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Production of biodiesel from microalgae

Nkongolo Mulumba
University of New Hampshire, Durham

PRODUCTION OF BIODIESEL FROM MICROALGAE

BY

NKONGOLO MULUMBA

B.S., Chimie Industrielle, Faculté Polytechnique
Université de Lubumbashi, 1999

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ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

This thesis has been examined and approved.

Dr. Ihab H. Farag
Thesis director
Professor
Department of Chemical Engineering

Dr. Leland S. Jahnke
Professor
Department of Plant Biology

Dr. Dwight Peavey
Senior Scientist
United States Environmental Protection Agency

Aug 13, 2010

Date

DEDICATION

To my sister Miandabu Mulumba Buela who left us prematurely,

To my parents Mulumba Wa Nkamba and Muadi Kabunvu,

To my daughters Muadi Nkongolo and Kabundi Nkongolo

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PRODUCTION OF BIODIESEL FROM MICROALGAE

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NKONGOLO MULUMBA

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Biodiesel production from microalgae is a promising technique, with advantages of high biomass yield with high lipid content. Challenges include effective techniques to harvest the grown microalgae, extraction of the algal oil and its transesterification to biodiesel. A microalgae strain was selected from 8 different species screened for growth rate and lipid content. A Tubular Photobioreactor was designed and constructed to study microalgae growth. Productivity of 1 g of dry algal biomass per liter of medium within 12 days was achieved, with lipid content up to 20 %. The observed 10 fold increase in biomass is higher than those reported for open ponds and helical photobioreactors. In situ transesterification of dry algae to fatty acids methyl esters (FAMEs) was achieved using ultrasonication. A Gas Chromatograph was used to analyze the FAMEs. Biodiesel produced through 20 minutes of in situ transesterification yields up to 3.679 mg of FAMEs per g of dry algal.

CHAPTER I

INTRODUCTION

1.1 BACKGROUND

Humankind depends on petroleum-based fuel as a source of energy since its discovery years before Christ (BC) era. It is believed that the first well of petroleum was drilled in China around 347 BC. Growing demands of this non-renewable source of energy, its depletion and cost, the full dependency of all humankind on its usage as one of the principal transportation fuel, motivate researchers to find an alternative fuel from renewable sources, capable to replace fossil fuels.

Biodiesel looms as potential replacements of petrodiesel (Chisti, 2008). It is a renewable and alternative fuel produced from a transesterification reaction between alcohol and fatty acids (FAs) from animal fat or vegetable oil in the presence of a catalyst (Sahoo and Das, 2009; Li et al., 2007). Chemically, biodiesel molecules consist of long chain of mono-alkyl esters. Biodiesel can be used directly (100% termed B100) in any diesel engines with no modification or blended with diesel fuel at different volumes ratio such as 5% biodiesel in mixture (B5), 20% Biodiesel in mixture (B20), 50% biodiesel in mixture (B50) and so on.

Biofuel technology is not new to humanity. Numerous attempts have been made in the past to produce or use biodiesel. In 1900, the French automobile company, Otto, ran a diesel engine with pure peanut oil. Twelve years later, Rudolf Diesel stated that "the use of vegetable oils for engine fuels may seem insignificant today, but such oils may become, in the course of time, as important as petroleum and coal products of the present time" (Knothe et al., 2005). The first transesterification reaction between vegetable oil and alcohol is believed to be carried by G. Chavannes, on August 31, 1937 at the University of Brussels in Belgium (Knothe et al., 2005). After World War II, several countries including France, Germany, Brazil, Japan and China became interested in the production of fuel from biomass.

Currently, several biodiesel producers use vegetable oil from food crops such as soybean, canola, and palm to produce a transportation fuel with chemical and physical properties similar to petrodiesel. However, the use of oil from food crops has created significant worldwide opposition due to the increase of food price and growing food shortage. The Food and Agriculture Organization (FAO) of the United Nations (UN) is persuading researchers to redirect biodiesel production to the use of non-edible crops. In addition, food based crops alone cannot produce enough biodiesel to satisfy the world demand. For instance, The United States alone demand about 60 billion of gallons of diesel per year; on the other hand, soybean, canola and palm produce only 50, 90 and 650 gallons of biodiesel per acre per year (Chisti, 2007 and 2008). Microalgae can potentially produce 5,000 – 15,000 gallons of biodiesel per acre per year (Brown et al., 1993; Mulumba, 2009; Ferrentino, 2007). Moreover, the exploitation of food based crops to produce

biodiesel requires not only vast arable lands but also it creates environmental concerns such as ozone depletion due to N_2O emitted by fertilizers, acidification of soil due to sulfur and nitrogen oxides from fertilizers as well as eutrophication and algal blooms due to pesticides (Mousdale, 2008). Hence researchers have turned their interest to non-food based crops such as microalgae and Jatropha as raw material for biodiesel production. This work focuses on the use of microalgae.

1.2 **BIODIESEL**

Biodiesel is a renewable alternative transportation fuel for diesel engines. It is produced through transesterification reaction between triglycerides (vegetable oil) or fatty acids with alcohol (methanol) in presence of catalyst (Li et al., 2007; Knothe et al., 2005; Chisti, 2007).

Biodiesel is recognized as a potential replacement for petrodiesel. Fossil fuel (petrodiesel) currently becomes insufficient to satisfy worldwide demand due to the depletion of its supplies and the significant contribution of greenhouse gases such carbon, nitrogen and sulfur oxides released during petrodiesel combustion (Brown, 1993; Chisti, 2008). In contrast, biodiesel is considered a carbon neutral fuel, i.e., there is no net emission of CO2 during its use. Other environmental advantages of biodiesel include no net emission of sulfur oxides (SOx), and nitrogen oxides (NOx) are significantly reduced in comparison to the emission from petrodiesel. In addition, Biodiesel is an alternative

fuel to meet the health effect requirements for the US Clean Air Act (US CAA) established in 1990 (Brown et al., 1993).

Biodiesel has been growing to be a vital alternative fuel to the United States (US) economy with a doubling production nationwide each year as shown in Figure 1.1. Furthermore, new plants or research centers for biodiesel production are built in US at fast pace (National Biodiesel Board, 2008).

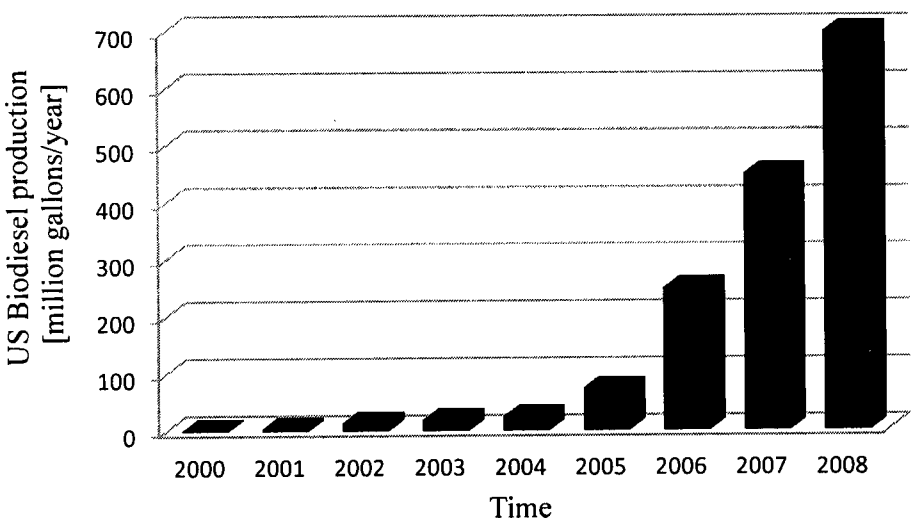
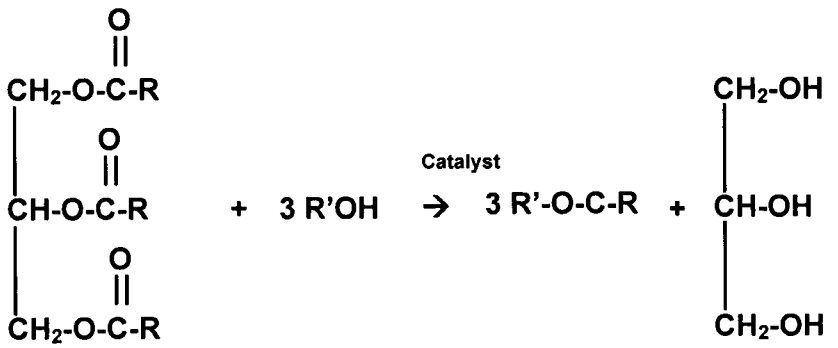


Figure 1.1: Estimate biodiesel production per fiscal year in the United States (US National Biodiesel Board, 2008)

Chemically, biodiesel is a mixture of Fatty Acids Methyl Ester (FAMEs) produced from a reaction of triacylglycerols or TAGs (vegetable oil or fatty acids) with methanol, termed transesterification. This reaction requires a catalyst, which could be an acid or an alkali. In most application sodium hydroxide, potassium hydroxide or sodium methoxide is used. These are less expensive and result in much faster reaction rate compared to

acids catalysts and biocatalysts (Manesh and Enayati, 2008). Reaction 1.1 shows a transesterification reaction for the production of fatty acid alkyl ester (FAAE) (Knothe et al., 2005; Ferrentino, 2007).

Reaction (1.1)



Triacylglycerols (TAG) Alcohol Fatty Acid Alkyl ester Glycerol

Where R represents chain of fatty acids in the TAG and R' represents the alkyl group in the alcohol molecule; for example, for methyl alcohol R' is CH_3^-.

The biggest challenge in biodiesel production is to find enough vegetable oil from non-food based crops as raw material. Biodiesel produced from food crops would not be competitive on price and quantity with petrodiesel. The price of soybean in the United States has significantly increased, from $5.80/bushel in 2005 to $9.80/bushel in 2009 as shown in Figure 1.2. This price increase is due to soaring demand for food and also its use in part as raw material for biodiesel production (National soybean index, 2009), which would make biodiesel fuel non-competitive to petrodiesel.

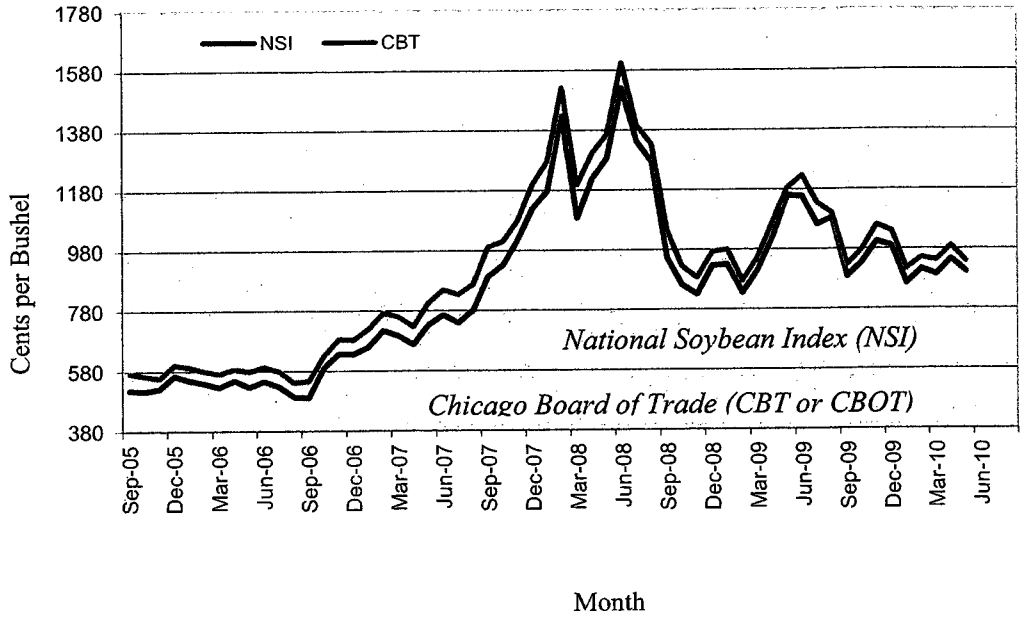


Figure 1.2: Soybean prices overtime tabulated by the National Soybean Index (NSI 2009)

1.3 **FOOD CROPS CHALLENGE**

1.3.1 FEEDSTOCK FOR BIODIESEL

Vegetable oil from food based feedstock is the classic raw material used in biodiesel production throughout the world. The major feedstocks for vegetable oil are: canola, soybean, palm, rapeseed and sunflower. The choice of vegetable oil for biodiesel depends on the availability as well as the price. European Union (EU), for instance, uses rapeseed and sunflower oil for biodiesel production; whereas US uses predominantly soybean oil and waste vegetable oil (WVO, also termed as used vegetable oil, UVO) (usually from cooking, characterized by the presence of free fatty acids, FFA) to produce biodiesel (Knothe et al., 2005; Chisti, 2007). The biodiesel yield varies widely based on

the crop used. Table 1.1 shows a variety of crops and their relative biodiesel production in gallon per acre per year. The crops are arranged in ascendant order based on the biodiesel production. It shows the great advantage of algae.

Table 1.1: Feedstock and biodiesel yield (Brown, 1994; Chisti, 2007; Khan et al., 2009)

Feedstock	Gallon of biodiesel per acre per year
Corn	15
Hemp	39
Soybean	50
Canola	90
Sunflower	102
Peanut	113
Olive	120
Rapeseed	125
Coconut	200
Jatropha	207
Palm	650
Microalgae	5,000 – 20,000

The United States alone consumes about 60 billion of gallons of diesel per year. Clearly, food crops cannot be used to provide the biodiesel needed to replace petrodiesel. All these food based crops can only be used to produce biodiesel that will be blended with petrodiesel. In addition, the price increase of soybean in the United States, as stated in section 1.2, will result in higher price of biodiesel, which will make it not competitive with petrodiesel. Therefore, the world will continue to face dependency on fossil fuel and suffer from greenhouse gas effects from petrodiesel believed to contribute to global warming.

On the other hand, Table 1.1 shows that microalgae can yield 5,000-15,000 gallons of biodiesel per acre per year. Obviously, microalgae loom as the only potential source of vegetable oil that can be economically used to produce biodiesel.

1.3.2 MICROALGAE

Microalgae are single cell photoautotrophic (capable of synthesizing own food from inorganic substances using light as an energy source) or photoheterotrophic (use light energy but cannot use CO_2 as source of carbon) microorganism. Photoautotrophic algal cells grow like plant through photosynthesis process, during which algal cells capture CO_2 and photons, and convert them into biomass rich in lipid. More than 3000 algal strains have been discovered and most of them live in aquatic habitat such as sea, rivers and oceans (Sheehan et al., 1998). Based on their habitat, microalgae are classified into fresh water and marine algae.

Algal cell may contain lipid up to 80% in mass depending on the strain, the growth medium composition and the culture conditions such temperature, pH, carbon dioxide and photonic energy absorption (Meng et al., 2009; Chisti, 2008). Table 1.2 shows a variety of microalgae, habitat and lipid content in g lipid per 100 g dry algae.

Table 1.2: Microalgae lipid content (Chisti, 2007; Bigogno et al., 2002)

Algal strain	Habitat	Lipid content in g lipid per 100 g dry algae
Chlorella sp	Freshwater	28-32
Amphidimium Carteri	Marine	>20
Tetraselmis	Marine	15-23
Dunaliella primolecta	Marine	23
Isochrysis sp	Marine	25-33
Thalassiosira Pseudonana	Marine	> 30
Nannochlorosis sp	Marine	31-68
Porphyridium cruenturn	Marine	>40
Schizochytrium sp	Marine	50-77
Monallanthus Salina	-	>20
Phaeodactylum	-	20-30
Neochloris oleoabundans	-	35-54
Botryococcus braunii	-	25-75

Being photosynthetic microorganisms, microalgae require water rich in nutrient, carbon dioxide and photonic energy for growth. They grow like other microorganism undergoing four growth phases: lag, exponential (growth), stationary, and death phase or lysis as shown in Figure 1.3.

Microalgae convert photonic energy, water and CO_2 to sugars; then sugars are converted to macromolecules such as lipids or/and triacylglycerols (TAG) as shown in reactions (1.2) and (1.3) below: two steps reactions.

$$CO_2 + \text{water} + 8 \text{ photons} \rightarrow \text{Sugars} + O_2 \qquad \text{Reaction (1.2)}$$

$$\text{Sugars} \rightarrow \text{TAGs} \qquad \text{Reaction (1.3)}$$

Lipids are believed to be the sustainable feedstock for biodiesel. In this process, microalgae are also sequestering the carbon from CO_2.

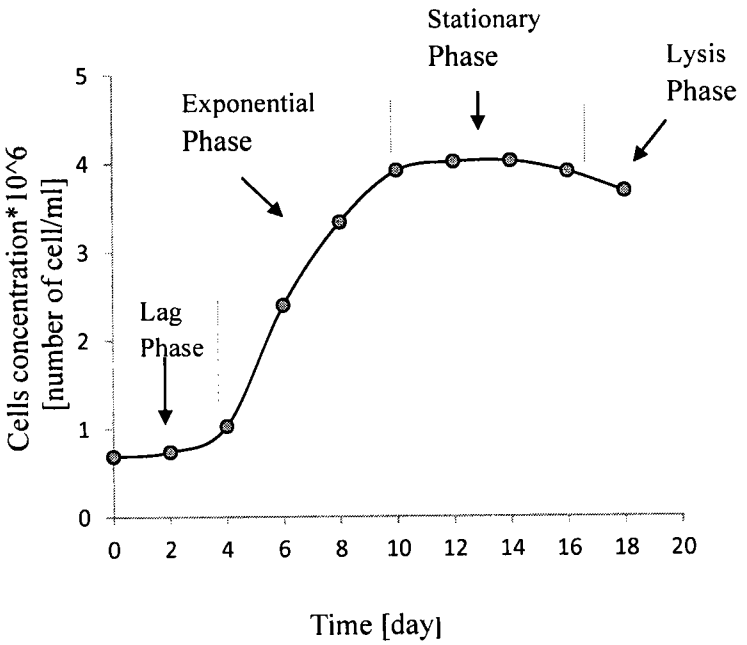


Figure 1.3: Algal growth phases. A *culture performed at room temperature with blowing air into the broth to supply CO_2* (Mulumba, 2009)

One of the biggest challenges in culturing microalgae for biodiesel is to find a suitable strain that grow fast and capable to produce as much lipid as possible during its culture. Another major challenge in algae culture is to design a cost effective photobioreactor, which mitigates contamination risks and enhances high growth. An additional challenge is the high cost of oil extraction from microalgae.

Even with all these challenges, biodiesel from microalgae looms as the only renewable biofuel that can substitute petrodiesel completely (Chisti, 2007 and2008; Brown et al., 1993). An additional advantage of microalgae is the capability to capture CO_2 and reduce greenhouse gas effects.

1.4 BIODIESEL PRODUCTION FROM MICROALGAE

Numerous studies have been conducted to explore the use of algal oil for biodiesel. Among them, the study conducted in 1993 by the National Renewable Energy Laboratory (NREL) of Colorado in conjunction with the United States Department of Energy (USDOE) to evaluate possibilities of producing biodiesel using algal oil (Brown et al., 1993). This study demonstrated the potentiality of algal cells to yield enough oil usable for biodiesel.

Certain strains of microalgae contain up to 80 % of vegetable oils as shown in Table 1.1. Algal cell can be cultured phototrophically in two systems: open ponds and enclosed photobioreactors (PBR). In this process, algae accumulate lipid (oil). At the appropriate time, algae are harvested and dried, and then oil is extracted. Figure 1.4 shows the different steps of biodiesel production from microalgae.

Fuls and Hugo (1984) concluded that the viscosity of vegetable oils and microalgal oils are usually higher (3 to 5 times higher) than that of diesel oils. Algal oils (lipid) can damage existing diesel engines, clog filters due to their high viscosity if they are used without modification. The transesterification of algal oils will reduce the original viscosity and increase the fluidity (Chisti, 2007). Alternatively, algal biomass can be converted directly into biodiesel using in situ transesterification without prior oil extraction. Both the in situ and the two step process are shown in Figure 1.4.

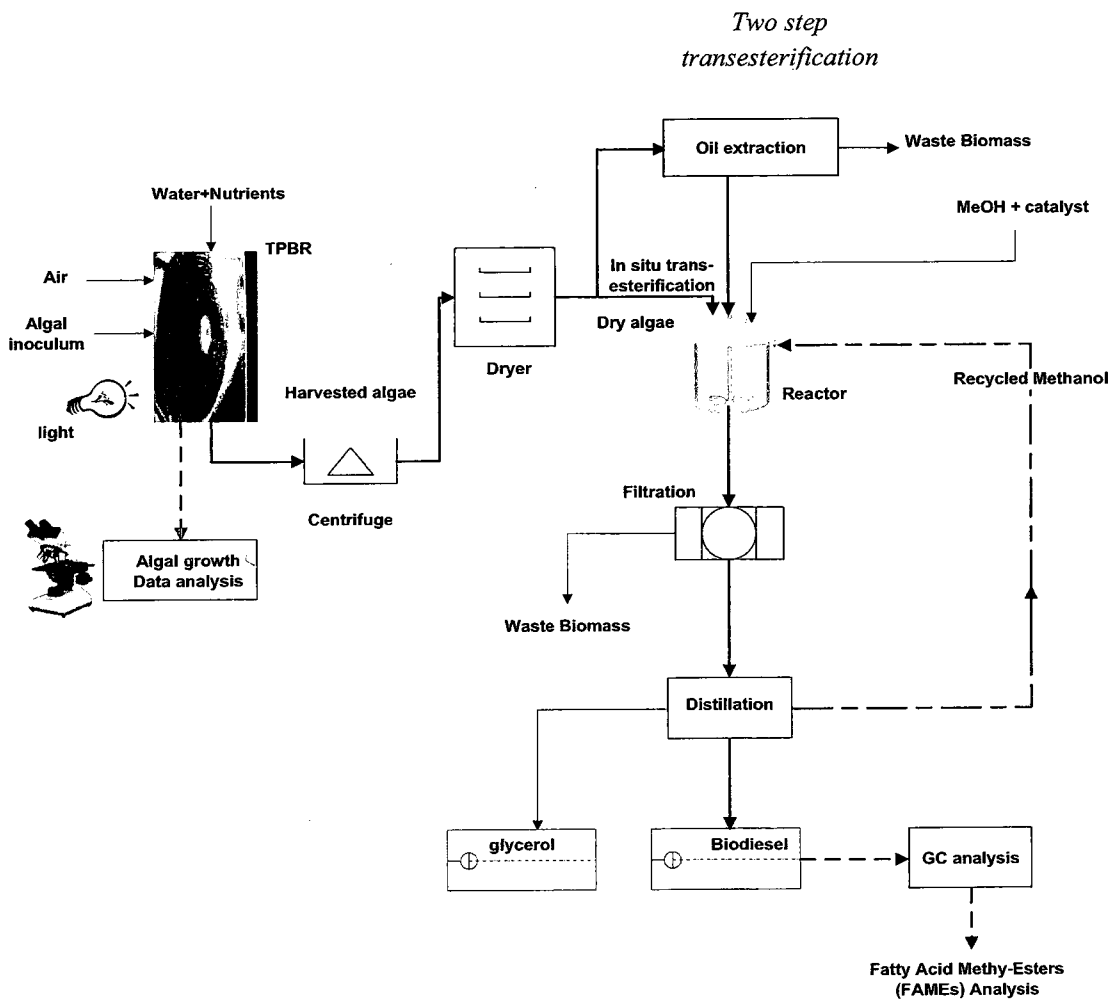


Figure 1.4: Different stages of biodiesel production from microalgae

It is important to note that alcohols are key components in the transesterification. Commonly used alcohols include methanol, ethanol, propanol, butanol and amyl alcohol. However, methanol is the most used alcohol for the transesterification of algal biomass in the United States (US). The US uses methanol because it is the least expensive alcohol, whereas Brazil uses ethanol for biodiesel, for it is less expensive than methanol (Knothe et al., 2005).

Another key component in the transesterification process is the catalyst, which could be an alkali (alkaline), acid or enzyme. Table 1.3 shows a comparison of these three catalysts.

Table 1.3: Advantages and challenges of catalysts used in transesterification (Knothe et al., 2005; Mousdale, 2008)

Catalysts	Advantage/Use	Challenges
Acid	Useful for the conversion of high free fatty acids (FFAs) feedstocks (like WVO) to esters (biodiesel)	Very low reaction rate converting triglycerides to FAMEs. (Knothe, 2005; Mousdale, 2008)
Alkali or Alkaline (most frequently used)	Higher reaction rates (4000 times faster) than acid catalyst	FFAs may react with alkali to from soap and water. More alkali is needed. FFA + KOH → K-Soap + water When FFA is greater than 5 %. The resulting soap will emulsify FAMEs and glycerol.
Enzymes	Good tolerance to FFAs in the feedstocks	Expensive, may not be able to provide high quality biodiesel to meet ASTM specification. May not be as fast as alkaline catalysts

The transesterification of algal oil is generally carried out by alkaline (alkali) catalyst such as KOH, NaOH or sodium methoxide (NaOMe). These catalysts present a high level of miscibility of vegetable oil with methanol or ethanol offering an advantage of a homogeneous solution, which result in more rapid process as stated in Table 1.3. However, a saponification reaction may occur during an alkali catalyzed reaction. The presence of soap in the biodiesel necessitates a washing process for its complete removal. If water is used to wash the soap, the biodiesel needs to be dried.

Xu et al. (2006) provided a comparison of biodiesel from algae petroleum diesel and the ASTM standards shown in Table 1.4.

Table 1.4: Algal biodiesel/petrodiesel properties versus biodiesel ASTM Standards (Xu et al., 2006)

Properties	Algal Biodiesel	Diesel fuel	ASTM Biodiesel standard
Density (kg/L)	0.864	0.838	0.84 – 0.90
Viscosity (mm^2/s, CST at 40 °C	5.2	1.9 – 4.1	3.5 – 5.0
Flash point (°C)	115	75	≥ 100
Solidify point (°C)	-12	-50 to 10	-
Cold filter plugging point (°C)	-11	-3 (max -6.7)	Summer max 0 Winter max -15
Acid value (mg KOH/g)	0.374	≤ 0.5	≤ 0.5
Heating value (MJ/Kg)	41	40 – 45	-
Heating value (cal/g)	9795.1	9556.2 - 10751	
Heating value (Btu/gal)	127,127	124,028 – 139,534	
H/C ratio	1.81	1.81	-

1.5 **PHOTOBIOREACTORS**

1.5.1 OPEN-PONDS

Open-pond is referred to any container widely open or lakes used to grow algal cells. Raceway ponds are the most used in an algal culture. They are made with recirculation

channels equipped with paddlewheel for medium mixing and circulation to prevent sedimentation. These channels are built in concrete or compacted earth or white plastic (Chisti, 2007). The Photosynthesis process in open-pond occurs in a cycle; algal cells capture photonic energy from sun during the day and cells undergo photosynthesis, which discontinues during night time. Figure 1.5 shows an aerial view of a raceway pond

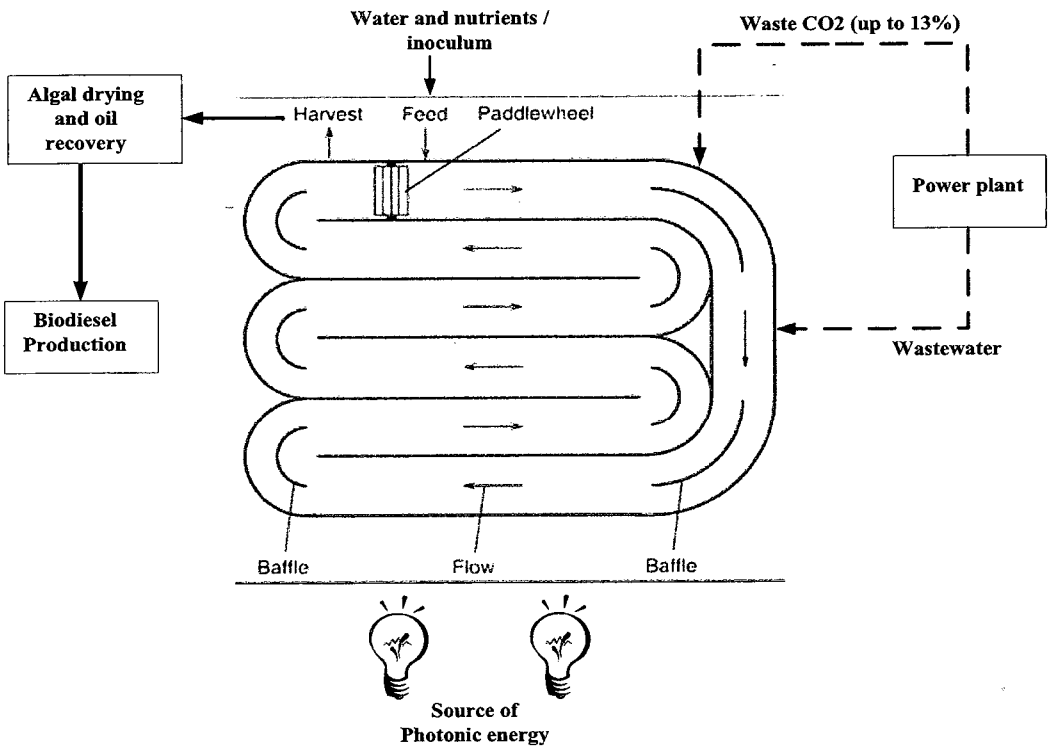


Figure 1.5: Raceway pond photosynthesis system. *The figure also shows the possibility of using wastewater and waste CO_2 (in flow gases) from a nearby power plant* (Chisti, 2007)

Raceway ponds are possibly more suitable for culturing microalgae for biodiesel production due to the lower operating costs relative to enclosed PBR. The pond has to be supplied with water, nutrient algae inoculum and CO_2. If the pond is located outdoor near a power plant, it is possible to use CO_2 from waste flue gas and the wastewater from

the plant, which may contain nutrients such as iron and nitrogen, as shown in Figure 1.5 (green line).

