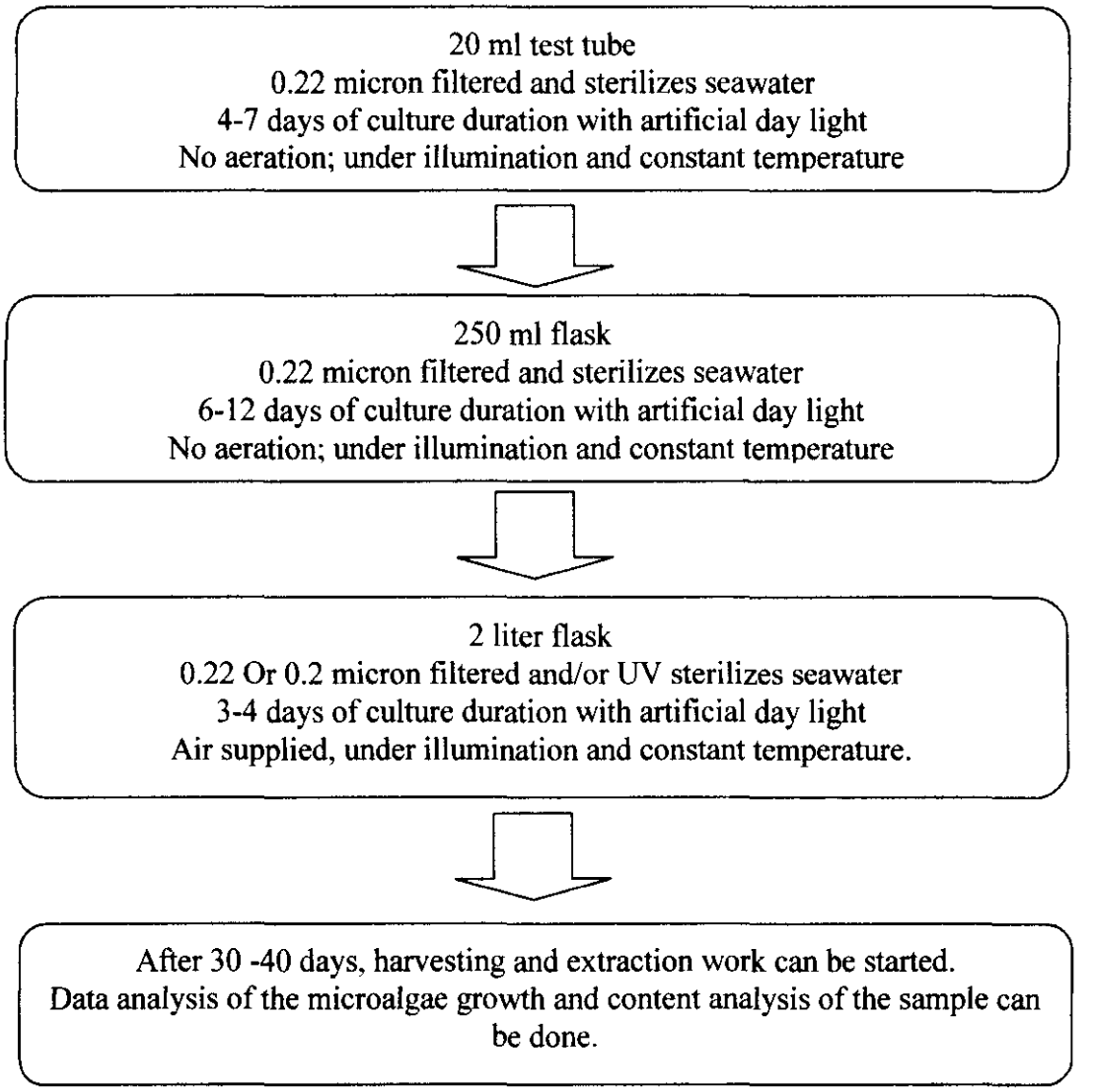


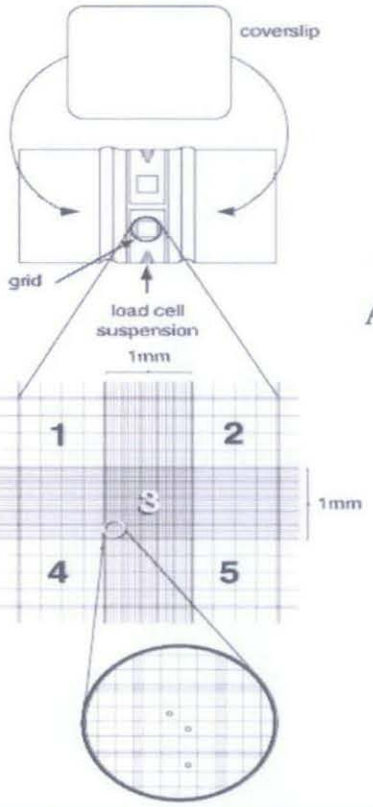
Figure 3.5 (a),(b) & (c) : *Chlorella sp.* , *Nannochloropsis sp.* and *Tetraselmis sp.*

Production of microalgae is in stage with ascending volume of culture media container. During the cultivation process, cell count is done every 3 – 10 day to monitor microalgae growth.