Culturing algae in Open Pond is more challenging. The following are technical issues that arise when using open-ponds: water evaporation, pH and temperature control, cells illumination and contamination risks by other organisms that overtake more often algal growth (Ugwu et al., 2007). Not only there is a need of understanding those issues, but also preventing them by using more efficient closed system such as tubular PBR that will enhance temperature and pH control as well as support healthy algal culture.

It is certain that the population on the earth increases at exponential rate with projection to reach 10 billion by 2040. This population requires approximately 5 billion acre of arable land allocated to grow their food and space to accommodate their living (Mousdale, 2008). Obviously, the use of open-ponds for fuel production will jeopardize human resource for basic needs, which is food and living space. Moreover, algal cells growth in open-ponds is often inhibited by photonic energy starvation. Richmond (2000) showed that 85% of algal cells in a raceway pond are in the dark at any instance. As result, photosynthesis process is very slow with a low growth rate for algal cells culture, which would not make biodiesel from microalgae a replacement for petrodiesel. Chisti (2007) stated that algae species cultured in Raceway Pond can produce oil at a rate of 0.12 Kg per $m^3$of pond per day. Therefore, there is an important need of different types of reactor for algae growth such as a closed PBR.

1.5.2 CLOSED PHOTOBIOREACTOR

Algae best grow and produces oil in a narrow temperature range. Thus night sky radiation, temperature variation (low temperature or high temperature) and excessive solar radiation will interfere with algae growth and lipid production. Therefore, algal cell can be cultivated in a closed photobioreactor (PBR). Virtually, any transparent container can be called PBR. Contrary to open-ponds (such as raceway) or tank, a PBR is a closed system that incorporates photonic energy to culture microorganism (algae). It is designed to mitigate culture vulnerability to contamination mostly caused by bacteria attacks, polluted air, rain and toxic debris. The productivity of closed PBR tends to be 5 times higher than open pond. Hence they have smaller "foot print" and allow the use of single microalgae species.

Photobioreactors may be classified based on their design/shape and on their operating modes. Designs based on the shape are: serpentine tubular airlift, flat plate, horizontal/circular/spiral/vertical tubular and cylindrical (Ugwu et al., 2007). Tubular PBRs usually consist of several straight transparent tubes, of 0.1 m diameter or less. This is important to ensure good light penetration. The microalgae broth is circulated through a reservoir which acts as a degassing column. An example of a tubular PBR is shown in Figure 1.6.

A second subdivision of photobioreactors is their operating mode: batch, semi-batch and continuous. Each photobioreactor presents advantages and drawbacks. These are detailed in Chapter 2, Section 2.2.

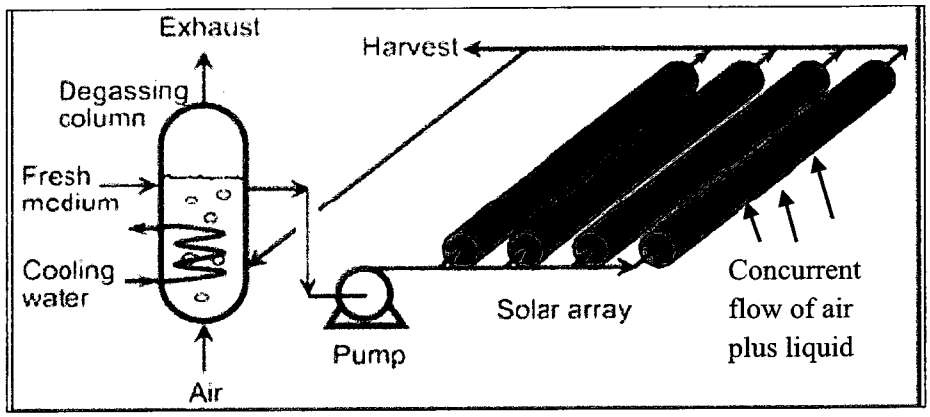
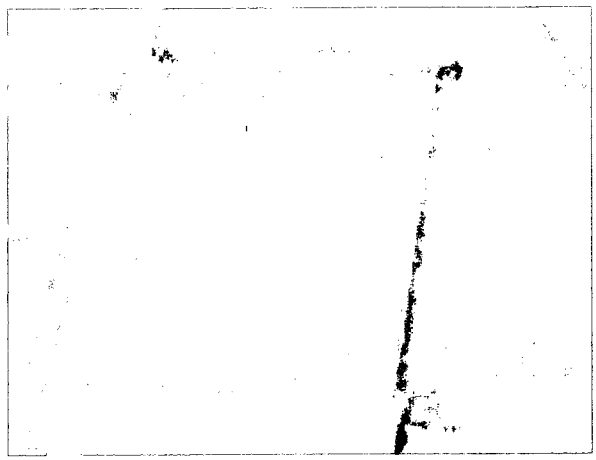


Figure 1.6: Tubular PBR (Chisti, 2007)

1.5.3 HYBRID SYSTEM

Open-ponds and closed PBRs can all operate as a combined (or hybrid) system to maximize algal biomass productivity. A common advantage in all PBRs is a high growth rate of algal cells, achieved by controlling culture temperature, mixing mechanism, carbon dioxide input as well as light intensity.

The Open-pond in Figure 1.7 has a temperature regulator that keeps pond temperatures well above the optimum minimum growing temperature for algae of 64°F. The hybrid system in Figure 1.7 can also use additional external temperature controls, if necessary, to cool the pond in the hot summer and heat the pond in winter conditions during extended sunless days to maintain maximum growth conditions (Green Car Congress, 2007).



http://www.greencarcongress.com/2007/05/greenstar_produ.html

Figure 1.7: 40000 L-Hybrid algae production system (HAPS) with incorporated temperature regulator (Green car demonstration 2007)

In a hybrid system that has combined open-pond/closed PBR, cultures are first grown in the closed system (closed PBR), where algae grow fast. Then algal culture is transferred in the open-pond when it reaches stationary phase. The yield in algal biomass will then increase in the open-pond. It is a significant advantage for hybrid system.

On the other hand, a culture transferred in an open-pond faces all risks of contamination, which could lead to culture collapse. A combined open-pond/closed PBR elongate growth period compared to the life of algae in single system PBRs. The elongation of culture period is believed to increase the lipid yield of long chains of carbon such as C18s (Miyamoto, 1997).

1.5.4 PBR ADVANTAGES

An earlier study done by NREL in collaboration with the United States Department of Energy (USDOE) showed that microalgae can yield about10, 000 gallons of biodiesel per

acre per year (Brown et al 1993). This estimate was based on algal cultured in open-ponds; consequently, the use of PBRs could even yield higher volume of biodiesel. The US alone consumes about 60 billion gallons of diesel per year. The production of an equivalent amount of biodiesel will require a significant amount of algal biomass rich in oil, which can only be achieved with a well-designed PBR for any algal culture. The detailed advantages of PBRs are further discussed in Chapter 2, Section 2.2.

1.6 **NUTRIENTS**

Each algal strain grows in a specific medium based on its ability to tolerate salinity, acidity or alkalinity. Algal media vary widely from fresh water with low salinity to medium highly concentrated in salt ions (Terry et al., 1986). For instance, the majority of Chlorella strains grow in fresh medium whereas Diatom strains (such as Thalassiosira) and some Chlorella termed Salina grow in medium with high salt concentration.
The three key nutrients that influence algae growth and lipid production are nitrogen, phosphorus and carbon. The metabolism of microalgae is strongly affected by limitation of these nutrients. Detailed of the nutrients used in this project are further discussed in Chapter 3, Section 3.2.

1.7 **MOTIVATION**

1.7.1 OPEN POND VERSUS PHOTOBIOREACTOR

In the United States (US), biodiesel is mostly produced from soybean oil. If the entire production of soybean is allotted to the production of biodiesel, it would cover only 6% of the US demand in diesel estimated at 60 billion gallons per year (Mousdale, 2008). Clearly, there is an imperative need for profound research on alternative and renewable source of raw material for biodiesel production such as microalgae cultivated in a PBR.

Due to their low costs, open ponds are widely used to phototrophically culture microalgae for biodiesel. Open-ponds are normally designed to be 30cm deep. Light penetrates roughly 3.5cm from the top. This means that 80% - 90% of the volume (dark zone) is not used for photosynthesis. Hence, they offer limited surface exposed to sunlight. In addition, they have poor mixing mechanism resulting in poor circulation of algal cells from dark zones to area exposed to sunlight. As a result, Open Ponds have a low daily productivity; roughly 0.12 kg of algae per m^3 per day (Chisti, 2007). The design of tubular PBR for microalgae culture is motivated by the incapacity of raceway ponds to grow algae rich in lipid at high growth rate in a short period of time. A grown algal biomass is to be used as a source of algae oil which is converted into enough biodiesel capable to replace petrodiesel.

The co-current flow of air/carbon dioxide in a tubular PBR facilitates culture mixing with long residence time during which algae cells capture CO_2 and multiply through photosynthesis. Different forms of tubular PBRs such as horizontal, inclined, serpentine and conical have been studied with satisfactory results (Ugwu et al., 2007). However, none of these studies have addressed all characteristics of algal cultures. It is important to conduct a study on a circular tubular PBR in order to assess algal productivity and the quality of the resulting algal oil/biodiesel.

1.7.2 TWO-STEP BIODIESEL PRODUCTION VERSUS IN SITUPROCESS

Algal culture is harvested after a broth reaches the stationary phase. This is characterized by a cessation of cells growth due to numerous factors such as insufficient photonic energy, excess dissolved oxygen (DO), insufficient nutrients and so on (Ugwu et al., 2007; Chisti, 2007; Ferrentino, 2007). After harvesting, algal biomass is dewatered by centrifugation then dried. Algae lipid (oil) is then extracted from dry algal biomass as shown in Figure 1.4. The algal lipid can be converted into biodiesel through transesterification as shown in Reaction 1.1. This two-step biodiesel production increases production cost, which will make the biodiesel price noncompetitive with petrodiesel.

The extraction of oil from any crops, algae included, requires significant amount of a relatively volatile solvent such as hexane. Hexane is widely used in the solvent extraction of vegetable oils. However, it has several disadvantages: It is a petroleum product that has become very expensive and may be of limited availability in the future;

it is extremely flammable and forms explosive mixtures with air; hexane vapors are toxic, recovery of hexane from oil and meal is energy intensive; and recovery of hexane during the conventional extraction process is incomplete, so some of the solvent must be continuously replaced. Clearly combining the oil extraction and transesterification steps into an integrated or in situ step will improve the biodiesel production economics.

1.7.3 TECHNICAL CHALLENGES

The following technical issues need to be addressed: the selection of the proper algae strain to be grown and harvested, the optimum harvesting time, the production of the dry biomass from the grown algae, the extraction of the algae lipid (oil) from algae and the transesterification of algal oil to biodiesel.

It is clear that despite the improvement in the algal biodiesel process there is a lack of basic information to make the algal biodiesel process economical. Thus there is a need to develop laboratory scale and bench scale setups to select algae and produce biodiesel

1.7.4 PRODUCTION COST

The cost of producing algae biomass and algae oil are crucial to the commercialization of algal biodiesel. Recent cost estimation of algae biodiesel production assumed:

CO_2 is available in abundance,

Dry algae contain 30% oil,

Oil recovery process is 50% of the final oil cost,

Preliminary estimates are given in Table 1.5.

Table 1.5: Algal Biodiesel cost estimate for annual dry algae production of 10,000 tons per year (Chisti, 2007)

	Raceway Ponds	Photobioreactors
Dry algae production cost $/kg	0.60	0.47
Algae oil cost $/liter	1.80	1.40
Recovered oil use $/liter	3.60	2.80

In the US the price of diesel is about $3/gallon or about $0.80/liter. The US price includes 20% (16 cents, tax), 52% (42 cents, crude oil price), 19% (refining expenses) and 9% (marketing and distribution) (Chisti, 2007). Excluding taxes and distribution, the petrodiesel price is $0.56/liter. Thus a reasonable target price of algae oil is less than $0.50/liter; for example, about $0.48/liter. The main challenge in algal biodiesel production is to reduce the cost of production of algal oil from $2.80 to $0.48 per liter. This investigation is looking into the use of a new photobioreactor (PBR) for algae growth and using a one-step (in situ) process for algae oil extraction and transesterification to biodiesel. Figure 1.8 displays the distribution of diesel production cost.

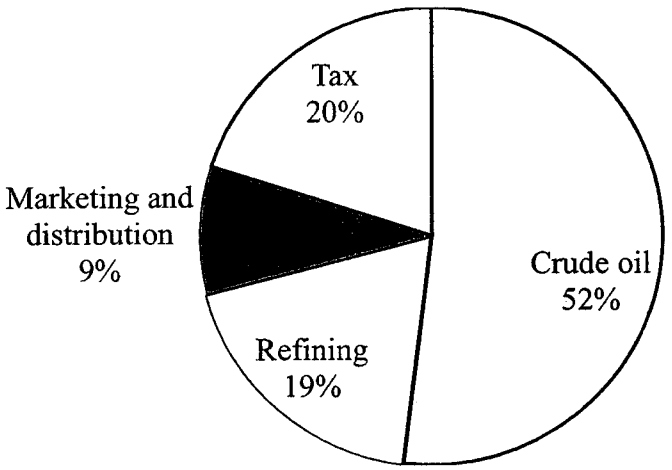


Figure 1.8: Petroleum diesel price Breakdown

1.8 **OBJECTIVES**

The goals of the present research are to design a PBR able to grow and maintain a healthy algal culture, then produce biodiesel from algal biomass through an integrated process called in situ transesterification. To accomplish these goals, the following objectives have been identified:

1. Select an algal strain based on growth rate and lipid content.
2. Select of appropriate instruments to monitor algae growth.
3. Develop a technique for harvesting algae, obtaining dry algae biomass, oil extraction and biodiesel production.
4. Design and construct a laboratory scale PBR for algal growth.
5. Assess the functionality of the PBR by culturing the selected algal strain followed by harvesting, drying, oil extraction and transesterification to biodiesel.
6. Develop a kinetic model of algae growth in batch reactor.

7. Produce biodiesel through in situ transesterification.
8. Perform lipid analysis of the resulting biodiesel.

1.9 APPROACH

1.9.1 OBJECTIVES 1 and 2: Selection of algal strain and appropriate instrument to monitor algae growth.

The first phase of this research is to select a suitable algal strain. Based on literature review, eight strains are selected and screened for high lipid content and high growth rate. These strains are cultured in 2 L-glass flask (batch PBR) and monitored over time by measuring lipid content and growth rate. A cell counting microscope and spectrophotometer are used to measure the concentration of algal broth overtime. Data obtained are used to monitor the growth rate.

The neutral lipid content for each algal culture was initially screened by Nile Red fluorescence in a spectrofluorometer (Cooksey et al., 1987). The Nile Red fluorescence technique only shows variations of lipid content within a culture, but it does not quantify the amount of lipid produced per broth. To quantify algal lipid per broth, the lipid content of algal cultures in PBR is measured by lipid extraction using Soxhlet extractor. Measurement details of this process as well as lipid content and growth rate measurement are further described in Chapter 3, Section 3.2 and Chapter 4, Section 4.2.

1.9.2 OBJECTIVE 3: Harvesting, oil extraction and biodiesel production

Once an algal broth reaches a stationary phase, it is then harvested by adding aluminum sulfate ($Al_2(SO_4)_3$) as flocculent, which precipitates algal cells. These cells are then separated from liquid by centrifugation, then vacuum dried by lyophilization. In this phase, an alternative harvesting process is explored to avoid the use of flocculent by direct centrifugation. Algal oil is extracted from lyophilized algae (dry algae) using a mix of polar and non-polar solvents: chloroform and methanol respectively. These two solvents have been replaced by normal hexane, which allows algal oil extraction within 5 to 6 hours using a Soxhlet extractor with no risk of transesterification at this stage. After solvent evaporation, the remaining algae oil is further converted into biodiesel through the transesterification reaction. Details of these four processes (harvesting, drying, oil extraction and biodiesel production) are given in Chapters 3, Section 3.2 and Chapter 4, Section 4.5.

1.9.3 OBJECTIVE 4: Design and construct a PBR

The focus of the second phase of this research is the design and construction of a PBR. This closed system consists of a 5 gal tank (main tank) connected to the PBR. The PBR consists of 150 ft Polyvinyl Chloride (PVC) tubes (ID = ¾ in) set in spiral (circular), which gives the nomenclature of tubular PBR. An inoculated medium is introduced to the reactor through the main tank, and then pumped through the tube along with air/carbon dioxide in a co-current flow during which algal cells capture photonic energy

(from a series of fluorescence lamps). The algae cells undergo photosynthesis and convert captured carbon dioxide and photons into carbohydrates, mainly lipid.

1.9.4 OBJECTIVE 5: Assess the functionality of the PBR by culturing the selected algae strain

In the third phase of this research, the selected strain is cultured in the PBR to evaluate its functionality in growing and maintaining a healthy culture. Algal concentration, pH and nutrient concentration of all cultures occurring in the PBR are measured over time using a spectrophotometer or microscope, strip pH paper, and colorimetric paper respectively. The details of measurement techniques are explained in Chapter 3, Section 3.2 and results are displayed in Chapter 4, Section 4.4.

1.9.5 OBJECTIVE 6: Kinetic study of batch PBR

The variation of culture concentration is monitored overtime using microscope or/and spectrophotometer. Data obtained are used to develop a kinetic model based on existing models such as the Monod equation. Details of the model are discussed in Chapter 2, Section 2.4 and Chapter 4, Section 4.5.

1.9.6 OBJECTIVE 7: In situ transesterification

The fourth phase of this research consists of production of biodiesel through the transesterification of algal oil while it is being extracted from algal biomass. This

combined process is known as in situ transesterification (Ferrentino, 2007; Mulumba, 2009; Haas and Scott, 2007). The in situ transesterification is done in a batch reactor where a sonicator breaks the walls of algal cells to release algal oil. This oil reacts with methanol in the presence of a catalyst such as potassium hydroxide (KOH) or sodium hydroxide (NaOH) to produce biodiesel (Schafer, 1998). The efficiency of the reaction as well as the amount of biodiesel produced are measured using a gas chromatographic (GC). This process is also compared to the traditional two-step process. Further details of this procedure and measurement technique are discussed in Chapter 3, Section 3.2 and Chapter 4, Section 4.6

1.9.7 OBJECTIVE 8: Lipid analysis of the resulting biodiesel

A sample of transesterified algal oil is analyzed using a Gas Chromatograph (GC). A calibration curve is generated to establish calculations basis for all FAMEs (biodiesel) compounds present in algae cultured in this project. The procedure for lipid testing using GC is further discussed in Chapters 3 and 4.

The four phase approach is summarized in Table 1.6.

Table 1.6: Project approach summary

Phase	Objectives	Descriptions
1	1,2,3 & 4	Algal strain selection and growth monitoring. Algae harvesting, oil extraction and biodiesel production
2	4	PBR design and construction
3	5 & 6	Algae culturing in PBR. Kinetics model development
4	7 & 8	In situ transesterification Lipid analysis by GC

CHAPTER II

LITERATURE REVIEW

2.1 MICROALGAE

2.1.1 INTRODUCTION

Microalgae are a single cell photoautotrophic or photoheterotrophic microorganisms that capture carbon dioxide and photonic energy to convert them into carbohydrates (mainly lipid) (Richmond, 2000). Photoautotrophic algal cells are like plants due to their ability to grow through photosynthesis process similar to regular plant. However, they have a simple structure compared to regular plant. In this investigation, our focus is on photoautotrophic algal cells.

Algal cells are aquatic organism. They grow well in water rich in nutrient in the presence of $CO_2$and photons. An additional advantage of algal cells is the high ratio body surface to volume. This latter advantage gives them the ability to consume enough nutrients during photosynthesis process and produce oil approximately 30 times more than terrestrial plants such as corn, soybean and canola (Khan et al., 2009). One the other hand, the production cost of algal oil (lipid) is extremely high compared to the common crop oils such as corn, soybean or canola oil. Microalgae play essential roles in aquatic

animals and plant. Marine microalgae are the major source of food and oxygen for aquatic organisms including human on the earth (Rao, 2006). Recently, microalgae have been targeted as the major source of oil that can be converted into transportation fuel able to compete with petrodiesel in quantity and quality.

2.1.2 CLASSIFICATION

Microalgae can be classified based on their pigmentation, growth conditions, cells wall structure and flagellation. There are six phyla of algae: cyanobacteria, green algae, red algae, diatomaceae, Eustigmatophytes and Prymnesiophyceae. A brief description of each phylum of algae is given in Table 2.1.

Table 2.1: Classification and description of microalgae phyla (Rodolfi et al., 2009)

Algal Phylum	Description
Green algae or Blue-green algae	Algal cells have green chloroplast that contains chlorophyll a and b. These cells have mitochondria. Some species have flagella.
Red algae or Rhodophyceae	Cells have chloroplast with chlorophyll a and d, and phycobillins. They have double cell wall, but do not have centrioles and flagella.
Cyanobacteria	Class of prokaryotic cells that contain chloroplast with no chlorophyll. These are bacteria; but they are assimilated to algae due their growth through photosynthesis process similar to microalgae. Some strains can grow in soil, marine or fresh water.
Eustigmatophytes	Class of eukaryotic algae which contain yellow-green chloroplast. They include strains growing in marine, freshwater or solid medium such as soil. Algae in this class have chloroplast containing chlorophyll a.
Prymnesiophytes or Prymnesiophyceae	Class of algae in chlorophyll a-c phyletic line. Some strains of this group have one or two flagella. For example, Pavlova strain has smooth flagella of equal length.
Diatomaceae	Class of Bacillariophyceae. Diatomaceae cells have chloroplast carrying chlorophyll a and c. They have hard wall due to the presence of silica. Most of these cells can be found in fresh or salted sea. Majority of diatom species live in cold water.

Khan et al. (2009) and Rodolfi et al (2009) studied a variety of microalgae. Table 2.2 list the biomass productivity, the lipid content and lipid productivity for each algal strain.

Table 2.2: Lipid content and productivity of different phyla of microalgae: *Algae cultured in 250ml flask at 25°C using air as source of CO_2 and day light fluorescent lamp for in two weeks culture period* (Khan et al., 2009; Rodolfi et al., 2009)

Algal phylum	Strains	Biomass productivity (g/L/day)	Lipid content % biomass	Lipid produc-tivity (mg/L/day)	Habitat
Diatoms	Chaetoceros muelleri F/MM	0.07	33.6	21.8	Marine
	Chaetoceros calcitrans	0.04	39.8	17.6	
	P. Tricomutum	0.24	18.4	44.8	
	Skeletonoma costatum CS 181	0.08	21.0	17.4	
	Skeletonoma sp.	0.09	31.8	27.3	
	Thalassiosira Pseudonana	0.08	20.6	17.4	
Green Algae	Chlorocuccum sp.	0.28	19.3	53.7	Fresh water
	Chlorella sp. F& M-M48	0.23	18.7	42.1	
	Chlorella sorokiniana	0.23	19.3	44.7	
	Chlorella vulgaris CCAP 11b	0.17	19.2	32.6	
	Chlorella vulgaris F&M-M49	0.20	18.4	36.9	
	Scenedemus quadricauda	0.19	18.4	35.1	
	Scenedemus F&M	0.21	19.6	40.8	
	Scenedemus sp	0.26	21.1	53.9	
	Tetraselmis suecica F&M-M33	0.32	8.5	27.0	Marine
	Tetraselmis sp. F&M	0.30	14.7	43.4	
	T. suecica F&M-M35	0.28	12.9	36.4	
Prymnesiophytes	Pavlova Salina CS 49	0.16	30.9	49.4	Marine
	Isochrysis sp. M177	0.17	22.4	37.7	
	Isochrysis sp. M37	0.14	27.4	37.8	
	Pavlova lutheri CS 182	0.14	35.5	50.2	
Red algae	Porphyridium cruentum	0.37	9.5	34.8	
Eustig-mato-phytes	Nannochlorosis sp.	0.19	28.4	53	
	Monodus subterraneus UTEX	0.19	16.1	30.4	Fresh water
	Nannochlorosis sp. CS 246	0.17	29.2	49.7	Marine
	Ellipsoidion sp	0.17	27.4	47.3	

2.1.3 CHLORELLA MICROALGAE

Chlorella is a microorganism of interest in this work due its growth rate and lipid content. Chlorella can be classified as a single cell and green algae based on the presence of green chloroplast as described in Table 2.1. Chlorella cells make wide range of lipid during their growth cycle. Khan et al. (2009) and Rodolfi et al. (2009) showed that some Chlorella strains are able to yield about 44 mg of lipid per day per liter of broth as displayed in Table 2.2.

2.1.4 ALGAE GROWTH

Algal cells multiply by cell division (mitosis). During the mitosis process, algal cells are divided into two identical daughter cells. Certain strains of algae such as diatoms Chaetoceros undergo gametes fusion through syngamy followed by mitosis (Rao, 2006). It is believed that the syngamy process enhance algal cell enlargement in volume.

Algal cells capture carbon from CO_2 in an intermediate step and transform it into complex carbohydrate molecules such as lipids, which are the raw material in biodiesel production.

Algal cells are either photoautotrophic or heterotrophic. Photoautotrophic cells capture carbon dioxide and photonic energy to convert into sugars (glucose), then lipid, as shown in Reactions 2.1 and 2.2.

$$6\ CO_2 + 6\ H_2O + 8\ \text{photons} \rightarrow C_6H_{12}O_6 + 6\ O_2 \qquad \text{Reaction (2.1)}$$

$C_6H_{12}O_6 \rightarrow$ TGA (or lipid) Reaction (2.2)

Heterotrophic cells, on the other hand, use other organic compounds as source of carbon and do not undergo photosynthesis.

In photoautotrophic algae, the photosynthetic reaction takes place within the chloroplast. CO_2, photons and water are key elements in algae growth process (photosynthetic reaction). It is important to note that algal cells can tolerate CO_2 up to certain concentration. The level of tolerance depends mostly on the algal strains. Kurano and Miyachi (2004) studied the impact of CO_2 fixation in algal culture and showed that algal culture of certain Chlorella strains were inhibited by CO_2 if its concentration exceeded 5%. However, certain Chlorella strains can reach high biomass productivity with CO_2 exceeding 10%. Khan et al. (2009) showed that Chlorella Kesslerican reach high biomass productivity of 87 mg per liter per day with supplied CO_2 exceeding 10%.

2.1.5 ALGAL LIPID METABOLISM

The term lipid is often used to mean fats. However, fat is a subgroup of lipids called triglycerides. Conglomerations of different fatty acids in algae form algal lipids. Fatty acids (FAs) are long hydrocarbon chain terminated with carboxylic acid group.

Fatty acids are classified into two categories: saturated and unsaturated. Saturated FAs do not contain multiple bonded carbons whereas unsaturated FAs contain at least one

double bonded carbon. Table 2.3 gives a list of some saturated and unsaturated FAs found in algal cells.

Table 2.3: Saturated and unsaturated FAs found in Algal cells (Matsumoto et al., 2009; Singh, 2010)

Category	FA Name	Formula
Saturated	Capric (10:0)	CH_3-$(CH_2)_8$-COOH
Saturated	Lauric (12:0)	CH_3-$(CH_2)_{10}$-COOH
Saturated	Myristic (14:0)	CH_3-$(CH_2)_{12}$-COOH
unsaturated	Myristoleic (14:1)	$CH_3(CH_2)_3$**CH=CH**$(CH_2)_7$COOH
Saturated	Palmitic (16:0)	CH_3-$(CH_2)_{14}$-COOH
unsaturated	Palmitoleic (16:1)	$CH_3(CH_2)_5$**CH=CH**$(CH_2)_7$COOH (= bond at C7)
unsaturated	Sapienic (16:1)	$CH_3(CH_2)_8$**CH=CH**$(CH_2)_4$COOH (= bond at C10)
unsaturated	Hexadecadienoic (16:2)	$CH_3(CH_2)_{10}$**CH=CHCH=CH**COOH
unsaturated	Hexadecatrienoic (16:3)	$CH_3(CH_2)_4$**CH=CH**CH2**CH=CH**CH2**CH=CH**-$(CH_2)_2$COOH
Saturated	Stearic (18:0)	CH_3-$(CH_2)_{16}$-COOH
unsaturated	Oleic (18:1)	$CH_3(CH_2)_7$**CH=CH**$(CH_2)_7$COOH
unsaturated	Linoleic (18:2)	$CH_3(CH_2)_4$**CH=CH**CH_2**CH=CH**$(CH_2)_7$COOH
unsaturated	α-Linolenic (18:3)	CH_3CH_2**CH=CH**CH_2**CH=CH**CH_2**CH=CH**$(CH_2)_7$ -COOH
unsaturated	Octadecatetraenoic (18:4)	CH_3CH_2**CH=CH**CH_2**CH=CH**CH_2**CH=CH** CH_2**CH= CH**$(CH_2)_4$-COOH
Saturated	Arachidic (20:0)	CH_3-$(CH_2)_{18}$-COOH
unsaturated	Arachidonic (20:4)	$CH_3(CH_2)_4$**CH=CH**CH_2**CH=CH**CH_2**CH=CH**CH 2-**CH=CH**-$(CH_2)_3$COOH
Saturated	Behenic (22:0)	CH_3-$(CH_2)_{20}$-COOH
Saturated	Eicosapentaenoic (20:5)	CH_3CH_2**CH=CH**CH_2**CH=CH**CH_2**CH=CH**CH2-**CH=CH**CH_2**CH= CH** $(CH_2)_3$COOH
unsaturated	Erucic (22:1)	$CH_3(CH_2)_7$**CH=CH**$(CH_2)_{11}$COOH
unsaturated	Docosapentaenoioc (22:5)	CH_3CH_2**CH=CH**CH_2**CH=CH**CH_2**CH=CH**CH_2-**CH=CH**CH2-**CH=CH**-$(CH_2)_3$COOH
unsaturated	Docosahexaenoic (22:6)	CH_3CH_2**CH=CH**CH_2**CH=CH**CH_2**CH=CH**CH_2-**CH=CH**CH_2-**CH=CH**CH_2**CH=CH**$(CH_2)_2$COOH
Saturated	Lignoceric (24:0)	CH_3-$(CH_2)_{22}$-COOH

The name of each FA is followed by the total number of carbon, and total number of double bonds; for instance, (16:1) indicates FA (palmitoleic or sapienic) of 16 carbons with one double bond.

Algal cells of each strain do not contain every single fatty acid displayed in Table 2.3. Matsumoto et al. (2009) stated that some Chlorella strains contain only C16s and C18s. For example, the FAs in Chlorella sp. strain are: C16:0, C16:1, C18:0, C18:1, C18:2 and C18:3.

Lipids are either polar or nonpolar. The polar lipids have a hydrophilic end, which make them soluble in water. But the nonpolar lipids have a hydrophobic end making them insoluble in water. The latter are compounds of interest in biodiesel production because they consist mainly of triglycerides. It is important to understand the metabolism of fatty acids in algal cells during their growth cycle. A better understanding of FAs metabolism facilitates to determine the best harvesting time (BHT) which corresponds to the cessation of growth culture. Therefore, unnecessary life time for algae cells in a growth reactor can be eliminated by harvesting algal broth once it reaches the BHT.

Rao (2006) observed a major change in fatty acids during growth phase. It is believed that the first pathway of FAs formation starts with carbon block construction resulting in short chain of saturated FAs such as C6:0, C8:0 and C10:0. These short chains change overtime and become complex and saturated chain of FAs such as C16:0, C18:1, C18:2 and C18:3 (Ferrentino, 2007). On the other hand, Wood et al. (1998) investigation showed that the majority of FAs in Chlorella are unsaturated. Their investigation implies that short chains of FAs are transformed into long and unsaturated chain of FAs.

Lipid metabolism in algal cells can be affected by culture conditions such as temperature, light and medium composition. Stitt and Hurry (2002), and Smith and Morris (1980)

demonstrated that low temperatures allow cells of high plants to make significant amount of fatty acids (FAs) during their growth cycle. It can be interpolated to low organism like plant (microalgae) that low temperatures would increase lipid content in algal cells. Rao (2006) stated that algal lipid content depend only on algal strain, culture medium and light intensity. In addition, Boyle-Roden et al. (2003) denoted the effects of medium composition on algal lipid content. Boyle-Roden et al showed that changing medium composition may increase or decrease a specific fatty acid; for instance, nitrogen source in culture media modifies fatty acids (lipid) composition in Chlorella cells depending on whether the metabolism is autotrophic or heterotrophic as shown in Table 2.4.

Table 2.4: Effect of nitrogen on FAs content in Chlorella grown photoautotrophically or photoheterotrophically (Boyle-Roden et al., 2003)

Fatty Acids	Autotrophic growth		Heterotrophic growth		
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	Urea
14:4	-	-	-	-	-
16:0	29.0	22.9	24.3	23.5	21.9
16:1	1.1	-	-	-	-
18:0	1.2	1.4	2.1	2.1	1.1
18:1	11.1	7.2	18.8	19.4	12.7
18:2	20.1	32.2	17.8	24.6	25.8
18:3	37.1	35.7	36.5	30.4	37.6

Results were expressed as percentage of total amount of fatty acids in Chlorella strain. Percentage less than 1 were not reported and were represented by (-).

Table 2.4 indicates that Chlorella strain cultured photoautotrophically using a medium that contained NH_4^+ as source of nitrogen (first case) produced algal oil that had the following composition for 100 g of total FAMEs: 29.0 g of 16:0, 1.1 g of 16:1, 1.2 g of 18:0, 11.1 g of 18:1, 20.1 g of 18:2 and 37.1 g of 18:3. Changing the source of nitrogen to NO_3^-, the same Chlorella strain cultured in the same conditions produced oil that had

the following composition for 100 g of total FAMEs. 22.9 g of 16:0 with 21% decrease compared to the amount of 16:0 produced in first case, no 16:1, 1.4 g of 18:0, 7.2 g of 18:1 with 35% decrease also compared to the first case, 32.2 g of 18:2 which increased significantly (60% compared to 20.1 of 18:2 produced in first case), and a decrease of 18.3 from 37.1 g to 35.7 g of 18:3. It can be clearly seen, from Boyle-Roden study, that the source of nitrogen had an effect on lipid composition.

Algae cells contain a range of fatty acids from Myristic acid (14:0) to Docosahexanoic acid (22:6). Bigogno et al. (2002) study of lipid metabolism in algae showed that low carbon fatty acids [(10:00) to (16:3)] are produced during lag phase and at the beginning of exponential phase, which agrees with Ferrentino (2007) observation stated earlier in this Section. Bigogno et al. (2002) also stated that C18:0 and higher are produced in the exponential and stationary phases. Triacylglycerols (TGA), which are the most important compound of interest in biodiesel production, are more produced in exponential phase. In addition, Bigogno et al. study demonstrated that the lipid content in algae is proportional to the age of culture. The longer a culture last, the higher is the lipid yield. For instance, Parietochloris incisa strain produced approximately 15% of fatty acids for 12 days of culture period. But this strain can produce approximately 27% if the culture is extended to 25 days.

Bigogno et al. (2002) and Singh (2010) studied the fatty acids composition of fifty different vegetable oils. They noted that the common fatty acids found in all vegetable oils are stearic (18:0), oleic (18:1), palmitic (16:0) and linoleic (18:2), which agree with

Bigogno et al. (2002) study of fatty composition in algae. Table 2.5 shows the fatty acids compositions in different vegetable oils and including Chlorella oil.

Table 2.5: FAs composition found in different vegetable oils (Bigogno et al, 2002; Singh, 2010)

Vegetable oil	FAs composition (number of Carbon: number of double bond)									
Soybean	16:0	18:0	18:1	18:2	18:3	18:4				
Corn	16:0	18:0	18:1	18:2						
Rapeseed	16:0	18:0	18:1	18:2						
Tallow	14:0	16:0	18:0	18:1						
Canola	16:0	18:0	18:1	18:2	18:3	20:1	22:0	22:1		
Jatropha C.	14:0	16:0	18:0	18:1	18:2	20:0				
Peanut	16:0	18:0	18:1	18:2	18:3	20:0	22:0	24:0		
Chlorella	16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3	20:4	20:5

Bigogno et al. found that fatty acids composition in Parietochloris incisa is approximately the same as in Chlorella strain. Fatty acids 16:1, 16:2 and 16:3 are about 1% of all FAs present in Chlorella strains.

The absence or low percentage of C10:0, C16:1, C16:2 and C16:3 in algal cells post harvesting may indicate that these FAs undergo biochemical transformation to form FAs with long chain of carbons such as C18:0, C18:1, C18:2 and C18:3 found in the majority of algae cells. The long chains of carbon in FAMEs (biodiesel) increase the cetane number with high heat combustion. Biodiesel from Chlorella would have high cetane number compared to biodiesel produced from soybean or corn.

2.1.6 EFFECT OF LIPID COMPOSITION ON BIODIESEL

Algal lipid compositions have direct effect on biodiesel properties. The fatty acid content of the lipid affects the properties of the resulting FAMEs. Figure 2.1 and Figure 2.2 show that:

1. As the number of saturated C in the FAMEs increases the cetane number and the heat of combustion increase. For example, the cetane number of 10:0 is 47.7 while for 18:0 is 86.9 as shown in Figure 2.1. Biodiesel with high cetane number is a better quality one. A cetane number > 40 indicates a good quality of fuel engine with high heat combustion.
2. The presence of unsaturated bonds in the FAMEs lowers the cetane number considerably. For example the cetane number of 18:0, 18:1 and 18:3 are 86.9, 47.2, 28.5 and 20.6 respectively. Hence the presence of unsaturated FAMEs lowers the quality of the biodiesel by decreasing the gel point for instance.

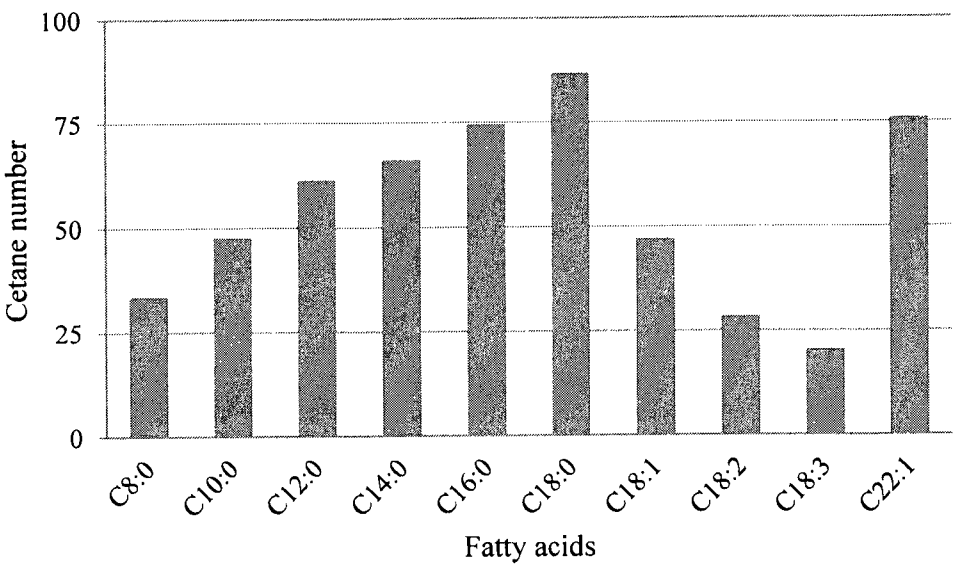


Figure 2.1: Variation of cetane number with fatty acids (Briggs et al., 2004)

However Figure 2.2 shows that the cloud point (in °C) or gel point of the FAME decreases as the number of unsaturated FAs increases. Lower cloud and gel points are highly desirable in cold climate like New England.

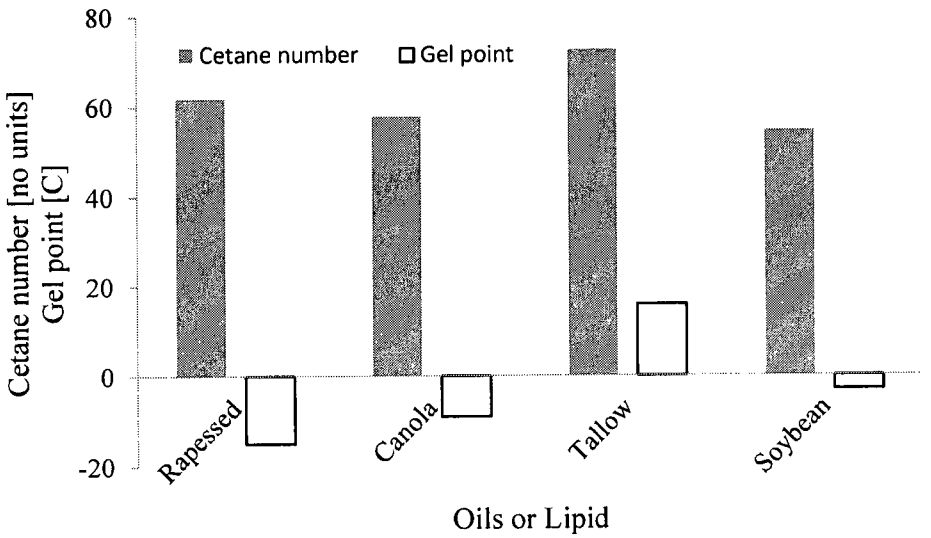


Figure 2.2: Variation of cetane number and gel point in vegetable oil. (Briggs et al., 2004)

2.2 PHOTOBIOREACTIOR

2.2.1 INTRODUCTION

Photobioreactors (PBRs) have been defined in Chapter 1 as any transparent, closed container that incorporates photonic energy to grow microorganism such as microalgae. PBRs type and shape have a significant role on the microalgae culture growth. The type or shape of PBRs can mitigate or eliminate culture contamination preventing culture

collapse, increase algal growth rate enhanced by the surface exposed to light, and provide efficient mixing in comparison to open-ponds.

2.2.2 PBR TYPES

Numerous types of PBRs have been designed in recent years and the most known are: tubular, flat panel, and column (Eriksen, 2008). These PBR are discussed in Table 2.6.

Table 2.6: Description of three most known PBR (Chisti, 2007; Eriksen, 2008)

PBR Type	Description
Tubular	TPBRs are composed of clear (transparent) tubes mounted horizontally, inclined or helical. A mechanical or airlifting pump is used to enhance Algal broth circulation in tubes. Broth is homogenized by pump shears and gas (CO_2 and O_2). The length of tubes is limited to avoid excess dissolved oxygen DO (O_2 poisoning) and CO_2decrease (CO_2 starvation). On the other hand, a long residence time, in a co-current flow, allows CO_2 to be in contact with algal cell for photosynthesis reaction. TPBRs offer large surface area exposed to light compared to open-pond or cylindrical reactor.
Flat panel	Flat panel photobioreactors consist of rectangular flat panel made with transparent material such as PVC or glass sheet to maximize light penetration. Several panels may be used to mount a reactor. They are most of the time set in series. Broth flows in thin layer across the panel or panels to ensure efficient mixing. One of the advantages of flat panel is that dissolved oxygen in flat panel is lower compared to the level of dissolved oxygen in a tubular reactor. It is desirable to design flat panel with thin panel because thicker panel cause light limitation resulting in culture collapse. Another advantage of flat panel is that cultures reach high density as a result of high growth rate.
Column	Column PBR consist of cylindrical tank (stirred tank) or bubble columns set vertically. In some cases, these are constructed with split cylinder or draft tube. The walls of the tank or columns are transparent as in any other PBR to enhance light penetration for photosynthesis reaction. The aeration (gas) is supplied at the bottom of the tank or on the impeller. It is important to note that column PBRs offer efficient mixing mechanism resulting in high growth rate.

PBRs for microalgae growth are mostly designed in a way to increase the distance of light penetration in algal broth as well as the area exposed to light rays in order to maximize photosynthesis process. On the other hand, light does not have any effect on microalgae strains that grow in the dark (heterotrophic cells).

All three types of PBR discussed in Table 2.6 offer considerable area exposed to light, which facilitate the photosynthesis process. Moreover, these three types of PBR offer good mixing mechanism compared to Open-Pond. The residence time for carbon dioxide is much longer in PBRs than open-pond, allowing a good absorption of carbon dioxide by algal cells.

All PBRs described in Table 2.6 can be illuminated with natural light (sun light) or artificial light (mostly fluorescent lamps). The light bulbs can be installed outside or inside of a PBR depending on its shape and size. It is believed that internal lighting offer maximum illumination to algae cells.

2.2.3 PBR ADVANTAGES AND LIMITATIONS

Each type of PBR offers advantages and limitations. The following lines give advantages and disadvantages of most known PBRs (Eriksen, 2008; Ugwu et al., 2007):

2.2.3.1 Open-Ponds

Raceway open ponds offer low cost for manufacturing and maintenance. They are easy to clean after each culture (Ugwu et al., 2007). The production cost for algae biomass ranges between $8-15 per Kg of dry biomass (Lee, 2001). However, they use large space

and expose algae to the surroundings with high risk for culture contamination. Control of culture growth conditions is limited, and water evaporation is a challenge. In addition, algae cells in open ponds are poorly exposed to light resulting in low biomass productivity of approximately 25 g/m^2-day compared to 72.5 g/m^2-day in an inclined TPBR made with tube of 2.5 cm internal diameter ID (Lee, 2001). A typical Raceway open pond is shown in Figure 2.3.

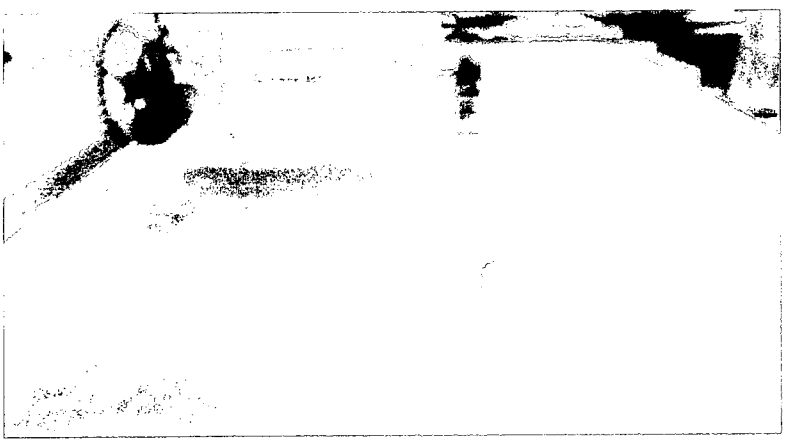


Figure 2.3: Raceway Pond for algae culture (Huesemann et al., 2009)

2.2.3.2 Tubular PBRs

Tubular PBRs are suitable for outdoor and can be used indoor with artificial illumination such as fluorescent lamp. Also they offer much larger illumination surface area compared to flat panel or column PBR. In addition, they are relatively cheap compared to flat panel PBRs. However, poor mass transfer between nutrient in medium and algal cells is the major limitation in tubular PBRs. Non-dissolved CO_2 and O_2 can be accumulated along the tubes resulting in culture growth inhibition (Richmond, 2000). Tubular PBRs offer a good mixing mechanism. Nevertheless, if the thin layer at the wall of the tubes is in laminar regime, cells may grow on the wall. This is undesirable because

the wall cells reduce light exposure and are hard to harvest. Further details of tubular PBRs are given in Section 2.2.4.

2.2.3.3 Column PBRs

Column PBRs offer smaller surface area exposed to light in comparison to the tubular PBR. The manufacturing of Column PBRs requires complex material such as glass, or acrylics which increase the cost of production (Chiu et al., 2009). Also, scale up of column PBR decreases the ratio of illumination surface area to the volume of the reactor. Column PBRs produce higher yield of algae biomass compared to open ponds, as shown in Table 2.7, and offer less space compared to open pond, Flat Panel or TPBR.

Table 2.7: Outdoor algae productivity versus PBR types (Lee, 2001)

Type of PBR	Size	Algae strain	Productivity (g/L/day)	Productivity $g/m^2/day$	Location
Open ponds	Depth: 13-15 cm	Spirulina platensis	0.18	27.0	Israel
	Depth: 1 cm	Chlorella sp	2.50	25.0	Czech
Horizontal TPBR	Tube ID: 2.5 cm	Spirulina platensis	1.60	27.8	Israel
Inclined TPBR	Tube ID: 2.5 cm	Chlorella pyrenoidosa	2.90	72.5	Singapore
Vertical Coil PBR	Tube ID: 2.4 cm	Tetraselmis chuii	1.20	-	Australia
Vertical Column PBR	Col ID: 2.6 cm	Isochrysis galbana	1.60	-	Israel
Flat Panel PBR	Width: 3.2 cm	Spirulina platensis	0.80	24.0	Italy

2.2.3.4 Flat Panel PBR

Scale up of flat panel PBRs is more complex compared to previous reactors. It requires additional compartments and widening of panel thickness. In addition, thicker panel

decrease light penetration distance, which may result in low yield of algal biomass. Flat panel PBRs offer large illumination surface area compared to open pond, and low oxygen buildup. These reactors enhance high biomass productivities. In addition, they are easy to clean and can be used indoor or outdoor (Ugwu et al., 2007). A sample of flat panel PBR is displayed in Figure 2.4.

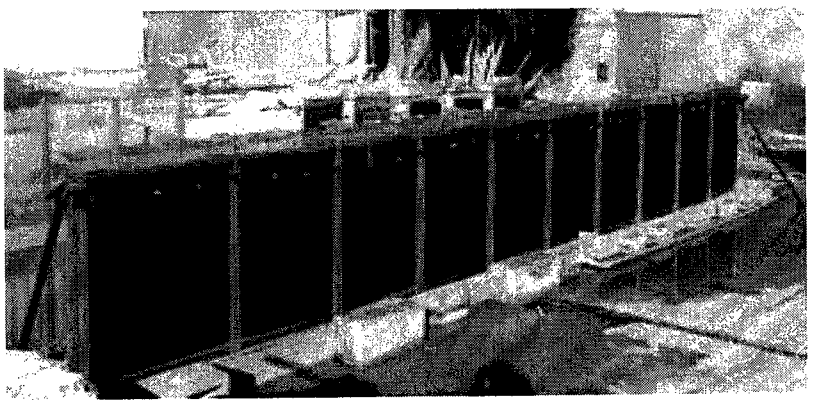


Figure 2.4: A 500L-Flat panel PBR, The Jacob Blaustein Institute for Desert Research laboratory (Richmond, 2000)

2.2.4 TUBULAR PHOTOBIOREACTOR

Tubular photobioreactors (TPBRs) are among the best PBRs for algae culture. TPBRs can be used indoor or outdoor with possibility of switching from natural photonic energy (sunlight) to artificial photonic energy using light bulbs or fluorescent lamps, for instance.

A TPBR is constructed with transparent tubes made with polyvinyl chloride (PVC)/plastic or glass. An air-lifting pump or a mechanical pump with low shear stress is used to propel broth culture into tubes. Mechanical pumps with high shear stress are avoided for microorganism cultivation because they can cause significant damage to algal

cells and prevent healthy culture (Chisti, 2007). As noted in Section 2.2.1, a tubular PBR presents a large surface area exposed to light.

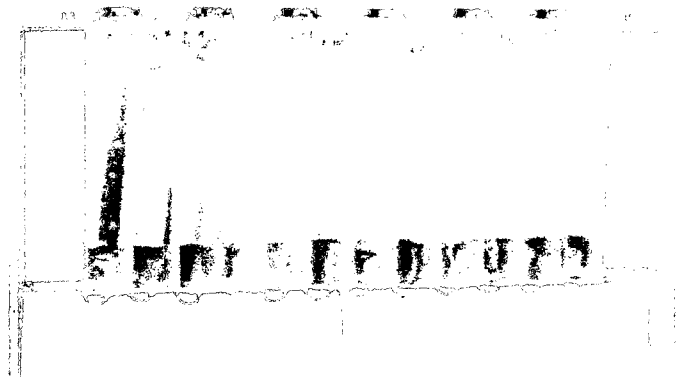
Tubes orientation depends on the manufacturer. The following orientations are the most applied to TPBR and they attribute their names to the PBR: horizontal, serpentine, vertical and inclined (Ugwu et al., 2007; Chisti, 2007; Lee, 2001). These TPBRs are described in as follow.

a. Horizontal TPBR consists of horizontal, straight and transparent tubes connected by U-bends. The tubes are made in PVC or transparent plastics. It is preferable to have tubes in glass; but glass-tubing present a breaking risk and their cost is higher compared to PVC. An example of a horizontal TPBR is displayed in Figure 2.5.

http://www.oilgae.com/ref/glos/tubular_photobioreactors.html

Figure 2.5: Horizontal TPBR

b. Vertical and serpentine TPBR are similar to horizontal TPBR, except that tubes are set vertically (as shown in Figure 2.6) or serpentine.



http://www.oilgae.com/algae/cult/pbr/typ/tub/tub.html

Figure 2.6: Vertical TPBR (oilgae club)

c. α-type of TPBR consists of combined horizontal and crossed tubes arranged in certain angles. The orientation of the tubing in α-type TPBR allows good liquid flow throughout the tubing and an excellent exposure to the light rays for photosynthetic reaction.

d. Coiled TPBR is composed with flexible tubes mounted in spiral around a vertical cylindrical frame as shown in Figure 2.7. Coiled TPBR presents an advantage of being illuminated from inside and/or outside the cylindrical frame. This advantage cannot be done with horizontal or vertical TPBR.

Figure 2.7: Coiled/helical TPBR from Murdoch University in Australia (Chisti, 2007)

It is important to note that sizes and orientations of tubes affect the growth of cells culture. Chisti (2007) studied the effect of tubes length on the health of broth culture. He demonstrated that the maximum rate of dissolved oxygen (DO) can reach 10 $g/m^3/min$ in a TPBR. Certainly, the level of DO in a TPBR is proportional to the sizes of tubes: both the tube diameter and the length. Thus, it is necessary to incorporate a degasification mechanism along tubes on a TPBR preventing excessive DO. The TPBR constructed in this work has a degasification mechanism placed at the cover of the main tank as explained in Chapter 3, Section 3.2.

2.3 TRANSESTERIFICATION OF ALGAL LIPID

2.3.1 CONVENTIONAL TRANSESTERIFICATION

In 1900, a French automobile company Otto used crude or virgin vegetable oil (lipid) from peanut to run a diesel engine at the Paris auto exposition (Knothe et al., 2005). Otto showed clearly that vegetable oil can be used as transportation fuel in diesel engines without modification. Virgin vegetable oil (VVO) has properties such as high viscosity that do not facilitate a proper functionality of petro-diesel engines. For example, a diesel engine can cease its normal operation due to filters clogging caused by VVO. Table 2.8 gives a list of known problems occurring in diesel engine when VVO is used without any modification.