3.5.5 Growth evaluation

The growth evaluation of the microalgae is done by performing the cell count. The equipments involved in this process are LEICA microscope with 40 x focusing power and a hemacytometer, that consists of a thick glass microscope slide with a rectangular indentation that creates a chamber. This chamber is engraved with a laser-etched grid of perpendicular lines. The growth of microalgae cells are tracked for about a month from day 0.



Deep = 0.1 mm

Area for 1 square= 0.04 mm^2

Figure 3.6: Hamocytometer and the laser etched grid

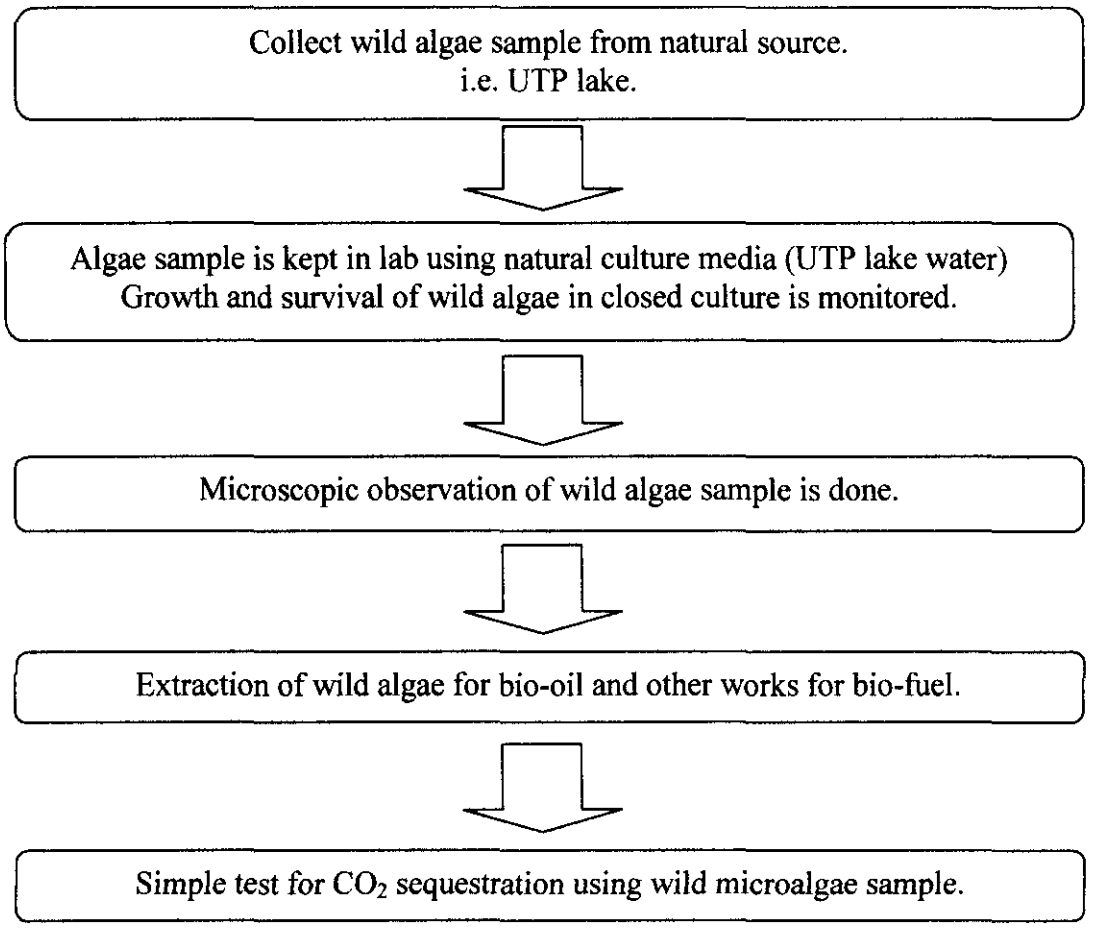
For the cell count, 10 readings are taken for each sample which are 5 readings from the upper part and another 5 from the lower part of the hemacytometer. Calculation of the cell count for each sample is as follows:

Average cell = Sum/10

$$Number\ Of\ Cells = \frac{Average\ Cells}{Deep\ x\ Area}$$

(Unit in cell ml^{-1}*)*

3.6 MIX CULTURE AND WILD ALGAE UTILIZATION



3.7 EXTRACTION OF CULTIVATED AND WILD MICROALGAE FOR BIO-OIL AND BIO-FUEL

3.7.1 Microalgae harvesting

The grown microalgae can be harvested in few ways. In this research, a simple harvesting is employed starting with centrifuging the sample. 45 ml plastic tubes are filled with *Nannochloropsis sp.* The sample is then centrifuged at maximum rpm for 13 minutes. As the microalgae are settled down by centrifugal force at the bottom of the tube, the clean water at the upper layer of the tube can be taken out. The wet cell mass is then kept in freezer before it will be bring back at UTP for extraction part.