All issues presented in Table 2.8 show that the need of transforming VVO prior being used in existing diesel engines. The common potential solution to all the issues presented in Table 2.8 is to "*transesterify vegetable oil into FAMEs or Biodiesel.*"

Table 2.8: List of problems caused VVO in diesel engines, and potential solutions (Singh, 2010)

Problems	Possible Cause	Potential solution
Short-term cold weather starting, plugging and gumming of filters, lines and injectors.	High viscosity, low cetane number and flash point, natural gums (phosphatides) and in VVO and other ash.	Preheat fuel before injection, *transesterify oil to FAMEs*; partially refine oils to remove gums by using 4 μm filter.
Engine knocking	Very low cetane number of certain oils and improper injection timing.	Adjust injection timing, use higher compression engines, and preheat fuel prior to injection. *Transesterify oil into FAMEs.*
Long-term coking of injectors on piston and head of engine.	High viscosity of vegetable oil, incomplete or poor combustion in the diesel engines.	Heat fuel prior injection, switch engine to diesel fuel when operation at part loads. *Transesterify oils into FAMEs.*
Carbon deposits on piston and head of diesel engines.		
Excessive engine wear.	High viscosity of oil, incomplete and poor combustion of vegetable oils, presence of free fatty acids VVO, and dilution of engine lubricating oil due to blow-by of vegetable oil.	Same as above row, plus increase motor oil changes, motor oil additives to inhibit oxidation
Failure of engine lubricating oil due to polymerization.	Collection of polyunsaturated vegetable oil blow-by in crankcase to the point where polymerization occurs.	Heat fuel prior injection, switch engine to diesel fuel when operation at part loads. *Transesterify vegetable oils to FAMEs*, increase motor oil changes, motor oil additives to inhibit oxidation

Potential solutions suggested in Table 2.8 indicate the need of chemical or physical transformation to adapt VVO to existing diesel engines. Chemical transformation occurs through two different reactions: transesterification reaction in which vegetable oil reacts with methanol in presence of a catalyst to produce biodiesel as shown in Reaction 1.1. Pyrolysis reaction in which vegetable oils undergo thermal decomposition or cracking in the presence of catalyst to produce FAMEs (biodiesel). Physical transformation is simply a blending of vegetable oils with petrodiesel to decrease VVO viscosity, for instance.

Transesterification is the most used method to convert vegetable oil into a convenient transportation fuel utilizable in existing diesel engines without any modifications. Transesterification reaction has been defined early in this Section as a reaction between alcohol (mostly methanol) and triacylglycerols (vegetable oil) in the presence of a catalyst as shown in Chapter I, Section 1.2. The transesterification reaction can be accelerated by an alkaline catalysts such as methoxide, NaOH, KOH or acidic catalysts such a diluted hydrochloric acid HCl and sulfuric acid H_2SO_4 (Manesh and Enayati, 2008).

The choice of alcohol type is based on availability and cost. Short chains of alcohols (C1-C4) are less dense with densities around 0.8 g/ml. They are suitable for transesterification reaction. In the United States, for instance, biodiesel producers utilize methanol because it is affordable and accessible. But in Brazil, ethanol is the most utilized alcohol in biodiesel industries due to its affordability and high accessibility to biodiesel producers (Knothe et al., 2005). Table 2.9 gives properties of most used

alcohols in biodiesel production. It is important to note that methanol has been used in the present work.

Table 2.9: Properties of alcohols used in transesterification reaction (Knothe et al., 2005)

Alcohol	Molecular weight	Boiling point [°C]	Melting point [°C]	Density [g/ml]
Methanol	32.04	65	-93.9	0.7914
Ethanol	46.07	78.5	-117.3	0.7893
2-propanol	60.10	97.4	-126.5	0.8035
Isopropanol	60.10	82.4	-89.5	0.7855
n-Butanol	74.12	117.2	-89.5	0.8098
2-Butanol	74.12	99.5	-	0.8080
Isobutanol	74.12	108	-	0.8018
Tert-butanol	74.12	82.3	25.5	0.7887

Knothe et al. (2005) noted that the presence of moisture in alcohol hydrolyzes FAMEs resulting in free fatty acids (FFA) and short (low) alcohol chains as shown in Reaction 2.2. The hydrolysis reaction between FAMEs with H_2O is complete at 99% in 4 hours at 32°C in the presence of an alkaline catalyst such as KOH and NaOH.

$$R\text{-}COOCH_3 + H_2O \rightarrow CH_3OH + R\text{-}COOH \quad \text{Reaction (2.2)}$$

or

$$\text{FAMEs} + \text{Water} \rightarrow \text{Alcohol} + \text{Acid}$$

Where R represents a FA radical.

Table 2.10 presents the advantages and disadvantages of methanol and ethanol when used as a reactant to produce biodiesel.

Table 2.10: Comparison of the two most common alcohols used in making biodiesel (Briggs et al., 2004)

Alcohol	Advantages	Disadvantages
Methanol	-Less expensive in the US. - More efficient reactant. - EPA study showed that rats can consume FAMEs with no adverse effects (Briggs et al., 2004)	- Toxic causing nerve deterioration due to prolonged exposure. -More poisonous than ethanol
Bioethanol	Environmentally safe (green chemical)	- Higher viscosity of biodiesel product. - Large scale use will require cellulose-based technology (Farell et al., 2006)
Ethanol	- Environmentally safe. - Preferred alcohol to use for cold weather operations.	- More expensive in the US. -Heavier for biodiesel - Transesterification reaction is less forgiving.

A catalyst plays an important role in transesterification reaction; it reduces the reaction time and increases FAMEs yield. The two most used catalysts in transesterification reaction are alkaline NaOH and KOH. Table 2.11 presents the advantages and disadvantages of these two alkaline catalysts.

Table 2.11: Advantages and disadvantages of NaOH and KOH in transesterification reaction (Briggs et al., 2004)

Catalyst	Advantages	Disadvantages
NaOH	- Less expensive in the US. - Useful for oil titration to check Free Fatty Acids (FFA).	- Hard to use - Does not provide a valuable byproduct
KOH	- Easier to use - Does a better catalytic job than NaOH. - Provides potash fertilizer as a byproduct.	- Use 40% more by mass than NaOH.

2.3.2 ALGAE HARVESTING

Harvesting is the process of separating algae from its growth medium. The challenge is that algae are in a dilute solution with an alga content of about 10 g/L. The high water content has to be removed to enable harvesting and oil extraction. Several processes have been developed for water removal such as centrifugation, filtration, and use of flocculent to decant algae cells followed by water removal.

Ferrentino (2007) used aluminum sulfate as flocculent for Chlorella cells sedimentation. Aluminum sulfate binds to algae cells in dilute solution and precipitates them at the bottom of a vessel creating two layers. The bottom layer contains sludge of algae. The top layer consists of clear medium with no algae. The top layer is simply discarded when using a toxic flocculent such as aluminum sulfate. However, if the separation of algae cells and medium is done by using a mechanical method (i.e., centrifugation), the top layer can be recycled for next cultures. In this work, aluminum sulfate has been used to precipitate Chlorella cells, followed by decantation and centrifugation in order to obtain Chlorella pellets. These pellets contain approximately 80% of water. These pellets are then frozen and placed into a lyophilizer for complete drying. The final product is dry algae ready for oil extraction or direct transesterification (in situ process).

2.3.3 OIL EXTRACTION

Vegetable oil is extracted from feedstock prior to be transesterified. The most used oil extraction technique consist of solvent extraction (using hexane) combined with

mechanical pressing. The same technique can be used to extract oil from dry algae. One of the disadvantages of solvent extraction technique is the release of significant amount of volatile solvent in the atmosphere which may contributes to the greenhouse effect. Ferrentino (2007) stated that approximately 4000 liters of hexane are released into the atmosphere to extract 4000 tons of vegetable oil. Certainly, oil production cost increases the price of biodiesel that makes it difficult to compete with petrodiesel price. All these elements motivate researchers to pursue alternative transesterification technique, which could potentially eliminate the oil extraction step by performing the transesterification process directly in crops or biomass. This type of transesterification process or one-step process is termed in situ transesterification.

2.3.4 INSITU TRANSESTERIFICATION

In situ transesterification (or one-step process) of oil rich crops is performed to produce biodiesel (biodegradable transportation fuel). Haas and Scott (2007) performed in situ transesterification process on soybean flakes using 0.1 N NaOH and methanol through a 16 hours reaction with 97 – 100% yield of fatty acids methyl esters (FAMEs) or biodiesel. Hass and Scott also demonstrated that in situ transesterification on flakes was efficient in a way that reduces about 60% of solvent compared to the conventional transesterification or 2-step process. They also discovered that removing moisture from soybean flakes reduces reaction time, which agrees with Knothe et al. (2005) statement.

The in situ transesterification presents a major challenge which is the release of lipid (oil) isolated in crops cells. Lipids (oils) are incorporated in cells crops (soybean, microalgae, corn, etc...). This isolation requires sophisticated techniques to release lipids or oil for the transesterification reaction to occur. Ferrentino (2007) applied ultrasonication to break algae cell-walls in order to release lipid (oil) and perform transesterification at the same time. Similar technique was used by Ji et al. (2006) to produce fatty acids methyl esters (FAMEs) from soybean flakes using methanol in the presence of NaOH as a catalyst. Ji et al experiment was carried out at 45°C using an ultrasonic set at 100 Watts. Thus the ultrasonication technique was used in this work to produce biodiesel.

2.4 KINETIC MODEL OF ALGAE

An important aspect of designing a PBR is the kinetic modeling of photosynthetic cell growth. Kinetic models or growth kinetics of algal cells are generally expressed by a rate of cell growth. A kinetic model can be affected by different parameters such as medium composition, environmental and/or growth conditions. Lee (2001) described growth kinetic as a result of several complex networks of biochemical/chemical reactions and transport phenomena involving numerous phases and multiple component systems. Monod model is a classic one, which is based on the specific growth rate (μ_{max}) of cell grown in any reactors. Equation (2.1) gives the empirical expression of the Monod equation or specific growth rate (inverse of time) (Lee, 2001).

$$\mu = \mu_{max} * \frac{C_s}{K_s + C_s} \qquad \text{Equation (2.1)}$$

Where C_s, K_s and μ_{max} represent the concentration of the limiting nutrient, the saturation constant and the maximum growth rate respectively.

However, growth limiting nutrients in culture medium have to be determined in order to use Monod model (Equation 2.1). If growth limiting nutrients are not determined, then there is a need to develop a kinetic model specific to a reactor, culture performed and strain in use.

Obviously, each growth phase has a different expression of growth rate; however, the number of cells or cells concentration in the lag and stationary phases are approximately the same resulting in negligible growth rates. Thus, the focus for a kinetic model is more in the exponential phase where cells multiply rapidly.

Huesemann et al (2009) studied biomass productivities in algae. They calculated the maximum specific growth rate (μ_{max} in 1/s) in the exponential growth phase of algae batch culture using Equation (2.2).

$$\mu_{max} = \frac{1}{\Delta t} * \ln\left[\frac{C_f}{C_i}\right] \quad \text{Equation 2.2}$$

Where Δt [s], C_f and C_i [gmol/L] represent respectively the length period of incubation time (exponential growth), the final and initial biomass concentrations over the Δt time period. Equation 2.2 is applied in exponential or growth phase only; therefore, the incubation period corresponds to the duration of exponential phase.

Huesemann et al also demonstrated that photosynthesis reaction can be a rate limiting in algae growth caused by illumination (light intensity) and carbon fixation. They demonstrated that photosynthetic oxygen evolution rate termed by P was a function of light intensity as shown in an empirical expression in Equation 2.3.

$$P = P_{max} * \tanh\left[\frac{a*I}{P_{max}}\right] \quad R_{dark} \quad \text{Equation 2.3}$$

Where P_{max}, I, R_{dark} and "a" represent respectively the photosynthetic oxygen evolution maximum rate, the light intensity, the rate of oxygen uptake in the absence of light and the initial slope of P-I curve as displayed in Figure 2.8. The initial slope in Huesemann model (Equation 2.3) indicates the photonic energy efficiency in photosynthetic activity. It indicates the minimum number of photons required to produce one molecule of oxygen during photosynthesis reaction (Huesemann, 2009).

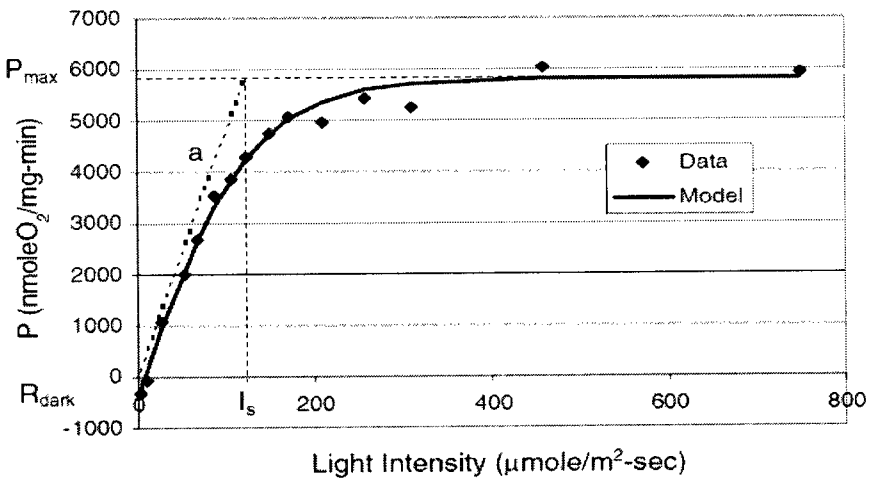


Figure 2.8: Photosynthetic oxygen evolution P versus light intensity (Huesemann, 2009)

Another kinetic model correlating growth rate to light intensity was developed by Chojnacka and Marquez-Rocha (2004). They conducted a study of photoautotrophic microalgae cultures which involved light as the source of photonic energy for photosynthesis reaction. Photosynthetic activity can be limited by light and CO_2. Light was a growth limiting in Chojnacka and Marquez-Rocha (2004) experiment. They considered light as physical substrate. Therefore, Monod model was applied in the absence of photo-inhibition to determine specific growth rate as shown in Equation 2.4.

$$\mu = \mu_{max} * \frac{I_o}{K_{I_o} + I_o} \quad \text{Equation (2.4)}$$

Where μ_{max}, I_o and K_{Io} are respectively the maximum growth rate, the incident light intensity and light saturation constant. The constant K_{Io} is expressed in the units of light intensity I_o. It can be seen that Equation 2.4 and 2.1 are exactly the same, except that light intensity I_o was substituted by substrate concentration C_s from Equation 2.1 to 2.4. If photo-inhibition is observed during photosynthetic activity, then Monod model (Equation 2.4) is replaced by Haldane model Equation 2.5 (Chojnacka and Marquez-Rocha 2004).

$$\mu = \mu_{max} * \frac{I_o}{K_{I_o} + \frac{I_o^2}{K_I} + I_o} \quad \text{Equation 2.5}$$

Where K_I represents the inhibition constant for incident light intensity. The constants K_{I_o} and K_I have the same units as I_o. All other parameters in Equation 2.5 were defined early in this Section.

In this work, growth limiting substrates were not determined. Moreover, the light intensity was found not to be limiting the photosynthetic activity in Chapter 4, Section 4.5. Therefore, Haldane, Monod and Huesemann models are not applied to microalgae culture growth performed in the TPBR constructed in this project. However, μ_{max} is evaluated using Huesemann approach in Equation 2.2, which allows calculating the culture doubling time and dilution rate.

2.5 GAS CHROMATOGRAPH ANALYSIS OF ALGAL OIL

Characterization of algal oil is needed to determine the fatty acid profile and the percentage of different fatty acids present in algal oils. Basically a gas chromatograph (GC) is used to separate the mixture of different FAME molecules based on their physical properties. A GC equipped with a narrow bore (0.1 nm ID) column (like the RTX-1 column used in our GC) is more attractive than conventional capillary column. This fast GC column provides faster analysis time and higher resolution. The Gas Chromatograph (GC) allows us to assign total carbon and total unsaturation number to each peak found in transesterified algal oil (Ferrentino, 2007)

The typical fatty acid compositions of lipids from various sources are available in the literature. For instance, Bigogno et al. (2002) studies provide composition of some lipids shown in Table 2.7. A typical GC chromatogram in lipid oil, performed in this work, is shown in Figure 2. 9.

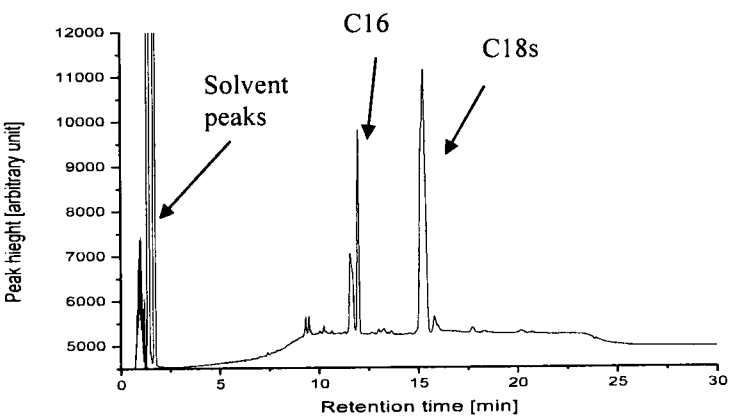


Figure 2.9: Chromatogram of transesterified algal oil

Carrapiso et al. (2000) used GC chromatogram to analyze fatty acids from Iberian pig subcutaneous adipose tissue. In their study, in situ transesterification of Iberian pig subcutaneous adipose tissue with methanol was performed to produce FAMEs. The transesterification reaction was conducted for 45 minutes using 5% HCl as catalyst to accelerate this reaction. Similar technique is used in this project to produce and analyze biodiesel from algal biomass.

2.6 CITED WORK SUMMARY

A brief summary of the literature review presented in this Chapter is given in Table 2.12.

Table 2.12: Summary of cited work in literature review

Research area	Cited work	Comment/Conclusion
A review on microalgae biotechnology	Amos Richmond 2000	Definition of microalgae
Microalgae characteristic	Khan et al. 2009 Rodolfi et al. 2009	Classification of microalgae
Biodiesel FAMEs	Matsumoto et al. 2009	Lipid composition in Chlorella
Microalgae culture and lipid metabolism	Rao 2006, Kurano and Miyachi 2004, Ferrentino 2007, Stitt and Hurry 2002, Boyle et al. 2003	Fatty acids formation: short chain of FAs are formed in earlier culture stage and long chains FA developed from short chains in latter culture stage (exponential and stationary phase)
Photobioreactor	Ferrentino 2007, Eriksen 2008, Chisti 2007, Lee 2001, Ugwu et al. 2007	Construction and classification of PBR
Transesterification of algal lipid	Knothe 2005, Manesh and Enayati 2008, Ferrentino 2007, Haas and Scott 2005, Carrapiso et al. 2000	In situ transesterification of algal biomass
Kinetic models of algae culture	Chojnacka and Marquez-Rocha 2004, Huesemann 2009, Lee 2001	Evaluation of maximum specific growth rate

The literature review supports the objectives of this research. There is a need for understanding algae culture in a PBR, developing a kinetic model of algae growth in a batch reactor, and comparing a two-step versus one-step (in situ) biodiesel production from microalgae. These are addressed in this investigation

CHAPTER III

EXPERIMENTAL PROCEDURES

3.1 MATERIALS AND REAGENTS

3.1.1 MATERIALS/INSTRUMENTS

The materials and instruments used in this work are described in Table 3.1

Table 3.1: Materials/Instruments used in this work and their functions

Material/Instruments	Functions /Comments
Bausch and Lomb Spectrophotometer	Measure the turbidity (turbidity) of algal solution
Cell disruptor sonicator	Break cells walls during in situ transesterification and provide heat to reagents for transesterification reaction
Varian SF-330 Spectrofluorometer	Measure fluorescence signal of algal solution corresponding to lipid content in algal
Vicon Sartorius Balance	Gravimetric measurement: dry algal biomass, nutrients, etc...
ALLIED-7393D Balance	
Oven: National Environment Incubator	Dry glassware and samples containing excess moisture
Corning magnetic stir Plate or Stirrer	Mix algal biomass with solvent during oil extraction and transesterification process
Water bath	Provide heat to flask during solvent evaporation
Labconco Freeze-dryer (lyophilizer)	Lyophilize slurry paste of algal cells to produce pellets
Beckmann Coulter Centrifuge (Allegra 25R)	Separate algal cells from media
Damon IEC B-20A centrifuge	

Table 3.1: Materials/Instruments used in this work and their functions (continued...)

Material	Functions/Comments
HP3396 Integrator	Collect GC raw data, integrate and plot chromatogram
Hewlett Packard HP 5890 Series II Gas Chromatograph (GC)	Data analysis
OriginPro software	Analyze GC data; adjust base, show peaks, etc...
HP Peak96 software	Transfer GC data from HP3396 integrator to PC
2 L- glass Buchner flask	Small photobioreactor to grow algal cells
Buchner funnel	liquid filtration
Cooke Light meter	Measure light intensity
250 ml glass separatory funnel	Separation of non-miscible liquid or liquid/solid
5 µL Hamilton syringe	FAME sample injection in GC
Restek RTX-1 column: 15 m, 5 µm df, 0.32 mm ID	Mounted in GC to analyze FAME compounds
Soxhlet extractor	Apparatus for algal oil extraction from dry algae biomass
Graduate cylinders	Liquid volume measurement
Brookfield viscometer	Liquid viscosity measurement
Whatman filter grade # 5, 0.25 µm	Filter samples post transesterification and during oil extraction

3.1.2 REAGENTS

Reagents used in this work and their functions are summarized in Table 3.2. Salts used as nutrients are not included in this table; they are shown in Table 3.3.

Table 3.2: List of reagents used in this work

Reagents	Use/Comments
Methanol high grade	Solvent for oil extraction and reagent for transesterification reaction
n-Hexane 98% purity	Solvent for oil extraction
Chloroform	Solvent for oil extraction and preservative for FAMEs
Potassium hydroxide KOH (pellet)	Catalyst for transesterification reaction
Hydrochloric acid HCl	Enhancement agent for oil removal during glassware cleaning
Reverse Osmosis (RO) water	Prepare nutrient solution
Aluminum Sulfate anhydrous $Al_2(SO_4)_3$	Flocculent to harvest algal broth
Sodium Sulfate Na_2SO_4	Drying agent, used to remove moisture in FAME and oil
Methyl Dodecanoate or Methyl Laurate C12:0	GC Standards for FAME analysis
Methyl Palmitate C16:0	
Methyl Stearate C18:0	
Methyl Oleate C18:1	
Methyl Linolate C18:2	
Methyl Linolenate C18:3	
Methyl Nonadecanoate C19:0	
FAME mix standard at 1000 µg/ml from SUPELCO containing the following fatty acid methyl ester: • Cis-11-Vaccinic Methyl ester (C18:1) 99% pure • Cis-9-Oleic Methyl Ester (C18:2) 99% pure • Methyl Eicosenoate (C20:1) 99.9% pure • Methyl Eicosadienoate (C20:2) 99.5% pure • Methyl Laurate (C12:0) 99.8% pure • Methyl Linolenate (C19:3) 99.6% pure.	
Methyl Cis-9-octadecenoate C19:1 at 0.874 g/ml	

3.2 **PROCEDURES**

3.2.1 MICROALGAE CULTURE

3.2.1.1 Algal Strain Selection

Eight microalgae strains were tested. Six were obtained from Professor Leland Jahnke laboratory, Plant Biology Department at the University of New Hampshire (UNH) in Durham, New Hampshire. The remaining two were purchased from the Culture Collection of Marine Phytoplankton (CCMP), Bigelow Laboratory for Ocean Sciences, Boothbay Harbor, Maine. The algae strains and their oil contents are shown in Table 3.3. These strains were selected based on information from the literature such as growth conditions capability and lipid content. The selection procedure is described in section 3.2.1.2.

Table 3.3: Summary of algal strains and their oil content (Khan et al., 2009)

Algal Strain	Oil content in %	Provider
Chlorella C2	~ 19	UNH Plant Biology Department Courtesy of Professor Leland Jahnke
Chlorella sp. (C1)	~ 19	
Isochrysis sp	22 - 27	
Thalassiosira Pseudonana	20.6	
Porphyridium cruentum	9.5	
Gymnodynium	-	
Pavlova	30 - 36	The Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP)
Diatom Chaetoceros	33 - 40	

3.2.1.2 Procedure

Each species was first inoculated in the Photosynthesis Laboratory at the UNH Plant Biology Department. These inoculations were cultured in small glass tubes of 20 - 50 ml. The growth culture for each strain was monitored by simple observation. After the inocula reached stationary phase, they were transferred to the Biodiesel Laboratory for screening.

The concentration of algal cells in each inoculum was determined before adding inocula to the media in 2 L glass flasks (small PBR). An aliquot of each strain was placed into a Bausch and Lomb spectrophotometer set at 682 nm of wave length for turbidity measurement. The turbidity or optical density (OD) or turbidity of the solution is assumed to be linearly proportional to the algae cell concentration (g of dry algal cell per liter).

The spectrophotometer (Bausch and Lomb) has a measurement limit in the range of 0.0 – 2.0. Any samples with a turbidity (turbidity) exceeding the instrument high detection limit of 2.0 was diluted with Salina solution (constituted with 0.1 M sodium chloride in water or virgin medium), and re-measured till the results fell within the instrument measurement ranges 0 - 2.0. The measured optical density was adjusted to the turbidity of the initial solution using to the dilution factor applied to each case. For example, the algae sample S1 volume is 5 ml and its turbidity is greater than 2. 1.0 ml of the sample S1 is diluted with 4 ml of Salina solution to obtain sample S2; the dilution factor in this case is 5. The resulting diluted solution S2still has a turbidity (optical density) greater than 2. 1.0 ml of sample S2 is diluted with 2.0 ml of Salina solution to obtain solution

S3. The dilution factor is 3. The resulting solution S3 has a turbidity of 0.8, which is within the instrument measurement ranges. The turbidity of the original (undiluted) algae solution S1 is calculated as follow.

Turbidity of undiluted solution S1 = (0.8)* (5)*(3) = 12

Each species of eight strains was cultured in a 2 L glass flask for two weeks. The cultures were not monitored daily. The main goal of this work was to choose a suitable strain that achieved high oil content and high yield of algal biomass within two weeks. After two weeks, broth concentration and oil content were measured using a Bausch and Lomb spectrophotometer and Varian SF-330 spectrofluorometer respectively.

An aliquot of 8 ml was placed into a Bausch and Lomb spectrophotometer set as aforementioned and the turbidity measurement was read. Then the sample was normalized to a turbidity of 0.05 with Salina solution followed by a lipid binding with Niles Red solution at 250 μg/ml in acetone as described in Cooksey method (Cooksey et al., 1987). The sample was placed into a Varian SF-330 spectrofluorometer for fluorescence reading. The spectrofluorometer reading was assumed to be proportional to the algae oil content. The Varian SF-30 spectrofluorometer was set at 525 nm for excitation wave length and 575 nm emission detector. Sensitivity and read mode were set at 100 and 0.25 as described by Ferrentino (2007). The full setting of The Varian SF-330 spectrofluorometer is displayed in Appendix I. Chlorella C2 was selected based on the combined results of oil content and algae concentration. The results are presented in Chapter 4, Section 4.2. This strain (Chlorella C2) was retained for the rest of the project.

3.2.2 TUBULAR PHOTOBIOREACTOR DESIGN

3.2.2.1 Experimental Setup

A laboratory scale tubular photobioreactor (TPBR) was designed and constructed as shown in Figure 3.1. The TPBR system consists of six main sections: 1- Tank, 2- Pump, 3- Mixing Nozzle, 4- Air/gas supply, 5- Transparent PBR Tubes and 6-Photonic source. These sections are described in this Section.