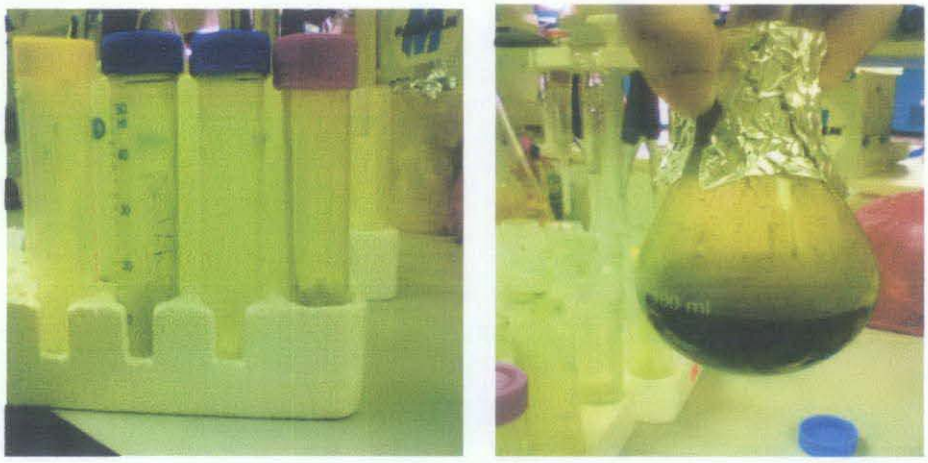
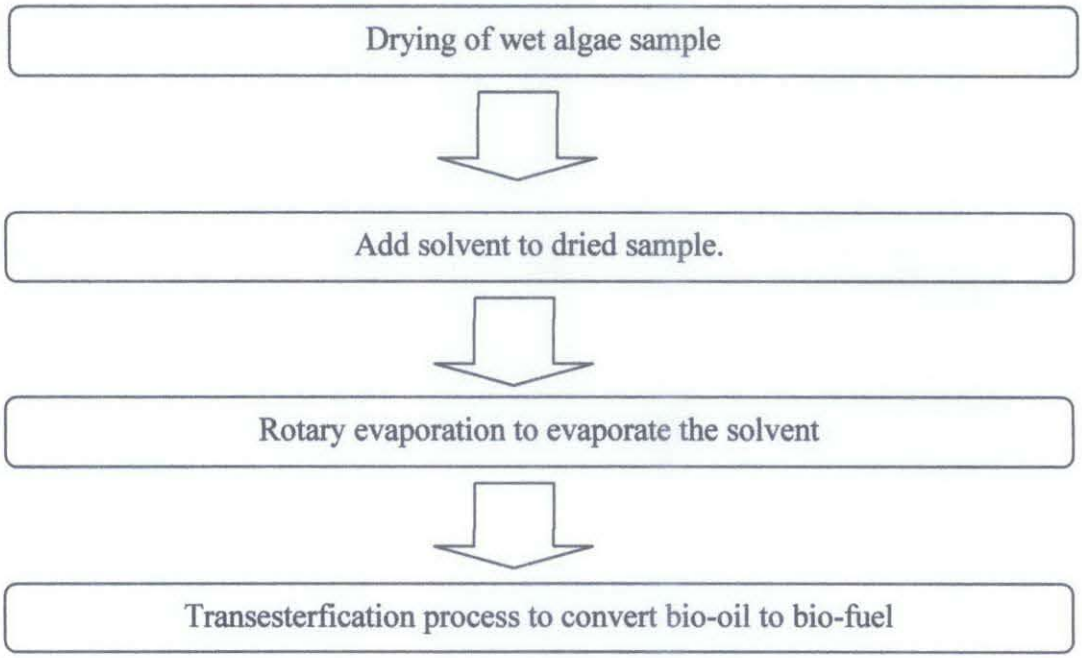


Figure 3.7 (a): Microalgae filled in 45 ml tubes.
Figure 3.7 (b): Microalgae collected in a 100ml flask before freeze-dried

3.7.2 Extraction

The extraction of microalgae is a continual work from harvesting work. The steps of this extraction work can be simplified in the flow chart below.



CHAPTER 4: RESULT AND DISCUSSION

4.1 Cultivation of Selected Microalgae Strains and Cell Growth Monitoring

Basically, there are 2 parameters tested for this research. The first part of the experiment will compare the growth rate of 3 different species of microalgae namely *Chlorella sp.* , *Nannochloropsis sp.* and *Tetraselmis sp.* . The growth rate of these species is measured using simple cell count method under the microscope using haematometer. In the second part of the experiment, the same procedure of cultivation is repeated with reduced nutrient supply in the culture media. This part aims to compare the survival of each strain when cultivated in harsh environment. The result gained from this experiment is represented below:

Day	Chlorella sp.		Nannochloropsis sp.		Tetraselmis sp.	
	A	B	A	B	A	B
0	9.75	6.70	7.00	5.85	1.20	0.95
3	14.40	7.15	11.00	11.70	1.70	1.40
13	19.50	7.40	15.05	15.25	2.20	1.90
21	24.00	9.05	19.40	17.70	2.90	2.20
28	30.40	11.70	24.50	21.00	3.55	2.85
36	34.35	14.70	30.10	24.10	5.25	3.15

Table 4.1: Overall Cell Count of Microalgae Strains
Culture A live in nutrient rich media while culture B live in limited nutrient media

4.1.1 Overall Algae Growth

Data collected above is cell count in determining cell growth of 3 different species of microalgae cultivated in 2 different culture conditions. Cell count is done within 36 days of algae cultivation. Graph below represent the growth rate of microalgae of respected species and culture condition. *Chlorella sp.* shown highest growth rate and doubling time when cultivated in culture A condition.

Chlorella sp. has relatively smaller cell size rather than *Nannochloropsis sp.* and *Tetraselmis sp.* This may be a factor why Chlorella sp. has higher cell growth rate than the other species. In culture condition B, Chlorella sp. didn't show high growth rate as in culture condition A. Main reason that maybe being the factor why the result show so is that culture contamination (by fungal, bacteria etc.) or the reduced concentration of CONWAY solution supplied. This shows that Chlorella sp. has high sensitivity to the culture condition as slight contamination or reduced nutrient supply will cause significant drop in growth rate of the species.

Nannochloropsis sp. and *Tetraselmis sp.* in other hand only shows slight reduction in reduction on supplied CONWAY nutrient. This may shows that both *Nannochloropsis sp.* and *Tetraselmis sp.* can tolerate rough culture condition despite its lower growth rate.

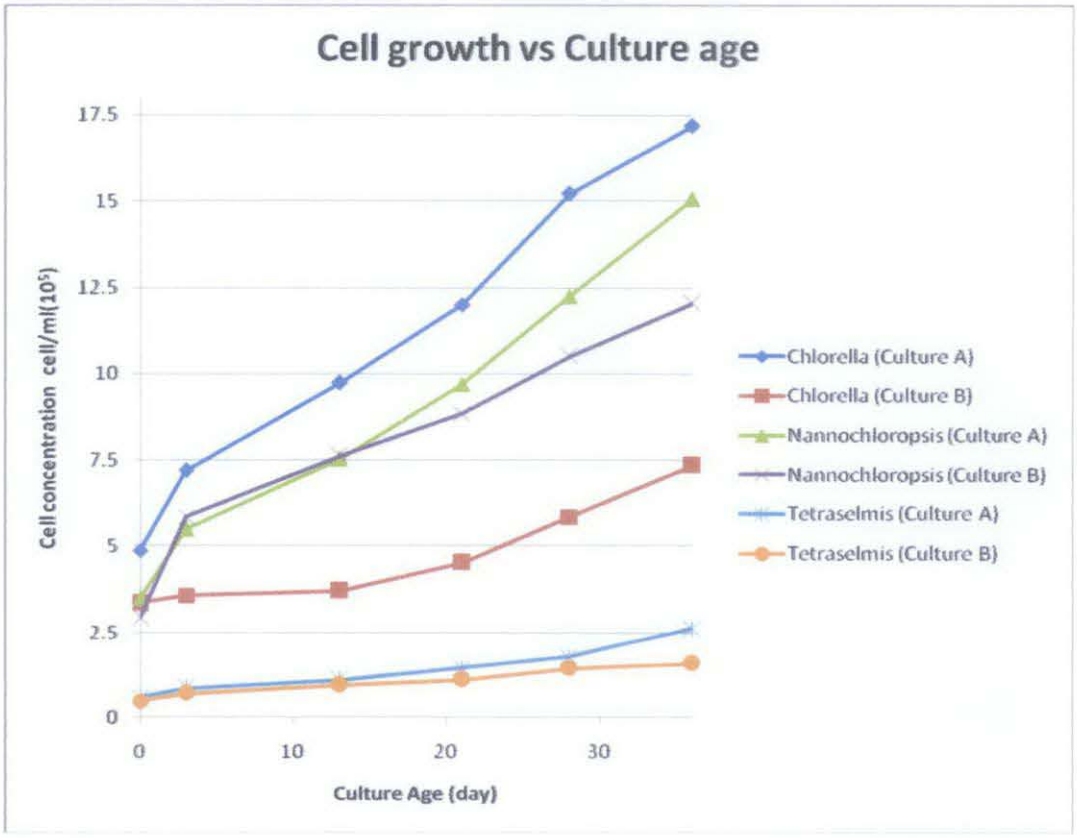


Figure 4.1: Cell Growth vs. Culture Age (days) for different microalgae strais in different culture condition