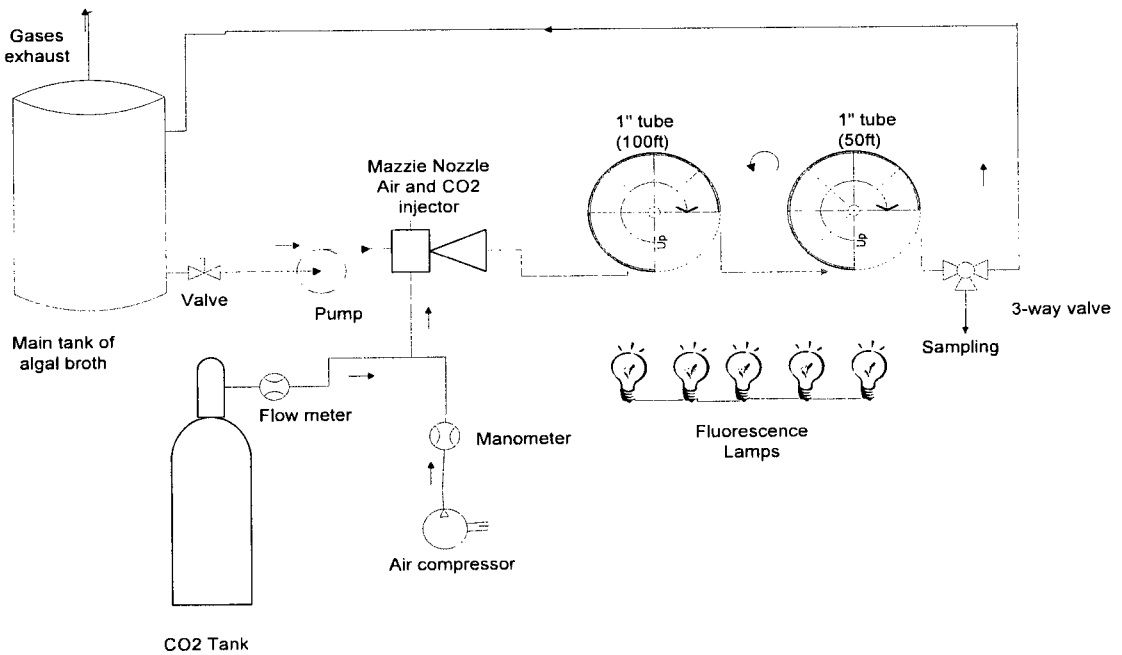


Figure 3.1: Flow chart of transparent tubular photobioreactor system

This TPBR was used to culture algal cells at high growth rate. Pictures of the constructed TPBR are displayed in Appendix II.

3.2.2.1.1 Main Tank

The main tank is a 5 gal container that can be a carboy or a bucket with cover. Both containers the 5 gal bucket and the carboy were used in this work. The 5 gal carboy was subjected of medium leaks on its connections. Thus it was discarded. The 5 gal cylindrical bucket with a cover made in clear polyvinyl chloride (PVC) was retained for the construction of the TPBR. The bucket has an external diameter of 304.8 mm (12") and a height of 368.3 mm (14.5"). It has two outlets and one inlet. The first outlet, located on the cover, serves to release insoluble gas such as CO_2, air and resulting O_2 from photosynthesis process. The second outlet located at the bottom of the bucket is connected to a compact ball valve made in PVC. Algae broth exited the tank (bucket) through this valve, which is connected to the pump by clear PVC tube 25.4 mm (1") external diameter, 304.8 mm (12") long. The medium re-entered the tank (bucket) at the inlet on the top of the tank after circulating throughout the tubular PBR.

3.2.2.1.2 Pump

A magnetic pump is installed in a line between the tank and the tubing. This line consists of a tube that has an external diameter of 25.4 mm (1") and a length of 556 mm (22"). The pump has a flow rate ranging up to 27 gal per minute, with a maximum head of 8383 mm (27.5') and a maximum pressure of 75 psi. The pump is powered up by 115V/2-1.6 Amp and 50/60Hz motor equipped with a vented shaded pole to prevent rising temperature. This pump has low shear stresses which prevent algal cells damage. The magnetic pump is manufactured by Gorman Rupp Industries (GRI), model 14100-015. It is important to note that pump flow rate is fixed; but a one-way valve is installed at the pump entrance point to regulate the liquid flow and direct the medium into the pump.

Since volume of the liquid in the main tank is 4 gal, the total volume in the system is 7.5 gal. At any moment 3.5 gal out of the 7.5 gal are exposed to the light or 47% of time, which is equivalent to 10 hours per day.

3.2.2.1.3 Mazzei Nozzle

The Mazzei nozzle was installed in the line to diffuse gas (air/CO_2) flow into medium line. The Mazzei nozzle is a high-efficiency, venture-type made in polypropylene (PP), differential pressure injector with internal mixing vanes. It creates a vacuum inside the injector body due to a sufficient pressure difference between the inlet and outlet ports (*www.Mazzei.net*, Mazzei Injector Company). Gas velocity and pressure in/out of the nozzle were not of interest in the present work. Thus they were not measured.

3.2.2.1.4 Gas Supply

Compressed air is introduced into the reactor via the Mazzei nozzle. A pressure gauge is installed in the air/CO_2 line to measure the air pressure entering the reactor. The operating gas pressure range is determined to insure parallel flow of air and culture medium. If the air pressure is too low, it will not enter the PBR. If the air pressure is too high, it will prevent the culture medium from entering the PBR. In both cases the algae will not grow. The operating pressure is determined by trial and error, varying the pressure gauge and observing the flow in the TPBR. The results of the gas pressure optimization are included in Chapter 4, Section 4.3. The picture of the pressure gauge is displayed in Appendix II.

Carbon dioxide was also supplied from CO_2 gas cylinder connected to the reactor in attempt to maximize photosynthesis process. However, it was observed that the

supplement CO_2 decreased the growth rate of the culture. Therefore the line supplying neat CO_2 was closed for the selected strain. However, the design allows the option of adding supplement CO_2 gas when using any strains that require an excess of CO_2. It is important to note that the compressed air contained about 300 ppm of CO_2. This amount of CO_2 in compressed air is sufficient to provide carbon needed for photosynthesis process.

3.2.2.1.5 Clear Tubing for Photobioreactor

Clear polyvinyl chloride (PVC) tube of 25.4 mm (1") external diameter (OD), 19.1 mm (3/4") internal diameter (ID) and 45.72m (1800" or 150') length is used to circulate algal broth throughout the reactor. These tubes consist of three pieces of 15.24 m (600" or 50') each connected each other with PVC female connectors of 19.1 mm ID, strengthened with clamps. These tubes can hold a total volume of 13.1 L (3.5 gal).

3.2.2.1.6 Photonic Energy Source

A total of 32 Phillips fluorescent lamps (J-12, 40W each) were used to supply photonic energy to the tubing. The PBR tubing is mounted on plywood (60 ft x 180 ft) painted in white to reflect the photon flux and maximize photonic energy turbidity. Light intensity was measured at the center and edges of the plywood using Cooke light meter. The measurement data are presented in Chapter 4, Section 4.3.

3.2.2.2 Batch System

A culture medium is manually transferred into the main tank. The pump is started immediately to circulate the medium throughout the tubing. The air, which contains CO_2,

nitrogen and oxygen, flows co-currently with medium through the entire cycle and go back into the main tank. Non-soluble gas (air and O_2) is separated from medium in the main tank and exit the tank via an outlet located at the tank top cover. The medium is pumped back into the reactor to continue the cycle. Before adding inoculum, the medium and air are circulated throughout the reactor for 1-2 hours to allow a complete dissolution of nutrients with the light source on. The light can be kept off without impacting nutrients dissolution.

Then inoculum is added to the medium in the main tank. The culture is then cycled throughout the reactor where algal cells are exposed to the photonic energy. Algal cells capture CO_2 and photon, and convert them into carbohydrate (lipid) through photosynthesis as shown in Reaction 2.1 and 2.2. It is important to note that the inoculation is done at 1:10 volumetric ratio. For instance, a batch of 20 L start with 20 L of medium prepared in a separate vessel (5 gal carboys) and poured into the reactor main tank. The medium is inoculated with 2 L of Chlorella Salina at 1 g/L.

3.2.2.3 Reactor Characterization

The fluid flow throughout the TPBR is characterized using water in lieu of the medium and air as source of CO_2 and O_2. The TPBR was tested to assess its stability and functionality. Several parameters were determined during the testing: flow velocity, air supply pressure, Reynolds number, and medium flow rate. It was determined that the maximum density of Chlorella medium used in this work was approximately the same as the density of water. Thus water was suitable for reactor testing process instead of medium. The testing of the TPBR with water should show the same TPBR functionality and performance as using algae solution.

3.2.2.3.1 Flow Rate

Water is pumped into the reactor at constant rate and circulated throughout the tubing. A3-way valve set in the liquid flow line is opened to fill a 2 L graduate cylinder with water. The filling time is recorded for each trial or filling. After performing several trials, an average time is estimated to calculate the flow rate using Equation (3.1). Results are displayed in Chapter 4, Section 4.3.

$$\text{Flow rate [L/s]} = \frac{2\ [\text{L}]}{\text{average time [s]}} \qquad \text{Equation (3.1)}$$

3.2.2.3.2 Air/Carbon Dioxide measurement

Air containing carbon dioxide is supplied to the reactor. A manometer is installed in the air supply line to measure air pressure before entering the reactor. The air pressure is varied by closing or opening the gas valve, which changes the air pressure. Results are shown in Chapter 4, Section 4.3.

3.2.2.3.3 Medium Velocity

Blue dye was introduced as a tracer into the water circulating throughout the reactor to measure liquid flow velocity. A 4 ft (1.2 m) portion of tubing that connected the two spiral parts was used to measure the liquid flow velocity. Time was recorded when blue dye passed the initial and end point of the1.2 m section. Average time was estimated and used in Equation (3.2) to calculate the liquid flow velocity (practical liquid flow velocity).

$$\text{Velocity [m/s]} = \frac{1.2\ [\text{m}]}{\text{average time [s]}} \qquad \text{Equation (3.2)}$$

This technique assumes highly turbulent flow so that the liquid has a uniform velocity profile at each point inside the tubing.

The liquid flow velocity can also be calculated using Equation 3.4.a (theoretical liquid flow velocity) from the flow rate measurement and the inside diameter of the tube d= 19.05 mm (¾").

$$v = \frac{q}{A} = \frac{\text{flow rate}}{\text{cross section area of flow}} \quad \text{Equation (3.4.a)}$$

Equation (3.4.a) becomes Equation (3.4.b) after substituting A by its expression ($\pi d^2/4$).

$$v = \frac{4q}{\pi d^2} \quad \text{Equation (3.4.b)}$$

Results are displayed in Chapter 4, Section 4.3. The results of liquid flow velocity calculated in Equation 3.2 can be compared to the results from Equation 3.4.b to assess the difference between theoretical liquid flow velocity and really or practical measurement.

3.2.3 CULTURE OF CHLORELLA IN TPBR

The selected strain of Chlorella was grown for an average of 2 weeks in 2 L flask using micro and macro-nutrient solution shown in Table 3.4. The culture served as inoculum for large culturing in the TPBR.

Table 3.4: Growth Medium Recipe

Chemical	Formula	Molecular weight	Required Concentration		Example of 20 L medium
Macro-nutrients					
Calcium Chloride	$CaCl_2*2H_2O$	147	0.2 mM	29.4 mg/L	588 mg
Boric Acid	H_3BO_3	62	0.13 mM	8.0 mg/L	160 mg
Potassium Nitrate	KNO_3	101	5.2 mM	525.2 mg/L	10,504 mg
Magnesium Sulfate	$MgSO_4$	121	5.0 mM	600 mg/L	12,000 mg
Sodium Phosphate	Na_2HPO_4	142	0.4 mM	76.8 mg/L	1,536 mg
Sodium Chloride	NaCl	58	0.1 M	5800 mg/L	116,000 mg
Micro-nutrients					
EDTA (Ethylenediamine Tetra acetic Acid)	$C_{10}H_{16}N_2O_8$	292	26.9 mg/L		538 mg
Ferrous Sulfate	$FeSO_4*7H_2O$	278	2.8 mg/L		56 mg
Zinc Sulfate	$ZnSO_4*7H_2O$	287	0.288 mg/L		5.76 mg
Molybdenum Oxide	MoO_3	144	0.125 mg/L		2.5 mg
Copper Sulfate	$CuSO_4*5H_2O$	250	0.075 mg/L		1.5 mg
Cobalt Chloride	$CoCl_2*6H_2O$	238	0.025 mg/L		0.5 mg
Manganese Chloride	$MgCl_2*4H_2O$	126	0.15 mg/L		3.0 mg
*Extra nutrients****					
Thiamine HCl (vitamin B1) at 100µg/ml	$C_{12}H_{17}ClN_4OS$	300.5	20 mg/L		400 mg
Vitamin B12	$C_{63}H_{88}CoN_{14}O_{14}P$	1353.9	0.5 mg/L		10 mg
Sodium selenite	Na_2SeO_3	173	80 mg/L		1600 mg

****Used for algae screening only.*

20 to 25 L of growth medium were prepared in separate vessel using reverse osmosis (RO) water and micro & macro-nutrient at the required concentrations as shown in Table 3.4. Extra nutrients (vitamins and silicate) were not required for this species, but these could be added as supplement. The medium was then poured into the main tank of the

TPBR and circulated throughout the reactor along with air in co-current flow. After a complete dissolution of nutrients, an inoculum from the 2 L flask was added to the medium at a volumetric dilution of 1:10.

Macro and micro-nutrients in Table 3.4 have been used in all media for algae culture in this project. Extra nutrients (sodium selenite, vitamin B1 and B12) were used in media for diatoms cultures during algae screening only. Vitamin B1 and B12 can be added to the fresh medium for Chlorella to enhance the growth. All media prepared in this work for the selected Chlorella strain did not contain any extra nutrients.

Culture growth was monitored every two days by measuring the cell concentration either by turbidity measurement using a spectrophotometer or by cell count using an optical microscope and a counting chamber. The pH of culture growth as well as the nitrite and nitrate ions concentrations were monitored using strip pH paper (with a pH range of 1-14), NO_3 strip paper (with concentrations range of 0 ppm - 200 ppm)and NO_2^- strip paper (with concentration range of 0 ppm -10 ppm). Monitoring results are displayed in Chapter 4, Section 4.4.2.

Algal cells growth was similar to growth of bacteria with different distinct growth phases: lag, exponential, stationary and death or lysis. At the beginning of the culture, in lag phase, cells were auto-adapted to the new environment. At this stage, cells multiplied at a low rate. The growth was unnoticed. The lag phase was followed by an exponential or growth phase. Cells are believed to grow at high rate in this exponential phase. The fast growth of cells in this phase attributed its name to the phase: growth phase. Cells

growth reached stationary phase at the end of growth or exponential phase, which is characterized by no-cells growth. The stationary phase is followed by lysis phase where cells started to cease living, as shown in Figure 4.5.

It is important to note that concentrations of sodium chloride (NaCl) and potassium nitrate (KNO_3) were doubled compared to concentrations shown in Table 3.4 in order to increase biomass productivity. Each algal culture in TPBR was grown for about 2 weeks until the color became dark green as shown in Figure 3.2. The green dark color indicates that the culture has reached its stationary phase and it is ready for harvesting.

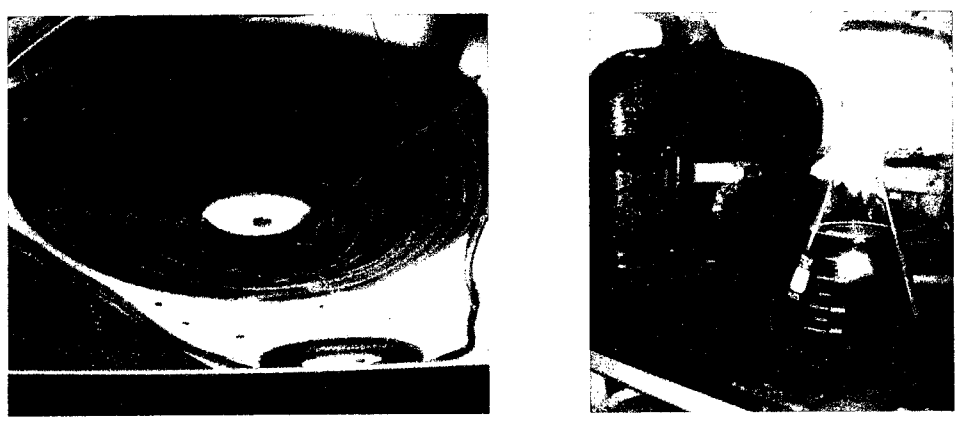


Figure 3.2: Algal broth in TPBR in its stationary phase.
Broth collected after 2 weeks of culture period prior harvesting.

Algae cultures in this work were harvested when cells concentrations become constant indicating that cultures reached stationary phase.

3.2.4 BIOMASS PROCESSING

Once the culture reaches a stationary phase, it was stopped and the broth collected into 5 gal carboy. A flocculent, aluminum sulfate anhydrous $Al_2(SO_4)_3$, was added to the broth at a concentration of 0.5 g/L (0.5 g of $Al_2(SO_4)_3$ per liter of algal broth) to precipitate algal cells. For example, 10 g of $Al_2(SO_4)_3$ were added to 20 L of broth solution to precipitate algae. Carboys were manually shaken to allow all cells to bind to $Al_2(SO_4)_3$, then left to settle into two layers for at least half hour for total precipitation.

The top layer consisted of relatively clear medium at low algae cell concentrations was gently discarded. The remainder, very condensed cell solution, was centrifuged using Beckmann centrifuge or Damon IEC B-20A centrifuge. The centrifuge produced algal paste or pellet, and a clear liquid. The clear liquid was discarded.

The algal paste or pellet was frozen using a mixture of dry ice, acetone and methanol in a Labconco jar. The frozen algae paste was then lyophilized using Labconco dryer (lyophilizer) for at least 48 hours depending on the level of moisture in the pellets and the size of the pellet. Dry algal biomass was then collected in pre-weighed dry jars. These jars were weighed again post-filling with dry algal biomass. The difference in weights prior and post dry algal biomass filling gave dry biomass weight as shown in Equation 3.3.

Mass of algal dry biomass [g] = mass of (Jar + dry biomass) – mass of (empty jar)
Equation (3.3)

The final concentration of algal broth was calculated using Equation (3.4):

$$\text{Final concentration [g of dry algae/L]} = \frac{\text{mass of algal dry biomass [g]}}{\text{medium volume [L]}}$$

Equation (3.4)

Results for different batches of algae culture are displayed in Chapter 4, Section 4.5.

3.2.5 LIPID EXTRACTION

Lipids were extracted from dry algal biomass using two different techniques.

1- Long protocol using modified Bligh& Dyer (Ferrentino,2007)

2- Short protocol using Soxhlet extraction apparatus.

Both techniques are based on solvent extraction. Lipid extraction was performed on different batches of samples cultured in TPBR. Prior to extraction, all glassware were washed with soap and water, soaked in 1 N hydrochloric acid (HCl) and then rinsed thoroughly with reversed osmosis (RO) water. All glassware in glass and metal were then baked in the oven at 120 °C for about 1hr. Baked glassware was left in the laboratory at ambient air to be cooled to room temperature. They were then rinsed with acetone, methanol and chloroform respectively to remove any potential oil that may affect results.

3.2.5.1 Long Protocol: Modified Bligh and Dyer

About 1 g of dry biomass was pulverized using a mortar and pestle. The powder was added into a 125 ml glass-beaker with a stir bar plus 10 ml of chloroform, 20 ml of methanol and 10 ml of RO water. The flask (containing chloroform, methanol, water, dry biomass and stir bar) was placed on a Corning magnetic stir plate (stirrer) for 24 hours. Sample was then filtered through 2.5 µm filter paper (Whatman grade # 5) using a 125 ml

Buchner flask, a 90 mm Buchner funnel and aspirator vacuums pump. The initial flask and the filter were rinsed with 10 ml of chloroform plus 10 ml of RO water to collect any remaining oil (lipid). Collected solvent mixed with algal oil (lipid) was transferred in a 250 ml glass separator funnel and settled for 1 to 2 hours to allow a total separation water/methanol (top layer) from chloroform/lipid (bottom layer).

The bottom layer was collected in a pre-weighted flask, followed by solvent evaporation using a water bath set at 40 – 45 °C under nitrogen or air blow. After a total evaporation of solvent, the remainder was algal lipid or oil. It was placed in the oven (National Environment Incubator) overnight at 45 °C to remove any remaining moisture. The lipid content (g of lipid per g of dry algae) was calculated using Equation (3.5):

$$\text{Lipid content} = \frac{\text{mass of (flask+lipid)}-\text{mass of (empty flask) [g]}}{\text{mass of dry algal biomass weight [g]}} * 100 \qquad \text{Equation (3.5)}$$

The results of different batches are displayed in Chapter 4, section 4.5.2.

3.2.5.2 Short Protocol: Soxhlet Extraction

Normal hexane (n-hexane) at 95% purity was used to extract oil from algal biomass. 250 ml of n-hexane was transferred into a 250 ml or 500 ml round bottom flask with 4 to 6 carbon chips. The flask was connected to the Soxhlet extractor, which contained glass-wool to prevent algal biomass from clogging solvent line or falling into the solvent (n-hexane) flask. 1 g to 5 g of algal biomass was added on the top of the glass-wool. The extraction was then connected to the condenser, which circulated cold water (at 4°Capproximately) from tap pipe. The Soxhlet set up is displayed in Figure 3.3.

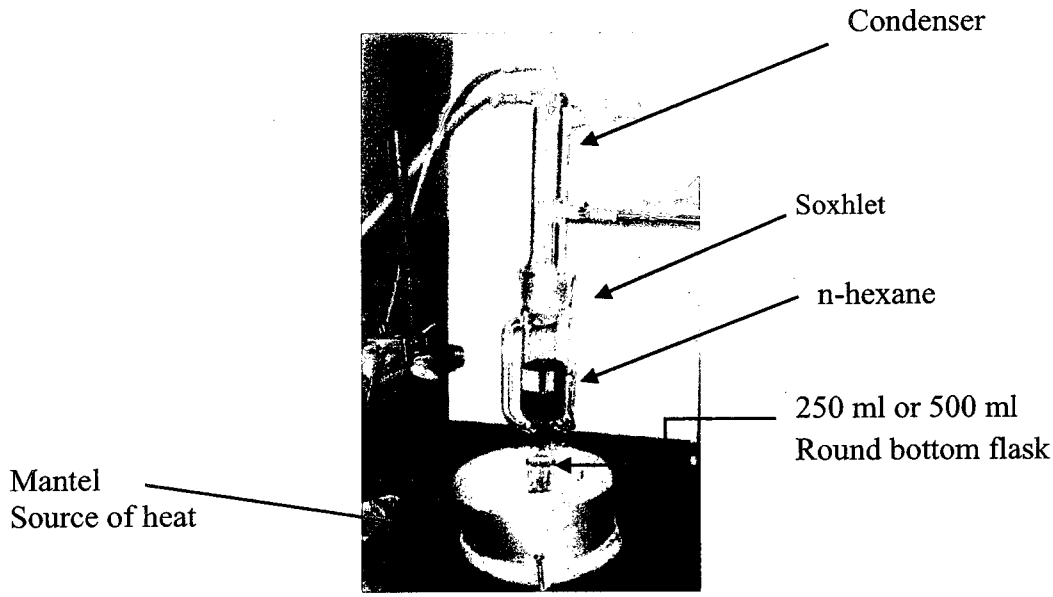


Figure 3.3: Soxhlet extractor setup

A water condenser was used during this process. Heat was provided via an electrical mantel for 5 - 6 hours followed by cooling at room temperature.

The mixture of algae oil/hexane was filtered through 0.25 μm Whatman filter grade number 5 using a Buchner funnel and flask to remove algae particles that went into the solvent during the oil extraction process. Sodium sulfate was used as a drying agent to remove excess moisture from the sample (mixture of algae oil/hexane). About 3 g of sodium sulfate Na_2SO_4 granular was added on the top of Whatman filter paper during the filtration process. Collected solvent/algal lipid was evaporated from the mixture using a water bath set at 40 - 45 °C. The Buchner flask containing algal oil was then placed in the oven overnight at 45 °C to remove remaining moisture. The lipid content was calculated using Equation (3.5). Once it was confirmed that the two protocols gave the same results, the long protocol was abandoned because it was time consuming and excessive labor.

3.2.6 TRANSESTERIFICATION

3.2.6.1 Conventional Transesterification

Conventional transesterification consists of a two-step process:

a) Extract oil from algal biomass as described in section 3.2.5;

b) Convert algal oil into biodiesel through transesterification reaction using methanol and an alkaline catalyst; for example, potassium hydroxide (KOH). Figure 3.4 displays the two steps process for biodiesel production from algal biomass.

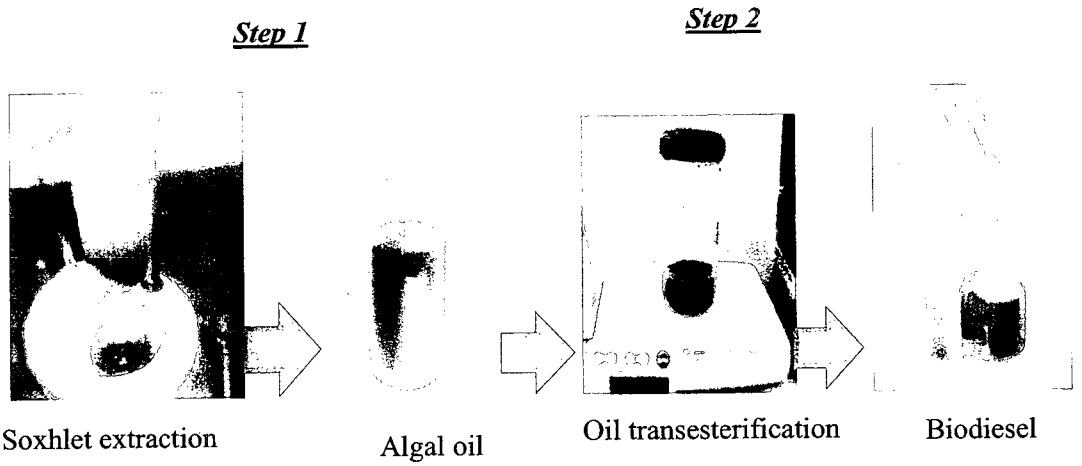


Figure 3.4: Biodiesel production in two-step: algae oil extraction followed by transesterification

After a partial segregation of the oil from solvent (n-hexane), the oil (mixed with remaining n-hexane approximately 1 ml) was mixed with 25 ml of 0.1 N KOH in methanol (CH_3OH) at 50 °C on a Corning stirring/heating plate for half hour. The reaction time (stirring/heating time) can vary depending on the size of the sample and the level of FAMEs yield needed. Transesterification reaction occurred during stirring/heating process. Transesterification product (FAMEs + remaining of algal

biomass + KOH+ methanol) was separated by filtration through a 0.25 μm Whatman filter paper grade number 5 with sodium sulfate anhydrous using Buchner funnel. Sodium sulfate anhydrous removes moisture from transesterified sample and the filter retains algae particles that were not captured during filtration post oil extraction. Excess solvent in transesterified sample was then evaporated to about 1 ml using water bath set at 35 °C – 40 °C and blowing air. 1 ml of final product was then transferred in a 10 ml graduate glass cylinder. The flask that served to evaporate excess solvent was rinsed with about 2 ml chloroform several times. This chloroform mixed with FAMEs was added to the 10 ml graduate glass cylinder till the final volume reached 10 ml mark. The final product (final volume of 10 ml) consisted of FAME, traces of n-hexane, methanol and chloroform. This final product was transferred into a 20 ml and it was ready for GC analysis. The GC procedure is described in section 3.2.6.3. The GC analysis results are presented in Chapter 4, Section 4.6.

3.2.6.2 In Situ ultrasonic assisted Transesterification

An aliquot of 1 to 5 g of dry algal biomass weighed in a 250 ml glass beaker was combined with 40 ml of 0.1 N KOH in methanol. The mixture was sonicated using an ultrasonic W375 Sonicator set to provide a power density of 9.4 W/ml with pulsed duty cycle of 50%. The reaction time (transesterification time) was varied for optimum biodiesel yield. The volume of methanol (0.1 N KOH in methanol) was kept constant at 40 ml for the completion of transesterification reaction. The amount of methanol was in excess compared to algal biomass amount. This one-step process is shown in Figure 3.5.