4.1.2 Culture A vs. Culture B

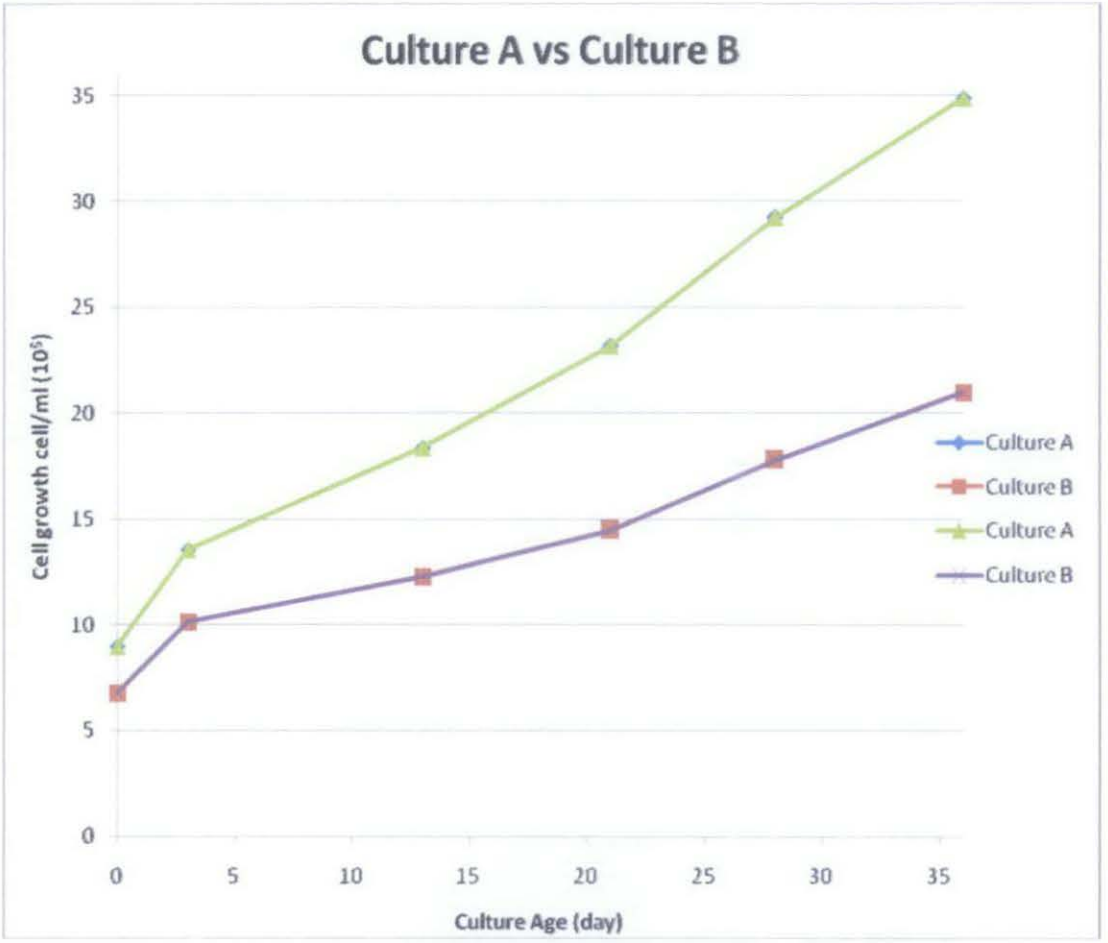


Figure 4.2: Cell growth of microalgae in different culture condition

Result shows the overall effect of nutrient content to microalgae growth rate. As mention before, some strain is more sensitive to nutrient supply change than other. But overall, reducing nutrient supply will cause reduction in microalgae growth. Higher resistance and less sensitive microalgae strain is more preferred to be utilized in CO_2 mitigation as they will be more practical and cost effective. Sensitive microalgae strain can easily collapse when come across infection and limited nutrient supply. This will of course increases cost as there will be a lot of parameter to be controlled in order to utilize the strain in CO_2 mitigation system.