One step process

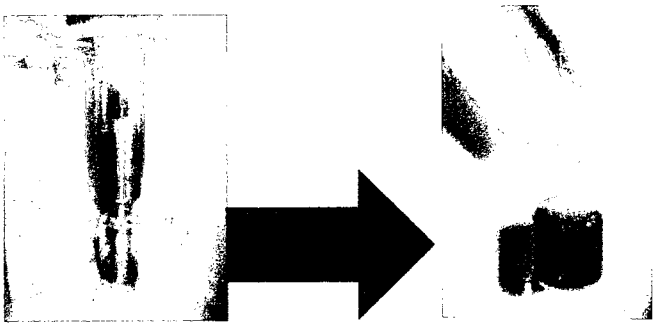


Figure 3.5: In situ (one-step) transesterification

At the end of the reaction, the mixture of Fatty Acids Methyl Esters (FAMEs), solvent (mainly methanol) and remaining of algal biomass was filtered through 0.25 μm Whatman filter paper grade number 5 using filtration apparatus mentioned above to remove the remaining of algae particles.

Excess solvent (methanol) was evaporated using a water bath set at 45 °C under blowing air. Air was used in this process to minimize production cost, even though it was not the focus of the present work. The final product after solvent evaporation was transferred in a 10 ml glass graduate cylinder. The Buchner flask used during solvent evaporation was rinsed several times with about 2 ml of chloroform to collect any remaining FAMEs from the Buchner flask. The collect FAMEs in chloroform were added to the final product in a glass graduate cylinder till it reached the 10 ml mark. The final 10 ml product was then transferred in a 20 ml clear glass bottle. The FAMEs samples were ready for analysis in the gas chromatograph (GC). The results of the one step in situ process are presented in Chapter 4, Section 4.6.

Figure 3.6 shows different steps for biodiesel production in the two-step process and the in situ or the one-step process.

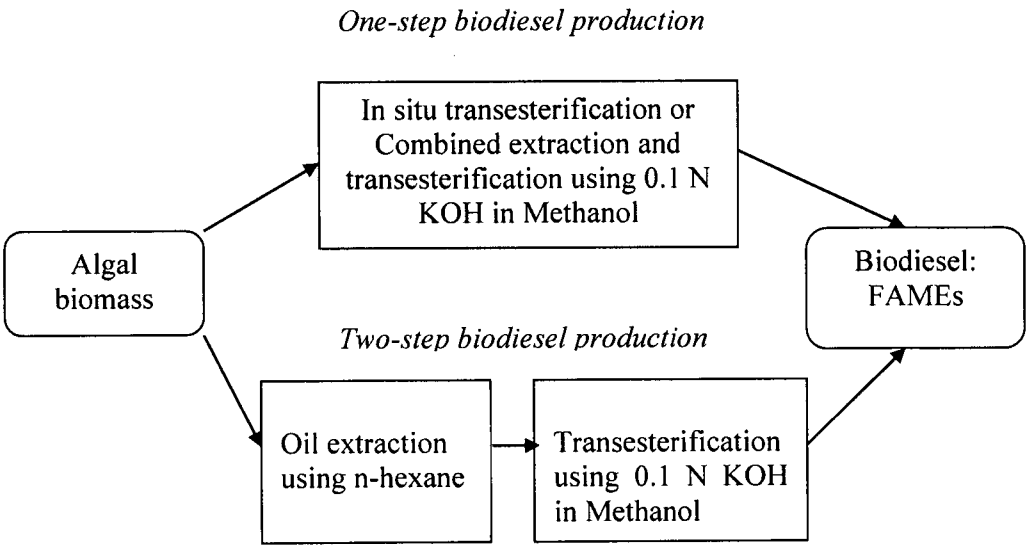


Figure 3.6: Comparison of one-step and two-step biodiesel production.

3.3 GAS CHROMATOGRAPH PROCEDURE

3.3.1 GC SYSTEM

Hewlett Packard HP 5890 Series II Gas chromatograph (GC) was used to identify potential FAMEs compounds in the transesterified algal oil samples (biodiesel). The GC had a cool on-column injection port. It was equipped with a flame ionization detector (FID) and RTX-1 fused silica fast column 15 m long, 5.0 μm df (film thickness) and 0.32 mm inner diameter (from Restek chromatography procedure). The GC was connected to

a 3396 HP integrator linked to a computer loaded with HP Peak 96 software. The settings of HP Peak 96 are displayed in Appendix III.

3.3.2 GC TEMPERATURE PROGRAM

The GC column is contained in an oven. The temperature of the column (or oven) is programmed into the GC. In our case the temperature program (often called GC method) is set as follows:

a. Injection port cool on-column at 40 °C.
b. Oven temperature profile: started at 40 °C and kept for 2 minutes (termed isothermal hold), then ramped to 230 °C at 8 °C/min. The final temperature was held for 20 minutes to bake any remaining material within the column.
c. The detector and oven track temperatures were set at 230 °C. It is important to note that the flame ionization detector was supplied with hydrogen gas to produce the flame. The GC had an internal flame igniter.
d. Helium was used as carrier gas flowing at 2.18 ml/min at 50 °C.

Figure 3.7 shows the variation of the column temperature versus time according to the above method.

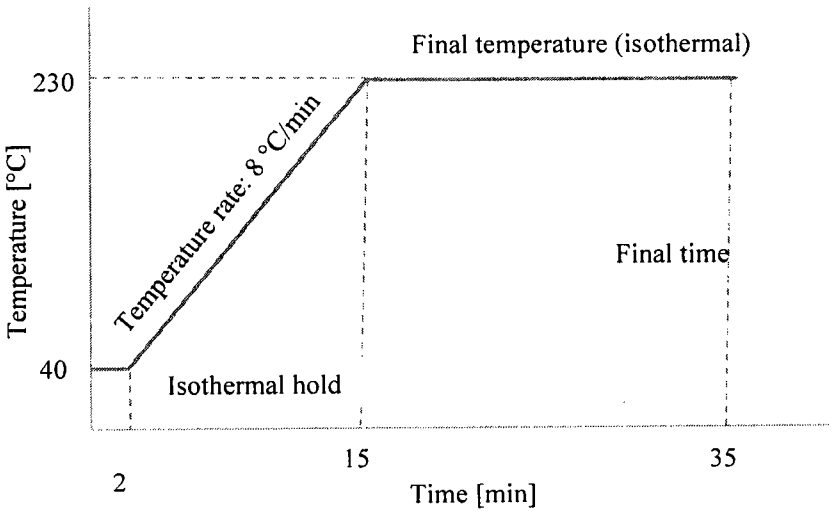


Figure 3.7: Temperature profile in the GC oven

3.3.3 SAMPLE INJECTION

1 μL of the liquid sample was injected into the cool on-column at 40 °C using a 5 μL-Hamilton syringe. The sample went through the total run time of 32 minutes with extra time used for GC column conditioning. Data were collected using HP Peak 96 software, and then transferred into a laptop equipped with OriginPro 8.1 software for analysis. Data were acquired in "asc" format. They were converted into "csv", and then transferred in OriginPro 8.1 for graphing and FAMEs analysis.

3.3.4 GC OPERATION AND DATA ANALYSIS

The GC operation was as follow:

a. First, a mixture of FAMEs standard in cocktail was prepared with C16:0, C18:0, C18:1, C18:2, C18:3 and C19:0 at 16.7 mg/μl each. This cocktail standard was run in

the HP 5890 Series II GC to identify retention time of each peak. Results of cocktail standard are displayed in Chapter 4, Section 4.6.

b. Second, a calibration curve was run using C19:1 standard (Methyl Cis-9-octadecenoate C19:1 at 0.874 g/ml) diluted in chloroform in serial of dilution ranging from 1:500 through 1:512000.

Results of calibration curves were analyzed in OriginPro and are displayed in Chapter 4 Section 4.6. A linear curve was established with coefficient "a" as a slope of the line using calibration curve data. Equation (3.4) represents the linear curve.

$$Y = a X \qquad \text{Equation (3.4)}$$

Where Y and X represent respectively the FAME concentration and the integrated area under standard peak. Equation (3.4) can be used to determine the concentration of any FAME found in algal samples analyzed in GC. It is important to note that Equation (3.4) gives an estimate FAME concentration, which may be biased by standard error in slope calculation. However, interpolation method can be used in lieu of Equation (3.4) to minimize the standard error. In this work, the interpolation method was used to determine the concentration of each significant peak found in the transesterified algal lipid.

Consider a FAME peak with an integrated area X corresponding to a concentration Y, if a value X is between two known peaks of the calibration curve (X_0, Y_0) and (X_1, Y_1), the concentration Y of unknown peak that has integrated area X is calculated using Equation 3.5 (linear interpolation) with an assumption that two close points in any function (curve) can be considered linear. X_i and Y_i represent an integrated area of peak i and its concentration respectively.

$$Y = Y_0 + \frac{(X - X_0) * (Y_1 - Y_0)}{(X_1 - X_0)} \quad \text{Equation (3.5)}$$

A calculation of concentration of unknown FAME peak with an integrated area of 1538.8 is illustrated below.

The GC standard C19:1 (Methyl Cis-9-octadecenoate at 0.874 g/ml) was diluted at 0.219 mg/ml which has an integrated area of 1932.4 (X_1=1932.2, Y_1= 0.219 mg/ml) and the same standard C19:1(concentrated at 0.874 g/ml) was diluted at 0.109 mg/ml corresponding to an integrated area of 1406.2 (X_0=1406.2, Y_0=0.109 mg/ml).

The concentration of unknown FAME peak with an integrated area of 1538.8 is found by substituting all terms of Equation 3.5.

$$Y = 0.109 + \frac{(1538.8 - 1406.2) * (0.219 - 0.109)}{(1932.4 - 1406.2)} = 0.137 \text{ mg/ml}$$

This unknown peak has been quantified and its retention time will be compared to those of GC standard C16:0, C18s and C19:1 in order to determine its identity.

A peak with a retention time which does not match any of the known GC standards run in this work will be labeled as unknown.

Total biodiesel produced per g of dry algae biomass can be estimated using Equation 3.6.

$$\text{Total biodiesel produced in 1g of dry algae} = \frac{V}{initial\ mass\ of\ dry\ algae} * \sum_{i=1}^{n} Y_i$$

Equation (3.6)

Where n, Y_i [mg/ml] and V [ml] represent respectively the number of peaks, concentration of FAME peak "i" and the volume of biodiesel plus solvent. In this work, the volume of biodiesel plus solvent is 10 ml. The total biodiesel produced in 1 g of dry algae represents also biodiesel yield based on the initial mass of dry algae used to

produce biodiesel through either the in situ process or the conventional process (two-step process).

3.4 **SOFTWARE**

3.4.1 JMP

JMP is a complex computer program developed to perform statistical analysis. It is also used to design different sets of experiments and analyze data. JMP is an interactive and comprehensive tool that links data to graphic for easy analysis. JMP version 8.1was used in this work to analyze transesterification results. The outputs of JMP are displayed in Chapter 4, Section 4.6.

3.4.2 ORIGINPRO

OriginPro is also a computer program that performs data analysis and graphing. It performs unique peak analysis and curve-fitting of data. OriginPro version 8.1 was used in this work to perform FAMEs peaks analysis and fit curves of several data displayed all across Chapter 4, Section 4.6. This program allows setting a baseline for FAMEs peaks and calculating the integrated areas under peaks as shown in Appendix IV.

3.4.3 PEAK 96

Peak 96 is a Hewlett Packard (HP) DOS based computer program that enhances communication between a computer (PC) and an integrator connected to the GC. It resides on the PC and has the capability of importing and exporting GC data from the HP 3396 integrator to a PC and vice-versa. It is also capable of storing data from the HP 3396 integrator. Peak 96 was used in this work to capture GC data from the HP 3396 integrator and transfer them to PC. These data were then exported to a laptop using a USB flash disk, for further analysis in OriginPro. The settings of this software are displayed in Appendix III.

3.5 **EXPERIMENTAL PROCEDURES SUMMARY**

Table 3.5 summarizes the methods and procedures used in this study.

Table 3.5: Summary of methods/procedures used

Property to be measured	Measurement	Equipment/software used
Algae growth	Algae concentration by measuring turbidity.	Spectrophotometer
	Cells count in algal solution.	Microscope and a counting chamber
Biomass yield	Mass of dry algae after harvesting per liter of algal broth	Centrifuge, lyophilizer and balance
Lipid content	Mass of dry algal lipid after extraction per mass of dry algal biomass	Soxhlet, oven (incubator), water bath, stirring plates, balance
FAMEs composition	Fatty acids methyl esters (FAMEs) concentration (μg/ml) per g of dry algal biomass	Gas Chromatograph, HP integrator, computer, JMP, OriginPro 8.1 and Peak 96 software

These methods were applied to reach the objectives of this research. The results obtained from these techniques are presented in Chapter 4.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 INTRODUCTION

The results for this work are divided in six parts. The first part presents the results for algae strains screening using appropriate measuring instruments. In the second part, the tubular photobioreactor (TPBR) constructed in this work and presented in Chapter 3, Section 3.2, will be characterized by assessing its functionality and defining its suitable operational conditions. Results obtained culturing algae in the TPBR will be displayed and discussed in the third part. The fourth part will show the results of harvesting technique, drying algal pellet and oil extraction from dry algal biomass. The results for maximum growth rate using Huesemann et al. approach as kinetic model for algae growth in a batch reactor will be showed in the fifth part. In the last part, the results of transesterification process for both conventional and in situ process will be displayed and examined. The results displayed and discussed in all these 6 parts represent responses to the 8 objectives addressed in Chapter 1, Section1.8.

4.2 PART I: ALGAL STRAIN SCREENING

4.2.1 CHLORELLA STRAINS SCREENING

Two strains of Chlorella were screened. An unidentified strain of Chlorella named Chlorella sp. (also called C1 earlier in this work) was screened for high lipid content and growth rate. Another Chlorella named Chlorella C2 was also screened for high lipid content and growth rate. Chlorella C2 was later termed as Salina due their ability to grow in medium containing high salt concentration especially NaCl and KNO_3. These two strains were obtained from Professor Leland Jahnke laboratory at the UNH Plant Biology Department. Chlorella sp. and C2 were cultured for two weeks in 2 L Buchner flask used as small PBR. These two cultures were performed in the same growth conditions set as follow. The temperature was not measured; however, it was assumed to be room temperature approximately 25 °C. The media cultures for both Chlorella strains consisted of fresh nutrient solution or fresh medium shown in Table 3.4 with no supplement of selenite, vitamins B1 and B12. The two flasks were exposed to 8 fluorescent light bulbs, which provided photonic energy 24 hours per day for the entire culture period. Air was bubbled in both flasks to homogenize algal broths and supply CO_2 for photosynthesis. The pH of these cultures started at 6 and increased until it reached 9 at the end of cultures.

As stated previously in Chapter 3, Section 3.2, the growth of the two algae culture was not monitored. The goal of this part of the work was to assess the extent of growth for these two strains cultured in same conditions. This was done by measuring the turbidity of algae broth (optical density) using a spectrophotometer at the beginning and at the end

of the two week culture period. Turbidity (optical density) of algae broth is proportional to the cell concentration of algae culture. Thus, a culture with high turbidity indicated a high growth rate for algae broths of same age. Figure 4.1 shows results of the two cultures as measured by turbidity or optical density.

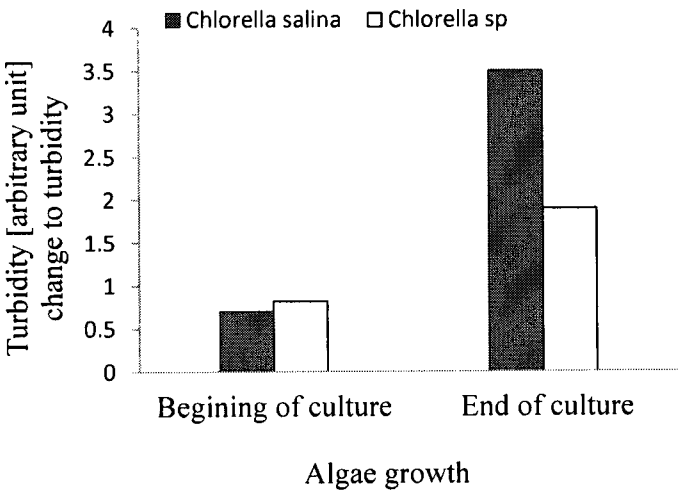


Figure 4.1: Comparison of algae growth for two chlorella cultures.

Figure 4.1 shows that Chlorella Salina has a higher growth rate compared to Chlorella sp by comparing the two algae broths turbidity at the end of two week culture period. The results of lipid content done using Red Nile Fluorescence technique were not conclusive; they are included in Appendix V.

However, Khan et al. (2009), Chisti (2007) and Bigogno et al. (2002) stated that the lipid content in dry algal biomass for Chlorella strains was approximately 20 % regardless of strain types. Therefore, Chlorella sp. was eliminated due to the lower turbidity of its broth compared to the Salina strain.

4.2.2 SCREENING OF DIFFERENT ALGAE STRAINS

Seven strains were cultured in small PBRs (2 L glass Buchner filter) for two weeks. These cultures were not monitored overtime. Nevertheless, growth rate of algae cells and their oil content were measured at the end of the two weeks culture period. Culture growth rate [cells numbers per unit time] was not measured, but the turbidity of each culture were measured at the end of the two weeks growth period using spectrophotometer with a wavelength set at 687 nm.

Results for oil content and turbidity at the end of two weeks culture period are displayed in Figure 4.2. All these cultures started with much diluted broth (1 ml of inoculum in 2 L of culture medium) with a turbidity of zero.

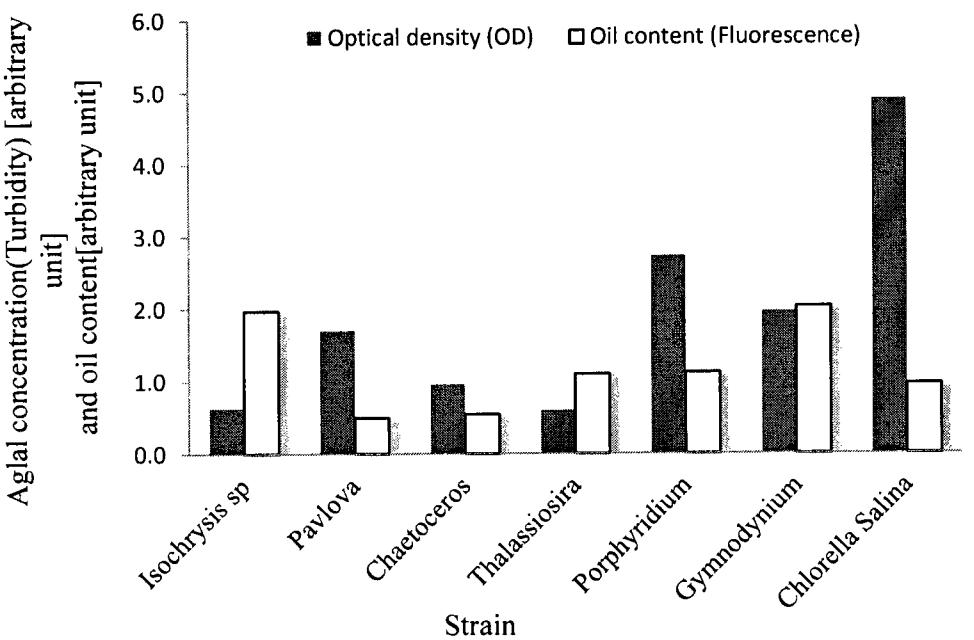


Figure 4.2: Algae concentration and oil content of seven algal strains

Oil content was measured from normalized algal broth using spectrofluorometer. The normalization was performed at turbidity of 0.05 with Salina solution.

Some algae strains from Figure 4.2 such as Chaetoceros, Isochrysis sp or Thalassiosira may grow fast if growth conditions were modified. For instance, diatom strain such as Chaetoceros grows faster in medium with high concentration of sodium chloride (0.5 M NaCl). But the medium used to culture Chaetoceros had NaCl at 0.1 M. However, growth conditions were kept uniform for all seven strains at the screening level. In addition, medium modification and/or variation of culture conditions such as temperature, amount of CO_2 and photonic energy supplied to algal cells were not the main goal of this work at strain screening level.

It can be seen from Figure 4.2 that Chlorella Salina has the highest turbidity (optical density), hence highest growth rate. But it has a low lipid content compared to Isochrysis sp, Gymnodynium, Porphyridium and Thalassiosira. Combined results of turbidity (optical density) and oil content, as seen in Figure 4.3, give high product for Chlorella Salina compared to the other six strains. In addition, Chlorella Salina has a good resistance to contamination resulting in less cultures collapse. Strains such as Gymnodynium, Porphyridium and Isochrysis sp. have high lipid content. However, their cultures tend to collapse rapidly in most cases and they are easily contaminated and overtaken by Chlorella traces. As a result, Chlorella Salina was retained for the rest of this investigation.

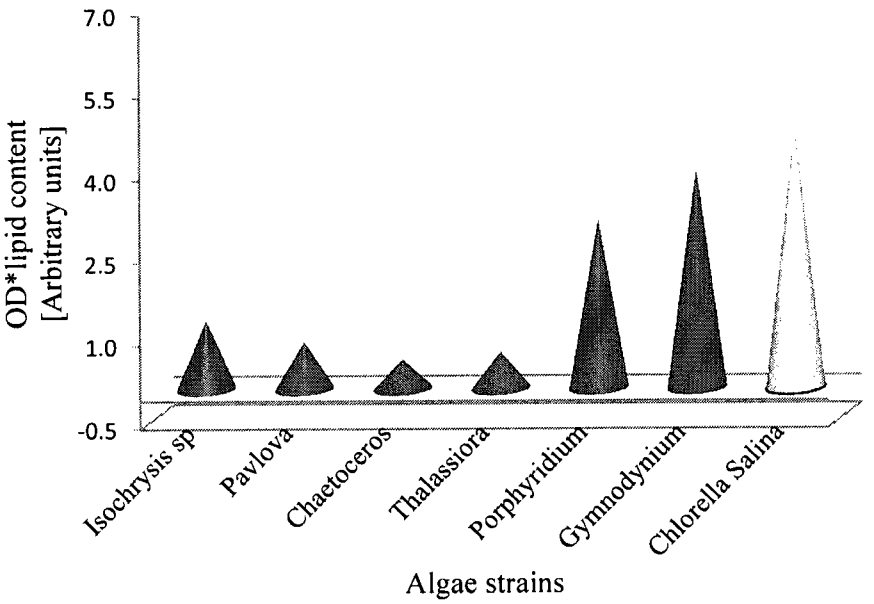


Figure 4.3: Combined results of algal concentration (turbidity or optical density) and lipid content. *All seven strains of algae were cultured in fresh medium, with air bubbling in the medium at room temperature. PBR (2 L glass flask) for all strains were exposed to fluorescent light bulbs for 24 hours a day.*

4.3 PART 2: TUBULAR PHOTOBIOREACTOR CHARACTERIZATION

Tubular Photobioreactor (TPBR) constructed in this work was characterized prior to being used for Chlorella Salina cultivation. The following parameters were determined for suitable functionality of the TPBR: light intensity, gas pressure ranges, medium flow rate, medium viscosity and medium velocity in tubing. Results are displayed and discussed in this section. After determining these parameters, the TPBR was operated more than a week without any problems. Then the TPBR was ready to be used for Chlorella Salina cultivation.

4.3.1 GAS PRESSURE

Compressed air supplied to the TPBR contained approximately 300 ppm of CO_2. The CO_2 was consumed along with water by Chlorella Salina cells, which transformed them into lipids essential to be used as raw material in biodiesel production process. The supplied air pressure was measured using a graduated manometer (pressure gauge) placed between air compressor and the reactor. Air pressure was varied by opening the air valve gradually in order to determine the suitable operating pressure ranges. The readings (measurements) for all pressures are presented in Table 4.1

Table 4.1: Air pressure readings with liquid flowing at 285.7 ml/s

Trial	Pressure [psi]	Observations
1	5.0	Small air bubbles observed in the reactor at supply point. It was assumed that air supplied at this pressure was not sufficient to provider CO_2 necessary for photosynthesis reaction.
2	5.2	Similar as trial 1
3	5.4	Reactor runs with steady liquid flow through tubing without culture interruption, air holding in tubing or air bubbles deficiency.
4	5.6	Similar as trial 3
5	5.8	Similar as trial 3
6	6.0	Flow of liquid ceases within (approximately) 3 hours of run
7	6.2	Flow of liquid stops 3 to 2 minutes after opening air line due to holding air in tubing.
8	7.5	Flow of liquid stops within a minute of opening air line.

The desired pressure range is defined such that liquid flow through tubing cannot cease due to excessive air in tubing or cause insufficient air in tubing with less CO_2 for photosynthesis reaction.

When the supplied air had a pressure < 5.4 psi, air was spread in small bubbles in the tubing and vanished within a few seconds. It was assumed that this pressure would not allow a healthy culture due to insufficient air in tubing with less CO_2 for photosynthesis reaction. This range of pressures was not suitable for this TPBR.

An air pressure > 5.8 psi resulted in building up air in the tubing preventing free liquid flow. This range of pressures was not used for this TPBR.

Air pressure ranging in the interval of 5.4 psi < P < 5.8 psi was ideal for a suitable functionality of this TPBR; not only it allowed sufficient CO_2 for photosynthesis process along the tubing, but it also prevented air holding up inside the tubing which facilitate a steady liquid flow in tubing. Air supplied at a pressure in the ranges of 5.4 psi < P < 5.8 psi enhances formation of small bubbles in the liquid flow, created by the Mazzei nozzle as discussed in Chapter 3, Section 3.2. Therefore, air pressure ranging in the interval of 5.4 psi < P < 5.8 psi, was retained for suitable run of the reactor without liquid flow cessation. A concurrent-flow of medium and air (gas) throughout the tubing was observed during the entire cycle (in and out of the tubing).

The separation of undissolved air from the liquid (medium) occurred in the main tank at medium returning point. The air went out of the main tank through an outlet and the medium was kept in the main tank continuing the circulation cycle. The summary of effects of air pressure on the operation of the TPBR is given in Table 4.2.

Table 4.2: Summary of effect of air pressure on the operation of the TPBR at room temperature, with a water flow rate of 285.7 ml/s

Air pressure	Observed effects
P < 5.4 psi	Small air bubbles, insufficient to provide CO2 necessary for photosynthesis reaction
5.4 psi < P< 5.8 psi	TPBR runs with normal flow of liquid and air
P > 5.8 psi	Liquid flow stops due to high air pressure

4.3.2 LIGHT INTENSITY

Light intensity is a vital part of algal cells growth during the photosynthesis process. In this project, light intensity was measured using light meter (Cooke) at three different area of the plywood (plw). Measurement picture is shown in Appendix II. Measurements in Figure 4.4 show that light rays have high intensity (of about 1200 fc) on the center of the plywood and less dense at the edges (around 600-900 fc). The average values at each point (left, center, right and outside) of plywood are displayed in Figure 4.4 and Figure 4.5.