4.1.3 Growth Rate Comparison for the Species Cultivate

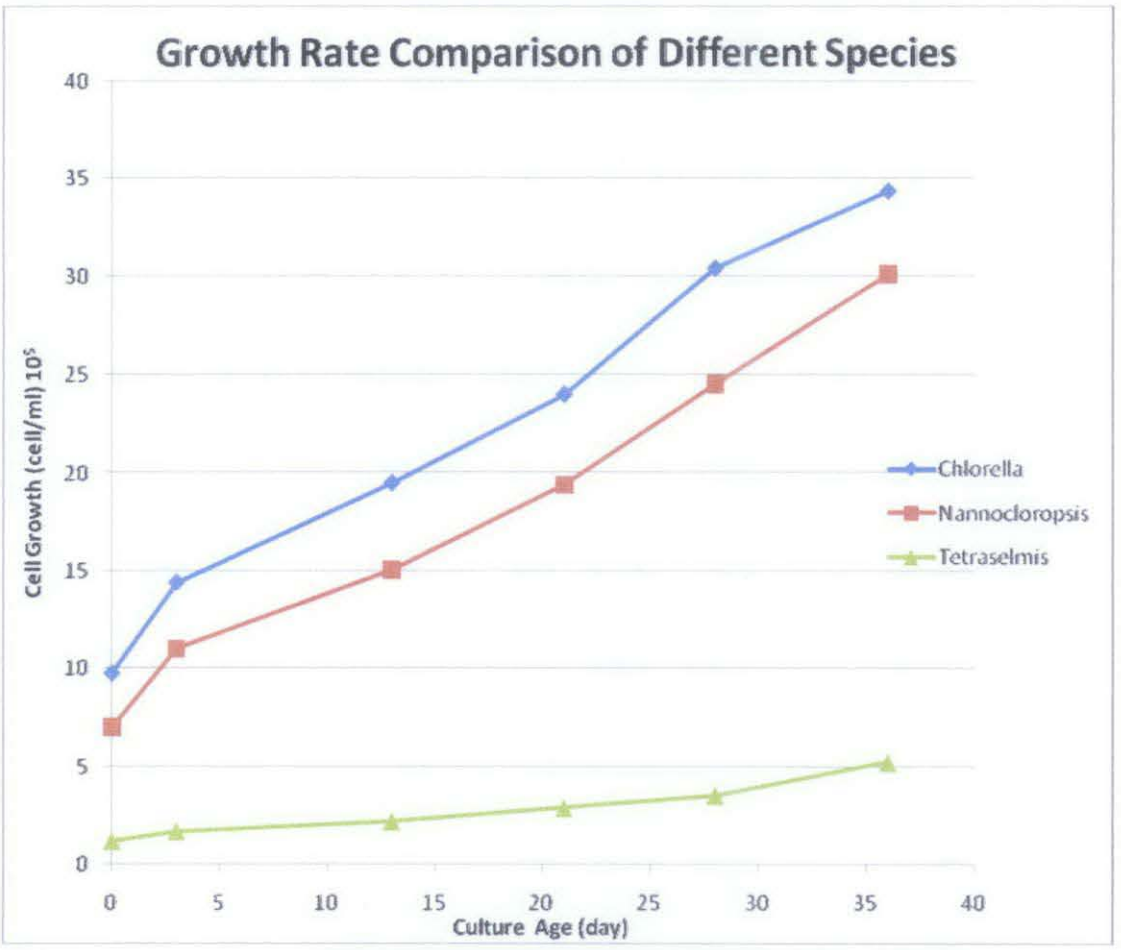


Figure 4.3: Cell growth rate of 3 different species in culture A

Cultivation in culture condition A has shows that *Chlorella sp.* has the highest growth rate compared to other 2 species. Chlorella sp. has smaller cell size compared to the other species. *Tetraselmis sp.* in other hand has relatively larger cell size thus maybe slowing its growth rate. But as mention before, there are lots of other criteria to select the best microalgae strain for the purpose of CO_2 mitigation system. *Tetraselmis sp.* has been proved to have higher resistance to infection [14] and nutrient supply changes. Furthermore it can be cultivated stably all year long. This may be a better criterion of a strain to be utilized in CO_2 mitigation system.

4.2 Extraction of Cultivated Microalgae and Wild Microalgae Sample

Extraction of the cultivated and wild microalgae is done via several steps. Centrifugation is one of the simplest techniques in microalgae extraction. Microalgae will settle down by centrifugal force applied to the culture. After removing the upper water layer, the bottom microalgae layer can be extracted for its bio-oil. Vacuum dryer in used to dried the wet harvested microalgae.

Hexane solvent is used to extract bio-oil from the microalgae sample while rotary evaporator is then used to remove the solvent to give concentrated bio-oil. This bio oil is then can be transformed into bio-fuel (ester) using Transesterfication using NaOH catalyst. Further content analysis and micro-activity analysis should be done for with proper equipment and better result.

Figure 4.4: Rotary Evaporator

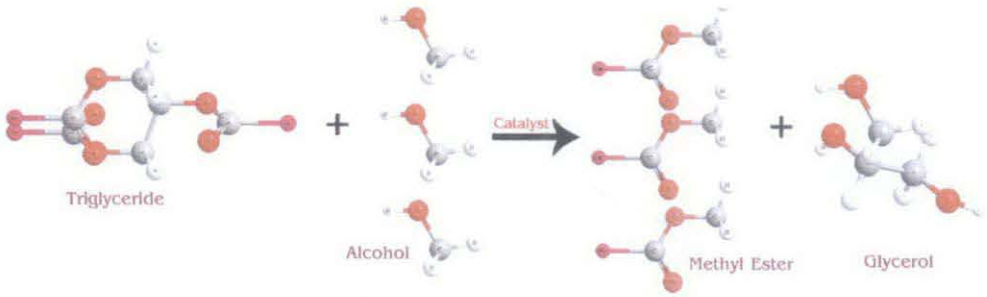


Figure 4.5: Transesterfication Process

4.3 Utilization of Wild Microalgae for CO_2 Removal and Bio-fuel Production

Different from cultivated microalgae, wild algae can be sampled on most of natural/artificial water supply i.e. pond, lake etc. sampling of wild algae are done by simply collect the water/algae-rich water supply i.e. UTP ponds.

Before utilization of wild algae can be done, some content analysis of the sample should be done. The extraction of wild microalgae sample for bio oil is done via same steps of those for cultivated microalgae. It is explain in section 4.2.

4.3.1 Microscopic Observation

In this FYP project, microscopic observation is among the test done to determine the content of the sample. The sample is taken from various UTP open water source such as UTP lake, drain and fish pool.

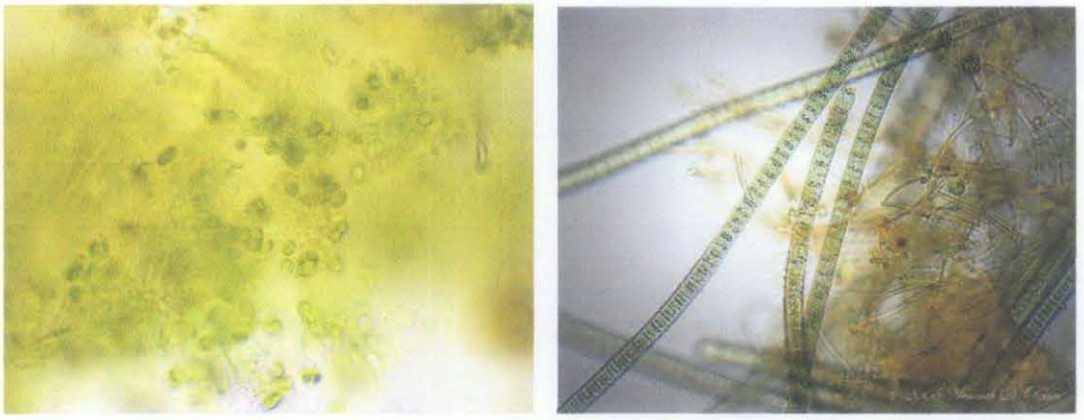


Figure 4.6 (a) and (b): Wild microalgae

Microscopic observation of wild microalgae sample is done to see and briefly determine the content of the sample. Few samples are observed and figure 4.6 is among the microscopic shot taken using the microscope. Brief information from the microscopic observation is;

Figure 4.6 (a) shows several colonies of microalgae from different species. Figure 4.6 (b) shows colonies of macro algae, bacteria and contamination.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The world now is facing the global scale energy and environmental crisis. The solution for all these crises may be held on a very simple and basic process call photosynthesis as they are the only process that can reverse the effect of environmental as well as produce energy. Microalgae are the simplest form of photosynthetic plant that can grow rapidly due to its simple structure. They have been investigated for the production of biofuels including bio-diesel, bio-syngas, bio-oil and bio-hydrogen. Microalgae fuel production is sustainable. It is possible to cultivate adequate microalgal fuel to satisfy the fast growing energy demand with the restrain on land and water resource. Microalgae production can be couple with CO2 mitigation and waste water treatment.

CO2 fixation using fast-growing microalgal species provides a very promising alternative for CO2 mitigation. This strategy is lies on the fact that cultivation of microalgae can be combined with CO2 fixation and waste water treatment in an economically feasible and environmentally sustainable manner.