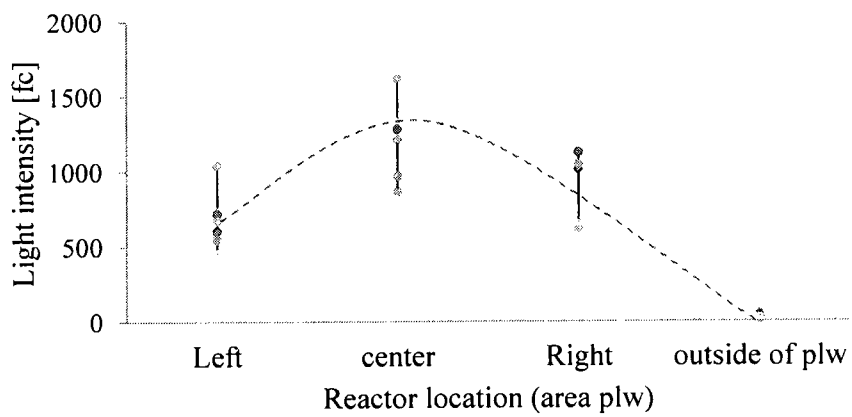


Figure 4.4: Light intensity at different area of plywood

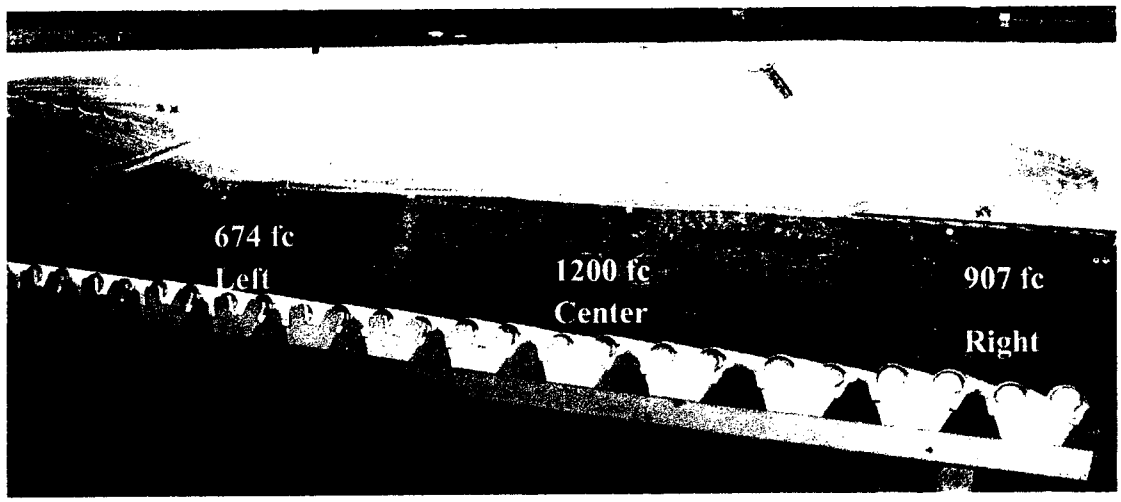


Figure 4.5: Average light intensity at different area of TPBR

The average light intensity on left side, center and right side of the plywood measure in foot-candle (fc) were 674 fc or 7254.9 lx, 1222 fc or 13153.6 lx and 907 fc or 9681.3 lx respectively. These light intensities are approximately 1/10 of the intensity of full, unobstructed sunlight (around noontime) in summer (10,000 fc).

It is important to note that approximately 2/3 of the external area of tubing (TPBR) was exposed to light provided by 32 fluorescent light bulbs 24 hours a day. As mentioned in Chapter 3, Section 3.2.2, the tubing for this reactor was mounted on plywood (plw) painted in white to enhance light reflection. It was assumed that reflected light rays were captured by algal cells to maximize photonic energy absorption.

The photonic energy was not optimized in this work. However, it could be suggested that the future project defines the optimum photonic energy amount required to culture different strains of microalgae using this TPBR.

One of the advantages of the TPBR compared to the raceway pond is the flexibility in using natural light or artificial photonic energy that allows the cultivation of algae in any seasons of the year (winter or summer) and overcome cool temperature and nighttime challenges. Thus, the TPBR could be used indoor or outdoor. In addition, algae strains that require intense light for a healthy growth still can be cultured in this TPBR by adding more fluorescent light bulbs or combining fluorescent light bulbs with other types of bulbs to provide desired light intensity.

On the other hand, adding more light bulbs could increase algae production cost. It is preferable to balance the cost of production in order to keep the price of biodiesel comparable with petrodiesel price. It was demonstrated in Section 4.5 of this Chapter that light used on this TPBR did not inhibit algae growth.

4.3.3 MEDIUM FLOW RATE

Flow rate measurements were performed using a 2 L graduate cylinder and a timer. A valve placed in tubing line served to collect water into a 2 L graduate cylinder. Table 4.3 displays filling time for different trials.

Table 4.3: Filling time of 2 L graduate cylinder, with air pressure set at 5.6 psi.

Trial	1	2	3	4	5	6
Filling time [s]	8	7	7	7	7	8

The average time of all ten trials was 7 seconds. Thus, the flow rate can be calculated using Equation 3.1.

$$\text{Flow rate [L/s]} = \frac{2\ [\text{L}]}{\text{average time [s]}} \qquad \text{Equation (3.1)}$$

$$\text{Flow rate [ml/s]} = \frac{2000\ [\text{mL}]}{7\ [\text{s}]} = 285.7\text{ml/s} = 0.0755\ \text{gal/s}$$

The TPBR was tested with lower liquid flow rates using a reactor bypass mounted on the return line into the main tank. The bypass line had a valve which allowed changing liquid flow rate to lower ranges (< 285.7 ml/s).

It has been observed that the TPBR could operate at liquid flow rates < 0.075 gal/s, if air is not supplied to the medium. Trials of flow rate using bypass are displayed in Table 4.4. It is important to note that the liquid flow stops when air is supplied to the reactor with liquid circulating at flow rate < 285.7 ml/s.

Table 4.4: Flow rate measurement using bypass line and valve

2L-graduate cylinder, filling time [s]	Flow rate [ml/s]	Comments
8	227	TPBR could operate with air supplied to the tubing
10	200	Liquid flow stops as soon as air pressure builds up in the tubing, which occurs within 5 minutes after opening the air valve.
12	166.6	
15	132.5	
23	87.1	

Thus, liquid flow rates comprised in the range of 227 ml/s – 285.7 ml/s were retained for a suitable operation of the TPBR allowing air/CO_2 supply through Mazzei nozzle. The summary of all flow rates tested for this TPBR and their effects on the reactor is given in Table 4.5.

Table 4.5: Summary of liquid flow ranges

Flow rate < 227 ml/s	227 ml/s < Flow rate < 285.7 ml/s	Flow rate = 285.7 ml/s
Liquid flow stops when air is supplied to the TPBR.	Normal liquid flow with air supplied at pressure ranging 5.4 - 5.8 psi, suitable for perfect run of this TPBR.	Maximum flow rate for the pump used in this work. TPBR operate without any problems.

4.3.4 MEDIUM VELOCITY

Measurements of the liquid velocity were performed on a 4 ft (1.2 m) straight horizontal section of the tubing (part of the TPBR). This section connected the two spiral sections of the tubing as shown in Figure 3.1. The residence time of the air bubble in this section was constant and equal to 2 seconds. Thus, fluid velocity could be calculated using Equation 3.2 assuming that the air and liquid move at the same velocity in the TPBR.

$$\text{Velocity [m/s]} = \frac{1.2\ [\text{m}]}{\text{average time [s]}} \qquad \text{Equation (3.2)}$$

$$\text{Velocity [m/s]} = \frac{1.2\ [\text{m}]}{2\ [\text{s}]} = 0.6\ \text{m/s}$$

The results obtained using an air bubble agreed with the results obtained by using blue dye in the water circulating throughout the reactor. Consistently, the dyed water ran through 1.2 m section in 2 seconds with water flowing at 285.7 ml/s or 0.075 gal/s in the TPBR. Liquid flows in this TPBR with a velocity of 0.6 m/s.

Theoretical flow rate can be calculated using Equation 4.1.

$$q = \frac{v * \pi * d^2\ [\text{m}^3]}{4\ [\text{s}]} \qquad \text{Equation (4.1)}$$

Where q, v and d are respectively, the theoretical flow rate, the medium velocity and the tubing internal diameter (ID) (0.019 m or 3/4 in).

$$\text{Hence, } q = \frac{0.6 * \pi * d^2 [m^3]}{4 [s]} = 171 \text{ ml/s or } 0.045 \text{ gal/s}$$

The theoretical flow rate (171 ml/s) is approximately 40% less than the actual flow rate (285.7 ml/s). The differences are due to unknown factors.

4.3.5 MEDIUM VISCOSITY

Medium viscosity was measured at room temperature using Brookfield viscometer and a 500 ml beaker containing about 150 ml of test sample. The measurements were taken daily for 8 days of Chlorella Salina culture period in the TPBR.

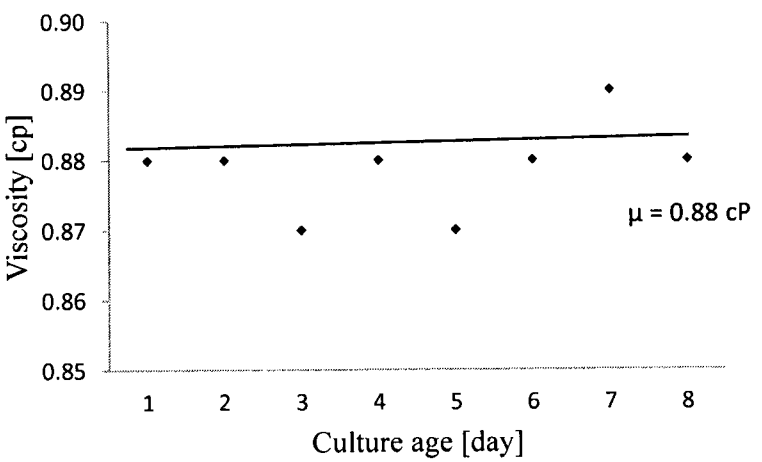


Figure 4.6: Viscosity of algal broth overtime

In Figure 4.6, clearly the viscosity of culture broth was relatively constant and close to the viscosity of water (about 1 cP at 20 °C). This is an indication that the culture was not dense enough to affect significantly the viscosity of medium.

4.3.6 FLOW REGIME

The liquid flow regime was determined by evaluating the magnitude of Reynolds number (Re) represented by Equation 4.2 and the results obtained in Section 4.3.5 and 4.3.4 of this Chapter.

$$Re = \frac{\rho.d.v}{\mu} \qquad \text{Equation (4.2)}$$

Where d, v, μ and ρ are the tubing internal diameter (d = 0.019 m or ¾ in), the liquid flow velocity (v = 0.6 m/s), the fluid viscosity (μ = 0.88 cP = $8.8*10^{-7}$ kg/m-s) and the liquid density (ρ = 1000 kg/m^3) respectively. It was assumed that the density of broth was approximately equal to the density of water, 1000 kg/m^3.

Hence Reynolds number (Re) can be calculated as followed:

$$Re = \frac{1000*0.019*0.6}{8.8*10-7} = 12{,}955$$

Clearly the Re is large enough indicating that the TPBR was operating in a turbulent regime.

4.4 PART 3: CULTIVATION OF ALGAE

4.4.1 OPTIMUM NUTRIENTS CONCENTRATIONS

Five different media were prepared in small photobioreactor (2 L Clear glass Buchner flask). The concentrations of potassium nitrate (KNO_3) and sodium chloride (NaCl) were

changed for all five media as shown in Table 4.6. Each flask was labeled with letters A, B, C, D and E. The cell growth was monitored by measuring the turbidity (optical density: OD) of algae solution. Concentrations of all other nutrients present in these five media were kept the same as indicated in Table 3.4 of Chapter 3.

Table 4.6: Composition of nutrient solution and KNO_3/ NaCl effect on cell growth

Flask	NaCl concentration	KNO_3 concentration	Observations
A	0.1 M	5.2 mM	Control with normal concentration for both NaCl and KNO_3.
B	0	5.2 mM	Very low growth rate, resulting in low yield of algal biomass compared to the control culture (flask A).
C	0.2 M	5.2 mM	Same yield as the control culture (Flask A).
D	0.1 M	0	No cells growth
E	0.1 M	10.4 mM	Excellent growth rate with algal biomass yield greater than the control culture.

In Figure 4.7, flask D that has nitrate (KNO_3) deficiency shows no cells growth. Similarly, sodium chloride (NaCl) deficiency (flask B) reduces cells growth rate. On the other hand, doubling KNO_3 concentration (flask E) increases the growth rate significantly resulting in higher yield compared to the control culture in flask A. Moreover, doubling NaCl concentration shows same algal biomass yield as the control in flask A. Thus Chlorella (Salina) grows fast in media with high concentration of KNO_3 and NaCl. This fact agrees with the name given to this species of Chlorella "Salina", which means this strain grows well in medium with high salt concentration.

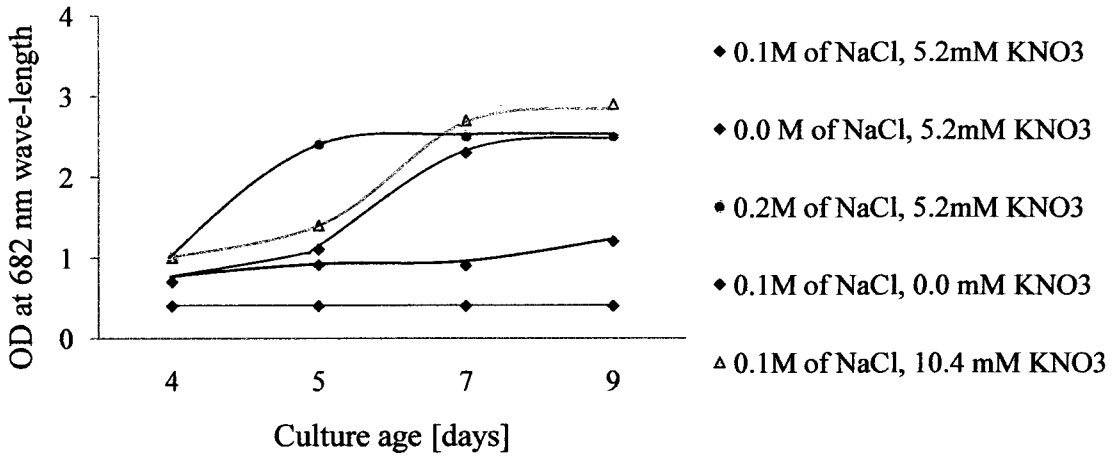


Figure 4.7: Effect of NaCl and KNO_3concentration on algal cells growth overtime.

The effect of nitrate and sodium chloride was done to assess the growth rate of cells culture. However, no relations between growth rate and oil content were established during this investigation.

The concentration of KNO_3 as set in this work agrees with the results of the Food and Agriculture Organization (FAO) of the United Nations (UN) as shown in Table 4.7 (Miyamoto, 1997).

Table 4.7: Effect of nitrogen on diatoms lipid content (Miyamoto, 1997)

KNO_3 concentration [mM]	Cell yield [g/L]	Lipid content [g/L]	Cell yield* Lipid content
0.9	0.39	42.4	16.54
9.9	2.5	32.9	82.25
9.9 with supplement feeding containing Nitrogen	2.6	33.6	87.36

Although the FAO results show that KNO_3 concentration is inversely proportional to algal lipid content (in g/L), the high concentration of KNO_3 increases significantly algal

biomass yield, which is also demonstrated in this work. In Table 4.7, the concentration of KNO_3 at 9.9 mM, which is approximately the same as the results of our investigation, showed a high product (cell yield * Lipid content) of 82.25. It can be inferred that high KNO_3 concentration is proportional to the product of combined cell yield and lipid content. Therefore, the concentration of NaCl and KNO_3 were doubled respectively at 0.2 M and 10.4 mM for the rest of this investigation.

4.4.2 ALGAL CULTURE IN TPBR

Several batches of Chlorella Salina were cultured in TPBR using medium containing nutrients displayed in Table 4.8.

Table 4.8: Composition of medium used to cultivate Chlorella Salina in TPBR

Chemical	Formula	Molecular weight	Required Concentration		Example of 20 L medium
Macro-nutrients					
Calcium Chloride	$CaCl_2*2H_2O$	147	0.2 mM	29.4 mg/L	588 mg
Boric Acid	H_3BO_3	62	0.13 mM	8.0 mg/L	160 mg
Potassium Nitrate	KNO_3	101	**10.4 mM**	1.05 g/L	21.0 g
Magnesium Sulfate	$MgSO_4$	121	5 mM	600 mg/L	12,000 mg
Sodium Phosphate	Na_2HPO_4	142	0.4 mM	76.8 mg/L	1,536 mg
Sodium Chloride	NaCl	58	**0.2 M**	11,6 g/L	232 g
Micronutrients					
EDTA (Ethylenediamine Tetra acetic Acid)	$C_{10}H_{16}N_2O_8$	292	26.9 mg/L		538 mg
Ferrous Sulfate	$FeSO_4*7H_2O$	278	2.8 mg/L		56 mg
Zinc Sulfate	$ZnSO_4*7H_2O$	287	0.288 mg/L		5.76 mg
Molybdenum Oxide	MoO_3	144	0.125 mg/L		2.5 mg
Copper Sulfate	$CuSO_4*5H_2O$	250	0.075 mg/L		1.5 mg
Cobalt Chloride	$CoCl_2*6H_2O$	238	0.025 mg/L		0.5 mg
Manganese Chloride	$MgCl_2*4H_2O$	126	0.15 mg/L		3.0 mg

Some batches were cultured simply to produce algal biomass. These latter batches were not monitored; therefore, there is no data for them. Data for monitored batches are reported in this section.

Figure 4.8 shows that Chlorella Salina cells grow like bacteria undergoing different growth phases: lag phase, exponential (growth) phase and stationary phase. If the culture goes more than 15 days, cells undergo lysis phase in which they cease.

It can be seen from Figure 4.8 that a healthy culture of Chlorella Salina can reach a concentration up to $60*10^6$ cells/ml.

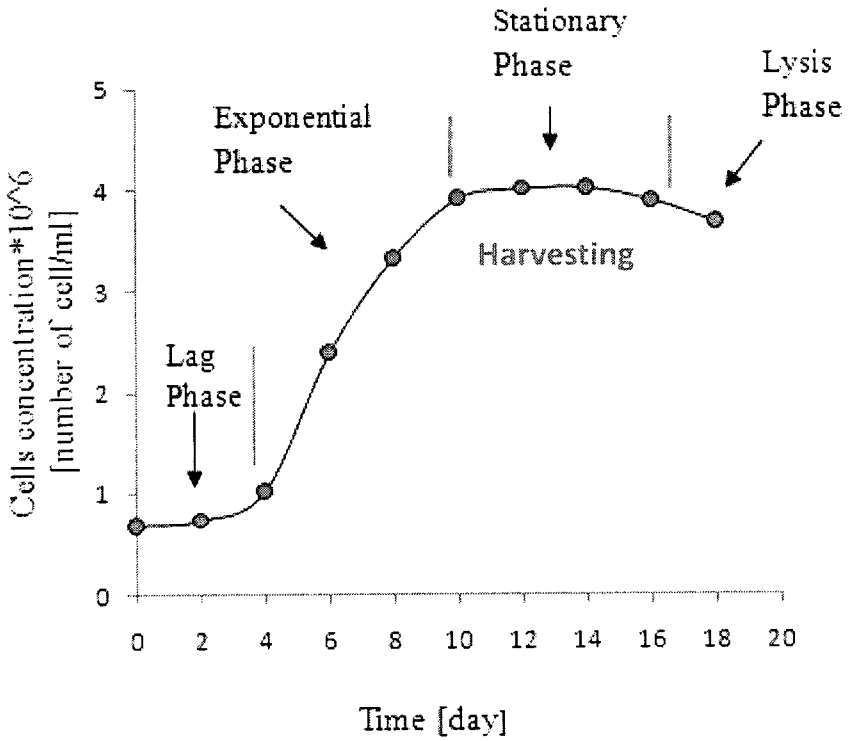


Figure 4.8: Growth phases of Chlorella Salina. *Culture performed at room temperature, medium composition from Table 4.8, inoculum added at ratio of 1:10 (Volume of inoculum: volume of medium)*

It is important to note that, in the present work, batch-cultures start much diluted with algal concentration of 0.1 g dry algae per liter of broth culture. They reach stationary phase with an average algal biomass concentration of 1.1 g dry algae per liter of broth culture. It can be seen from Table 4.8 that broth culture final concentration to initial concentration ratio, in this investigation, is approximately 6 times the ratio for culture of Arthrospira Platensis done using a Helix photobioreactor and medium of the following composition. 17 g/l $NaHCO_3$, 1 g/l K_2SO_4, 0.01 g/l $FeSO_4.7H_2O$, 0.08 g/l EDTA, 2.5 g/l Na NO_3, 0.5 g/l K_2HPO_4, 0.2 g/l $MgSO_4.7H_2O$, 1 g/l NaCl, (1 m/l) of (2 g/l H_3BO_3, 0.08

$CuSO_4.5H_2O$, 0.2 g/l $ZnSO_4.7H_2O$) and 23 mg/l NH_4VO_3, 44 mg/l $Co(NO_3)_2.6H_2O$ (Nerantzis et al 2000).

Even though the composition of medium and the species used in the Helix PBR were different to the medium and species used in this work, this work shows high yield in algae biomass by comparing the ratios final to initial algae concentration. This work yields a ratio (final to initial broth concentration) of 11 and Nerantzis et al (2000) study yields a ratio of 2, as shown in Table 4.9.

Table 4.9: Chlorella Salina culture characteristics comparison (Nerantzis et al 2000)

	This work	Helix PBR
Final/initial algae concentration	11	2
Lag phase [days]	3	5
Growth or exponential phase [days]	6	6
Stationary phase [days]	3	9

Although Chlorella Salina cells resist attacks of bacteria and other algal strains, it can be seen from Figure 4.9 that there were unknown limitations from time to time causing culture collapse. For example, batch 3 in Figure 4.9 experienced lysis within 5 days of inoculation.

Similarly, the maximum culture concentration in batch 2 was low, approximately $20*10^6$ cells/ml compared to the culture concentration in batch 1, which was three times the concentration of batch 3. Clearly cells in batches 2 and 3 suffered from unknown causes. Causes of cultures failure or poor cells growth were not investigated in this project. Nonetheless, these are important issues requiring a particular attention in future work.

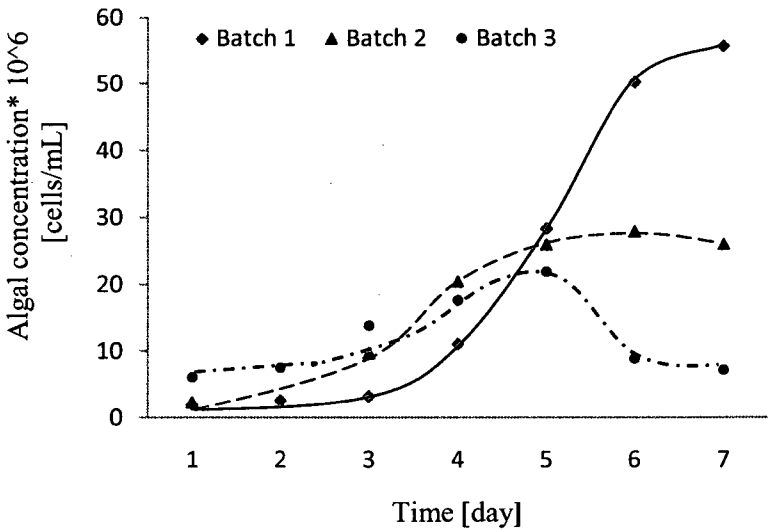


Figure 4.9: Chlorella Salina concentration versus time in 3 different batches over time. *Batches cultured with medium at concentrations displayed in Table 4.8, room temperature for a week culture period.*

4.4.3 BROTH CUTLURE pH

In Figure 4.10, pH started approximately at 6, then increased till it reached a plateau at 9 as (hydroxyl ions) OH^- are released in solution during cells growth.

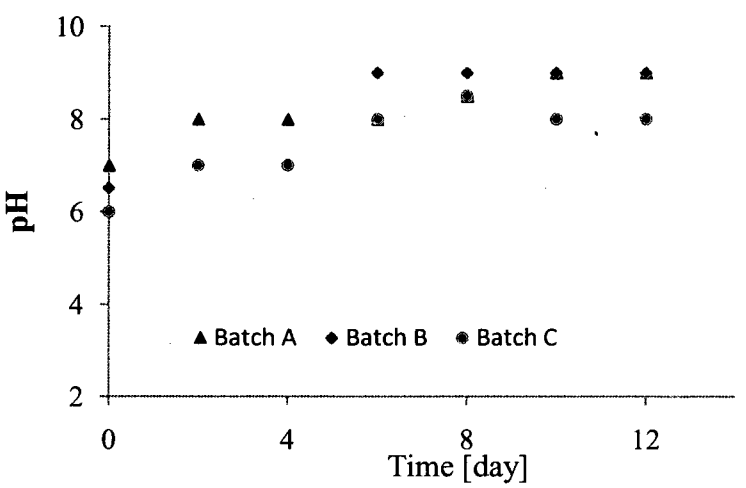


Figure 4.10: pH of 3 batches of Chlorella Salina overtime.

These 3 batches were cultured at different time in the same conditions, room temperature, and medium composition from Table 4.8, inocula from same source at 1:10. Clearly, the pH of Chlorella Salina culture reaches a plateau after a week of culture period as seen in Figure 4.10. Chojnacka and Marques-Rocha (2004) showed that OH^- ions are released in the reaction of H_2O and HCO_3^- (occurring due to the presence of CO_2 in H_2O) as shown in Table 4.10.

In this investigation, pH was not adjusted during cells cultures in the TPBR. It is likely that the increase in alkalinity indicates that Chlorella Salina undergoes at least one of the metabolisms described in Table 4.10. It can be inferred from Figure 4.8 that Chlorella Salina culture reaches a stationary phase after a week of culture period in TPBR. This may imply that when Chlorella Salina cells reach the stationary phase, photoautotrophic or mixotrophic metabolism ceases indicating the end of Chojnacka and Marques-Rocha (2004) reactions.

Table 4.10: Cells growth metabolism (Chojnacka and Marques-Rocha 2004)

Metabolism	Chojnacka and Marques-Rocha Reactions	Comments
Photoautotrophic	$H_2O + HCO_3^- \rightarrow C + ½\ O_2 + 3\ OH^-$	Both reactions release OH^- resulting in pH increase
Mixotrophic	$aHCO_3 + bCH_2O \rightarrow cC + 3OH^- + dCO_2$	

C represents the algal biomass in the two Chojnacka and Marques-Rocha reactions of Table 4.10

4.4.4 NITRATE AND NITRITE CONCENTRATION MEASUREMENT

The nitrate (NO_3^-) and nitrite (NO_2^-) ion concentrations were measured with strip paper indicating the concentrations ranges for both the nitrate and nitrite ions in culture

solutions. The concentrations limits on these strip papers for both NO_3^- and NO_2^- were respectively (0 ppm - 200 ppm) and (0 ppm - 10 ppm). The results in Table 4.11 showed that KNO_3 were in excess and was not consumed completely by Chlorella during its culture period.