5.2 Recommendation

Microalgae are an active research that is being actively studied by lots of researcher around the globe. Malaysia has lots of microalgae species and excess to freshwater, lake water, seawater and another source of water. It is a lost that there is only little organization that takes the research in microalgae seriously. UTP as a university that have the closest link to PETRONAS, national fuel company should take the first step to develop full scale facility for the microalgae research. The great potential of microalgae and its versatile field should be explored and developed as they may hold the key for all environmental and energy crisis around the globe.

Some improvement that can be done in term of research procedure and scope of studies includes;

1. Include more parameters including culture media content, culture temperature and more microalgae potential species.
2. For CO_2 removal system, CO_2 inlet concentration should be varied to study tolerance of microalgae species for high CO_2 concentration inlet gas. Simple run using real exhaust gas (high temperature and mixed gas content) should be done to determine whether the system can be used for real application.
3. Several cultivation systems should be developed to monitor microalgae growth rate in different cultivation system i.e. open system, batch system, continuous system etc.
4. For higher level research, bio-technology approach such as genetic altering process can be done to produce microalgae strains that has high growth rate, high resistance to bacteria and infections, high CO_2 uptake as well as high oil content.

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APPENDIX A

Cell Growth of Cultivated Microalgae

Cell Growth of Cultivated Microalgae

	8/12/2009		11/12/2009		21/12/2009		29/12/2009		5/1/2010		13/1/2010	
Chlorella												
A	18	15	26	30	33	36	42	63	69	59	77	56
	18	22	20	29	39	41	49	36	51	66	49	59
	18	17	30	28	45	43	39	43	72	43	58	87
	22	13	32	34	49	30	49	51	80	73	82	86
	27	25	30	29	31	43	53	55	42	53	65	68
B	14	13	10	17	9	12	8	9	7	16	6	17
	10	14	15	14	15	10	16	11	17	6	18	14
	11	17	15	15	17	15	19	16	21	17	23	18
	14	11	16	11	16	24	16	24	16	40	16	56
	12	18	11	19	11	19	27	35	43	51	59	67
		195		288		390		480		608		687
		134		143		148		181		234		294
Nannochloropsis												
A	19	15	17	29	26	39	47	36	61	34	65	60
	7	14	24	20	32	26	45	37	50	39	66	59
	14	15	22	22	28	32	32	42	42	41	51	58
	14	6	22	23	26	24	27	37	56	43	68	47
	16	20	20	21	37	31	44	41	65	59	65	63
B	11	12	29	24	36	33	35	32	44	41	53	50
	12	8	20	26	29	29	38	36	39	45	43	54
	9	10	24	31	33	40	42	41	49	47	55	51
	10	13	30	16	35	25	33	34	42	43	51	52
	18	14	14	20	23	22	32	31	35	35	34	39
		140		220		301		388		490		602
		117		234		305		354		420		482
Tetraselmis												
A	5	2	3	5	4	7	6	6	7	5	8	7
	3	3	5	3	6	5	3	4	9	6	9	9
	1	0	2	2	3	3	5	6	12	4	14	11
	0	2	1	2	6	2	4	9	6	9	9	14
	4	4	6	5	3	5	11	4	4	9	8	16
B	3	2	4	2	5	3	6	4	7	5	8	3
	3	1	2	5	4	6	6	7	8	8	10	9
	1	2	4	3	3	4	2	5	4	6	6	7
	2	3	1	2	2	3	3	4	4	5	5	2
	1	1	3	2	5	3	3	4	5	5	7	6
		24		34		44		58		71		105
		19		28		38		44		57		63

APPENDIX B

Sample of Calculation for Cell Count

Chlorella sp. **Culture A**

Day 14 (29/12/2009)

Above	Below
33	36
39	41
45	43
49	30
31	43

$$AverageCell = \frac{\sum cell}{10}$$

$$AverageCell = \frac{33+39+45+49+31+36+41+43+30+43}{10}$$

$$AverageCell = 39$$

Cell Concentration

$$NumberOfCell = \frac{AverageCell}{Deep \times Area}$$

$$NumberOfCell = \frac{39Cells}{0.1mm \times 0.04mm} \times \frac{1000mm^3}{1ml}$$

$$NumberOfCell = 9.75 \times 10^6 Cell\ ml^{-1}$$

APPENDIX C

Sample Calculation of Doubling Time

Chlorella sp. **Culture A**

From day 14 to 29

$$MaximumGrowthRate, \mu_{max} = \frac{\ln x - \ln x_0}{t - t_0}$$

Where,

$x = GrowthAtTime\ t$

$x_0 = GrowthAtTime\ t_0$

$$\mu_{max} = \frac{\ln x - \ln x_0}{t - t_0} = \frac{\ln 1.2 \times 10^7 - \ln 9.75 \times 10^6}{29 - 14}$$

Doubling Time

$$td = \frac{\mu_{max}}{\ln 2 \times 100}$$

$$td = \frac{0.01384}{\ln 2 \times 100}$$

$$td = 1.997 \approx 2 days$$