Table 4.11: Nitrate and Nitrite concentrations of four batches of Chlorella Salina cultured in TPBR, *fresh media with double concentration of NaCl and KNO_3, compressed air supplied to provide CO_2, light supplied 24hours for the entire culture period of 2 weeks.*

Time [day]	NO_3^- concentration $*10^3$ [ppm]				NO_2^- concentration [ppm]			
	Batch				Batch			
	A	B	C	D	A	B	C	D
0	0.2	0.16-0.2	0.2	0.2	5-10	1-3	1-5	5-10
2	0.16-0.2	0.2	0.2	0.16	10	5-10	1-5	5-10
4	0.16-0.2	0.2	0.2	0.16	10	5-10	1-3	5-10
6	0.2	0.2	0.16-0.2	0.2	5-10	10	5-10	3
8	0.2	0.16-0.2	0.16	0.2	5-10	10	5-10	5-10
10	0.16-0.2	0.08-0.16	0.16-0.2	0.16-0.2	3	5-10	5-10	5-10
12	0.16-0.2	0.16-0.2	0.2	0.2	5-10	10	1-5	5-10

4.5 PART 4: ALGAE BROTH HARVESTING

4.5.1 BIOMASS CONCENTRATION

Chlorella Salina cultured in TPBR yield an average of 1.0 g of dry algal biomass per liter of broth as displayed in Figure 4.11. The 4 batches in Figure 4.11 were inoculated at 1:10 ratio with algal seed solution (inoculums) at 1 g dry algae per liter of algal broth. To illustrate, a batch of 20 L was inoculated with 2 L of algal solution at 1 g/L resulting in

broth culture initially at 0.1 g dry algae per liter of algal broth. At the end of two week culture period, the culture reached a concentration of 1.1 g/L. The concentration increased10 times from 0.1 g of dry algae per liter of algae broth to 1.1 g of dry algae per liter of algal broth. These results agreed well with the results of the Food and Agriculture Organization (FAO) of the United Nations (UN) when the light intensity was 5000 lumens or 465 foot candle (fc) (Miyamoto, 1997)

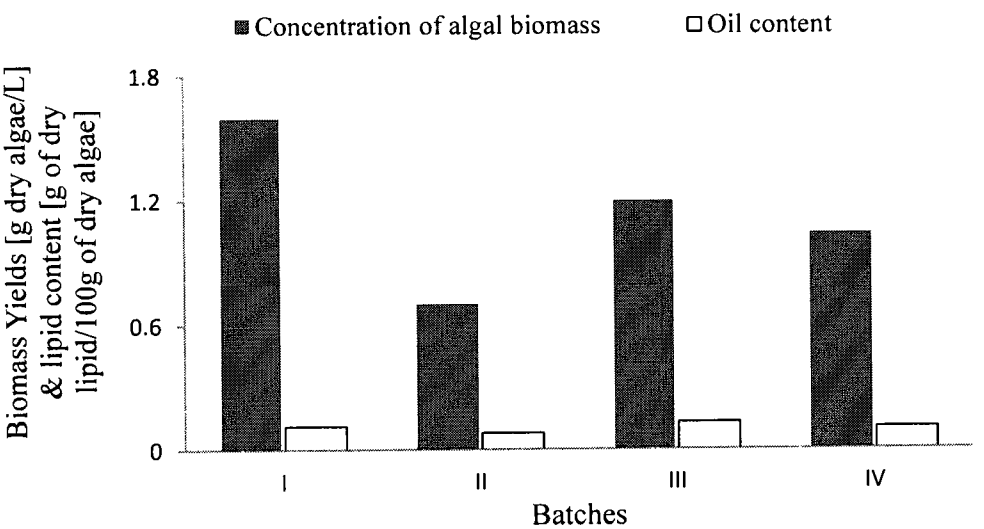


Figure 4.11: Algal biomass yield and lipid content after 12 days of culture period. *Culture done at room temperature, normal light 24 hours a day for the entire culture period, air entering the TPBR at 5.6 psi.*

The four batches in Figure 4.11 were cultured in the same conditions with inoculum from same source. Batch-I showed higher biomass concentration than the other three batches. Causes affecting growth rate of batches II, III and IV were unknown. However, culture contamination and level of dissolved oxygen (DO) cannot be ruled out as causes of low growth rate in the three batches.

4.5.1.1 Light effect on growth culture

Once a batch-culture become dense, it can be assumed that a dark color decrease light flux which will slow the growth with a risk to enter in to a stationary phase prematurely. Oswald empirical formula (Anderson, 2005), Equation 4.3, can be used to assess the effect of light on algae growth inhibition. This equation gives the maximum distance d_p (in cm) of light penetration.

$$d_p = \frac{6000}{C} \quad \text{Equation (4.3)}$$

Where C is the broth concentration in mg of dry algae per liter of broth culture. The average value of C (from Figure 4.11) was 1.1 g of dry algae per liter of broth or 1100 mg/l. Hence d_p is:

$$d_p = 6000/1100 = 5.5 \text{ cm.}$$

The maximum distance of light penetration is greater than the external diameter of tubes (1 inch or 2.5 cm) in the TPBR.

d_p= 5.5 cm >>> external d = 2.5 cm.

Therefore, light was not an inhibitor of Chlorella Salina growth in the TPBR.

4.5.1.2 Culture Dilution Rate

In Figure 4.11, the culture age of all 4 batches was 12 days. This was cells residence time in the reactor. Thus, a dilution rate D can be calculated using Equation 4.4.

$$D = \frac{1}{\text{Residence time}} \quad \text{Equation (4.4)}$$

$$D = 0.08 \text{ day}^{-1}$$

The productivity of Chlorella Salina in TPBR can then be calculated using Equation 4.5

$$P = C*D \qquad \text{Equation (4.5)}$$

Where C and D are the average increase in biomass concentration and the dilution rate respectively. The gain in concentration of algal biomass was 1.0 g dry algae per liter of broth culture (from Figure 4.11) and dilution rate was 0.08/day. Hence, 0.08 g dry biomass of Chlorella Salina could be produced in the TPBR per liter of algal broth per day.

$$P = 0.08 \text{ g/day-L or } 80 \text{ mg/day-L}$$

Huesemann et al. (2003) screened marine microalgae for maximum flue gas CO_2 biofixation potential. Two of the marine microalgae studied were Chlorella Salina and Chlorella Sp. Their dilution rate were in the range of 0.4 – 0.9 per day (larger than our work of 0.08 per day). Their results indicate a Chlorella Salina productivity of about 850 mg/L-day or 0.85 g/L-day at a dilution rate of 0.4 per day. Extrapolating their results to a dilution rate of 0.08 per day will bring their productivity closer to the present work. Table 4.12 compares results of this work with those of Chisti photobioreactor (PBR) and raceway pond (Chisti 2007).

Table 4.12: Comparison of results based on TPBR producing 1 g of algal biomass per liter in 12 days (**Chisti, 2007)

Variable	This work	PBR Facility**	Raceway Pond**
Biomass concentration [Kg/m^3] or g dry algae per liter	1.1	4.0	0.5
Productivity [$g * L^{-1} * day^{-1}$]	0.08	1.5	0.1
Dilution rate [day^{-1}]	0.08	0.4	0.2

As seen in Table 4.12, the productivity in this work is approximately the same as in raceway pond; however, the biomass concentration is twice the concentration in raceway pond. The TPBR showed 100% increase in biomass yield (about 1.1 g of dry algae per liter) compared to the yield of 0.5 g of dry algae per liter from raceway pond (Chisti 2007).

It is important to note that all Chlorella named Salina or sp are not exactly same strains. Nomenclature Salina indicates only that the species tolerate high concentration of salt. Similarly, sp. indicates that the species have not been identified yet. It can be inferred that Chlorella strains studied by Huesemann et al (2003) or Ben-Amotz and Gilboa (1980) may not be the same strains as the one selected and cultured in this investigation.

4.5.2 LIPID CONTENT

In Figure 4.12, lipid (algae oil) was extracted from eight different batches of dry algal biomass using Soxhlet apparatus. Batches 1 through 5 had an average of 9 g of lipid per 100 g dry algae biomass. Batches 6 and 7 had almost twice as much lipid as batches 1-5. Lipid in Batches 6 through 8 were extracted from more than 2 g of dry algal biomass, which implies that the amount of dry algal biomass as well as extraction method at laboratory scale affect lipid content results. Results of batches 6 and 7 showed that the TPBR can be used to culture Chlorella Salina able to yield up to 20% in mass of algal lipid. These results agreed with Bigogno et al (2002) results.

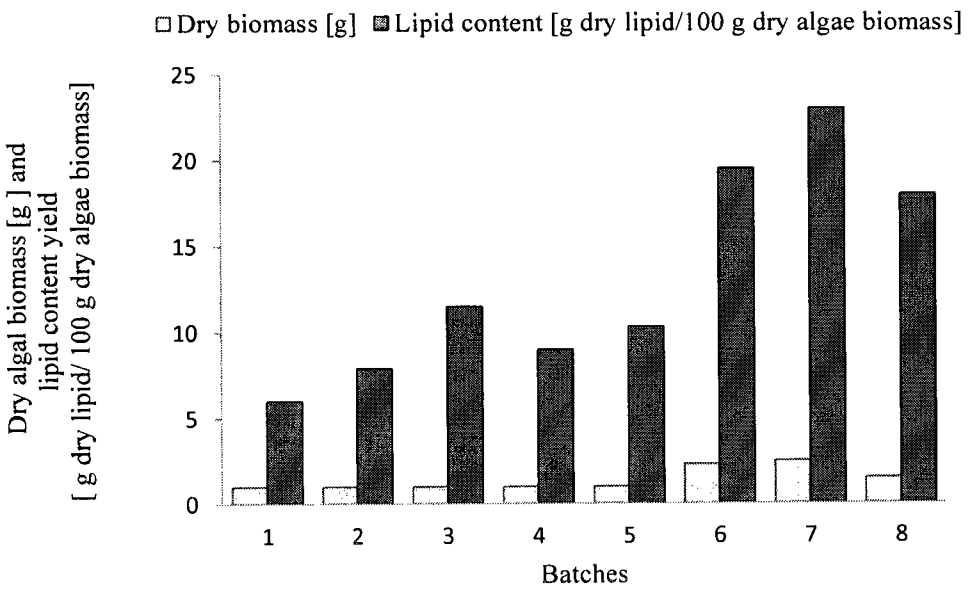


Figure 4.12: Lipid content extracted using Soxhlet apparatus

4.6 PART 5: KINETIC MODEL

A kinetic model for cells growth consists of an expression defining the variation of cells concentration with time. This expression can be determined if at least one of the following is cells growth limiting: one nutrient in broth culture, light intensity and/or CO_2 concentration. In this investigation micro-nutrients and macro-nutrients were not totally consumed over the two weeks culture period. In addition, it was proved in Section 4.5.1.1 of this Chapter that light was not a limiting factor on cells growth. Also Table 4.11 showed that NO_3^- and NO_2^- were in excess. They were not completely consumed over the two weeks culture period. However, the specific maximum growth rate μ_{max}

could be calculated using Equation 4.6, Huesemann approach (Huesemann et al 2009). This approach is applicable in exponential (growth) phase only.

$$\mu_{max} = \frac{1}{\Delta t} * \ln(\frac{C_f}{C_i}) \quad \text{Equation (4.6)}$$

Where C_f and C_i are respectively algal broth final and initial concentrations in exponential (growth) phase, and $\Delta t$is the exponential growth time.

Equation 4.6 was simplified to estimate the maximum growth rate μ_{max} using the following assumptions:

a. There was no significant cell growth in the lag phase resulting in constant concentration; thus cells concentration at the end of the lag phase was the same as the initial concentration.
b. These cultures were harvested when they reached stationary phase. It can be assumed that the final concentration of the culture was the same as the concentration at the end of exponential phase.
c. Equation 4.6 was linearized throughout exponential phase and rewritten as follow:

$$ln(C_f) = \mu_{max} * \Delta t + ln(C_i) \quad \text{Equation (4.6)}$$

Where Δt was constant and equal to 6 days (data from Table 4.9) and C_i was 0.1 g/L.

Table 4.13: Maximum growth rate of batches with initial concentration of 0.1 g/L

Batch	Biomass concentration [g/L]	μ_{max} [day^{-1}]
I	1.6	0.46
II	0.7	0.32
III	1.2	0.41
IV	1.04	0.39
Mean	1.1	0.4

Hence, the average maximum growth rate from Table 4.13 was:

$$\mu_{max} = 0.4/\text{day}$$

Ben-Amotz and Gilboa (1980) studied the growth of several microalgae on enriched sea water at 20 °C. They reported the following results for Chlorella Salina strain: cell length 8μm, cell width 6 μm, maximum growth rate μ_{max} = 2/day and cell maximum concentration $8*10^6$ cells/ml. The present work results show a maximum growth rate that is5 times less compared to Ben-Amotz and Gilboa results. The differences may be due to culture condition, reactors used as well as the inoculums used (Chlorella Salina strains).

The average doubling time can then be estimated as follow.

$$\text{Doubling time} = \frac{ln\,2}{\mu_{max}} = 1.7 \text{ day}$$

Thus Chlorella Salina concentration doubles every two days when cultured in the TPBR constructed in this work using modified fresh medium of Table 4.8. But Ben-Amotz and Gilboa results showed a doubling time of 0.3 day or 8 hours.

It can be suggested that a detailed study be conducted in the future to determine growth rate for Chlorella Salina or other species by culturing them in the TPBR.

4.7 PART 6: LIPID ANALYSIS OF BIODIESEL

4.7.1 FATTY ACIDS METHYL ESTERS (FAMEs) PEAKS IDENTIFICATION

The mixture FAMEs standards (cocktail) was prepared by pooling 100 μL of each of the following standard: C16:0, C18:0, C18:1, C18:2, C18:3 and C19:0 resulting in a final

concentration of16.7 mg/µl each. 1 µL of the cocktail standard was injected in the HP 5890 Series II Gas Chromatograph (GC) in order to identify the retention time of each fatty acid methyl ester (FAME) present in the mixture.

Figure 4.13 shows show retention times of C16:0, C19:0 and all C18s. C18:1, C18:2 and C18:3 were not well separated in this method. Their peaks overlapped in one peak when there were in a mixture.

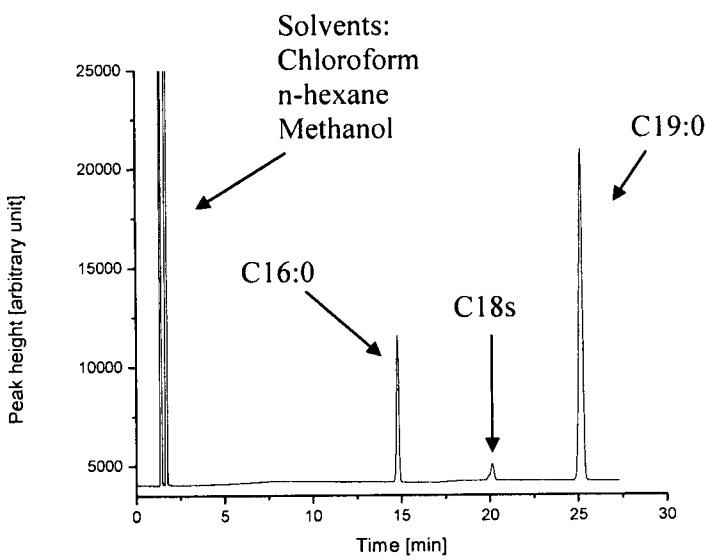


Figure 4.13: FAMEs peaks of cocktail standard. *1 µL of cocktail standard injected into the GC, GC data analyzed in OriginPro.*

Each of these GC standards (C16:0, C18:0, C18:1, C18:2, C18:3 and C19:0) was analyzed individually prior to be mixed in a cocktail. The retention times of these peaks are shown in Table 4:14.

Table 4:14: Retention time of main FAMEs standard analyzed individually. *OriginPro output of the data initially analyzed in GC.*

Fatty Acid Methyl Ester: FAME	Retention time [minutes]
C16:0	14.9
C18:0	20.9
C18:1	21
C18:2	21
C18:3	21
C19:0	21.4

Results displayed in Table 4:14 and Figure 4:13 indicate that all C18s peaks (C18:0, C18:1, C18:2 and C18:3) have a retention time of 21 minutes, same for all three peaks. Therefore, a FAME peak with a retention time of 21 minutes will be identified as C18 with no specific number of double bond. Similarly C16:0 has a retention time of 14.9 minutes; thus a FAME peak with a retention time of 14.9 minutes (approximately 15 minutes) will be identified as C16 with no specific number of double bonds. The C19:0 standard has a retention time of 25 minutes when analyzed in a cocktail. However, C19:0 analyzed individually, showed a retention time of 21 minutes, which was the same as the C18s FAMEs retention time.

Furthermore, it was observed that a biodiesel sample (produced in this work) contained an unknown FAME peak with the same retention time as C19:0. The C19:1 standard analyzed individually has a retention time of 21.5 minutes as shown in Figure 4:15, which is also closed to C18s retention times. Thus using C19:0 or C19:1 as an internal standard to analyze biodiesel produced in this work, produce overlapped peaks (C18s, C19 and the unknown peak). Overlapped peaks made the results hard to analyze.

Therefore, C19:0 and C19:1 standards were not used as internal standard for quantitative analysis of biodiesel samples. As a result, a calibration curve was established (using C19:1 standard) as a basis for quantitative analysis of FAMEs in this investigation.

These retention times may shift if the GC column is truncated for maintenance, or if the GC setting is changed. For example, increasing the temperature ramp from 8 °C/min to 15 °C/min shifts the retention time of all FAMES with approximately 5 minutes less than the initial retention time. The order peaks retention time stays the same.

4.7.2 CALIBRATION CURVE

Methyl Cis-9-octadecenoate or C19:1, GC standard, at 0.874 g/ml was analyzed in GC at different concentrations in order to generate a calibration curve. This calibration curve served as basis for the calculations of FAMEs concentration. Different concentrations of calibration curve were obtained by diluting C19:1 in Chloroform. Table 4.15 displays the different concentrations of C19:1 with their integrated area under FAME C19:1 peak.

Table 4.15: Different concentration of C19:1 standard in chloroform and their integrated area under the FAME peak.

Dilution factor	Area [Arbitrary unit]	Concentration [mg/ml]
500	12035.17	1.748
1000	8746.5	0.874
2000	3282.4	0.437
4000	1932.4	0.219
8000	1406.2	0.109
16000	667.6	0.055
64000	208.7	0.014
128000	94.5	0.007
256000	48.9	0.003
512000	35.5	0.002

Figure 4.14 shows an example of a C19:1 peak. This peak shows a retention time of 21 minutes in the HP 5890 Series II GC column.

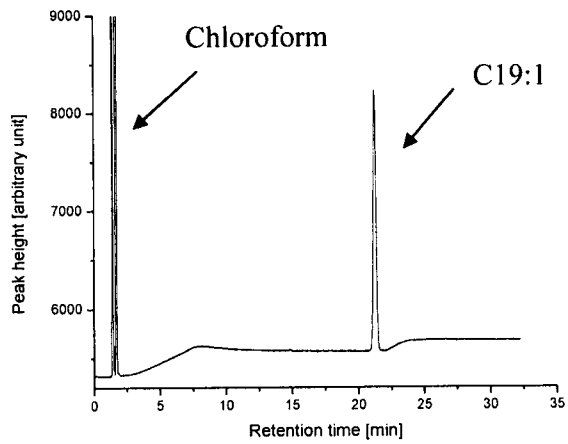


Figure 4.14: Peak of C19:1 standard
1 μL of C19:1 standard injected in the GC, data analyzed in OriginPro.

Data from Table 4.15 represent a linear function defined as an equation of a line (an integrated area under the peak C19:1 as function of C19:1 standard concentration) with a slope of $1.3* 10^{-4}$ as showed in Figure 4.15.

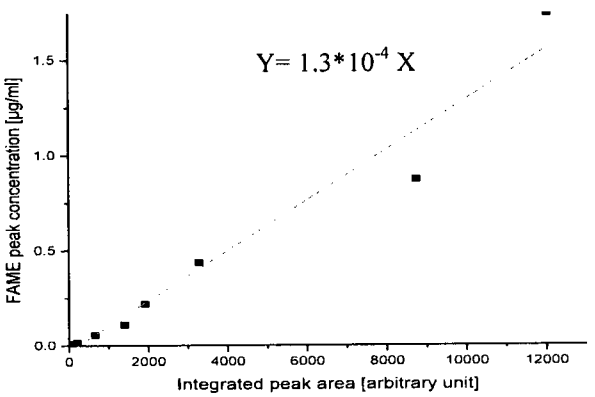


Figure 4.15: Calibration curve of C19:1 standard.

The concentration of any FAMEs peaks can be determined using Equation 4.7. or by interpolation using data from Table 4.15.

$$Y= 1.3*10^{-4} X \qquad \text{Equation (4.7)}$$

Where X represents the integrated area under FAME peak with unknown quantity (species) and Y is the concentration of the unknown species. Equation 4.7 gives approximate results due to high magnitude of the slope standard error. Thus, the interpolation method, using data from Table 4.13, was used to quantify FAMEs found in biodiesel samples. The area of the peak with unknown concentration has to be within the range of calibration curve data. For instance, a concentration of peak with an area of 5 cannot be evaluated accurately using the calibration curve data in Table 4.15. Similarly, a concentration of a FAME peak with an area of 40000 cannot be evaluated precisely using data of Table 4.15. If the area of the FAME peak is out of the calibration curve

range, it is recommended to run a new curve with extended data point or dilute the sample if it has a huge area under the FAME peak.

4.7.3 CONVENTIONAL TRANSESTERIFICATION: TWO-STEP PROCESS

The conventional transesterification refers to two-step process. In this process, oil is extracted from algae biomass first, and then converted into biodiesel as described in Chapter 3, Section 3.2.6. 1 μL of a transesterified sample was injected into the Hewlett Packard HP 5890 Series II Gas Chromatograph (GC). GC data were analyzed in OriginPro 8.1, which generated integrated area under FAME peak corresponding to FAMEs concentration.

Figure 4.16 displays an example of a chromatogram for a biodiesel sample produced in two-step process.

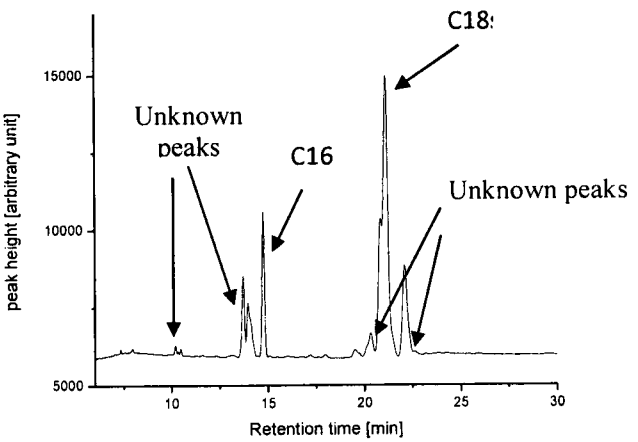


Figure 4 .16: FAMES produced through transesterification in two-step on a stirrer *9.6 g of dry algae biomass at room temperature, reaction time 6 hours.*

The C16 peak and C18s are well identified due to their retention times. C18s peak consists of overlapped peaks; they are identified as C18 without specifying the presence of double bonds. Figure 4.16 shows C16, C18s and unknown FAMEs peaks. The concentration of these peaks can be determined by interpolation using the calibration curve. C18s peaks overlapped. Thus their concentrations were evaluated as one peak. It can be seen from both Figure 4.15 and 4.16 that Chlorella Salina produced more C18s compared to other FAMEs present in the sample.

Biodiesel produced in two-step process contains up to 2.315 mg of fatty acids methyl ester per g of dry algae for a process performed in 24 hours (oil extraction and transesterification). Figure 4.17 shows that54% of FAMEs produced is C18s, which agrees with results found by Ferrentino (2007).

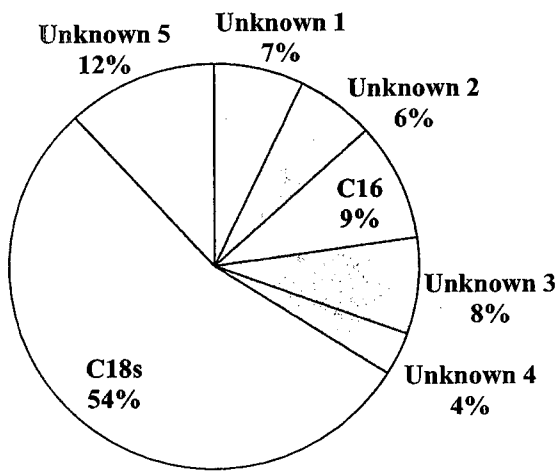


Figure 4.17: Composition of FAMEs produced in two-step on a stirrer.
9.6 g of dry algae biomass used at room temperature on one trial

It is important to note that the two-step process takes more than 20 hours to produce biodiesel: from lipid extraction to biodiesel production. The two-step process uses

solvent (n-hexane: 250 ml per trial) for lipid extraction, followed by 0.1 N KOH in methanol for lipid transesterification. Obviously, the conventional transesterification is time consuming and uses excessive solvent (n-hexane and methanol), which are toxic. Two batches of algal oil were transesterified through the two-step process. The oil batches were extracted from two different cultures of dry algal biomass. One sample yielded 2.315 mg of total FAMEs per g of dry algal biomass and the other one yielded1.189 mg of total FAMEs per g of dry algal biomass. FAMEs yielded through the two-step process in this work are lower compared to Ferrentino (2007) results of 11 mg of FAMEs per g of dry algae. The difference may be due to the algae strains, their lipid content, culture conditions as well as transesterification time.

The two-step process for these two samples was performed in the same conditions. 40 ml of 0.1 N KOH in methanol were mixed with algal oil (stored in 10 ml of n-hexane) using a magnetic stir plate for 6 hours at room temperature. The difference between these two runs can be due to the amount of algal oil in each algae batch. FAMEs yield per batch can be improved by a long transesterification (two-step process). The purpose of the two-step process in this work was to compare the FAMEs yield against the one-step process yield.

4.7.4 TRANSESTERIFICATION IN SITU: ONE-STEP PROCESS

4.7.4.1 One-step transesterification with no sonication

In this process, biodiesel is produced directly by reacting algal biomass with KOH in methanol. Figure 4.18 shows that fatty acids methyl esters (FAMEs) peaks produced

using one-step transesterification are similar to those produced in two-step transesterification.

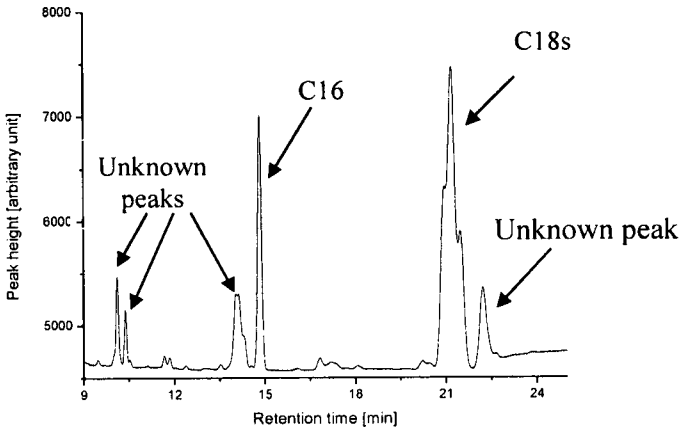


Figure 4.18: FAMEs produced through one-step process on a stirrer.
2.6 g of dry algae transesterified in 30 minutes transesterification

The results in both the one-step and two-step transesterification show that Chlorella Salina grown in this project is rich in C18s fatty acids or lipid (oil). Thus, C18s are the dominant FAMEs followed by C16 as shown in Table 4.16.

Miyamoto (1997) stated that lipids with high number of carbon are formed toward the end of exponential phase during algae culture. The formation of lipid with long chains of carbon extends in the stationary phase. Miyamoto also stated that light enhances the formation of polyunsaturated fatty acids C16 and C18 as well as other lipid such as mono and di-glactosyl-diglycerides, sphingolipids and phosphoglycerides in Chlorella vulgaris and Euglena. It may be inferred that extending stationary phase would increase lipid content in Chlorella Salina. This hypothetical observation would require profound study in future work.

Unknown FAMEs are found also in biodiesel produced through in situ transesterification. Thus one-step process is comparable to the two-step process. It can be seen from Figure 4.18 that sample transesterified in one-step process (in situ transesterification) has a good resolution on unknown peaks (with high magnitude) compared to the sample transesterified in the two-step process, Figure 4.16. The unknown FAMEs peaks considered in this work are the follow: small peak with retention time of 14 minutes termed as unknown 1 and another small peak with retention time of 22 minutes termed as unknown 2. The unknown peaks with retention times of 10, 12, 16 and 19 minutes were very small with areas values out of calibration curve range. Thus those peaks were not quantified.

None of the GC Standards analyzed during this investigation had the retention times of 14 and 22 minutes. As a result, the two unknown peaks (unknown 1 and 2) were quantified, but not identified. Hence, they did not have a specific number of carbons, or number of double bonds attributed to them. It can be assumed that the unknown FAMEs peaks with retention time less than 15 minutes contain chain of carbon less than 16 (C16). Similarly, the unknown peak with retention greater than 21 minutes has long chain of carbon greater than 19.

Table 4.16 lists the yield of each FAME found in the transesterified algal sample through the one-step process. The total FAMEs yields of all 4 runs of Table 4.16 are displayed in Figure 4.19.

Table 4.16: Identified FAMEs biodiesel produced through transesterification in situ using a magnetic stir plate.

Batch	Reaction time [hr]	FAMEs concentration [mg of FAME per g of dry algal biomass]				
		Unknown 1	C16	C18	Unknown 2	Total FAMEs
Run 1	24	0.01	0.046	3.048	0.029	3.133
Run 2	1/2	0.023	0.029	0.136	-	0.188
Run 3	1/2	0.147	0.163	0.777	0.079	1.166
Run 4	1/2	0.092	0.106	0.501	0.030	0.729

The differences in FAMEs yield for runs 2, 3 and 4 (from Table 4.16) can be attributed to the presence of moisture, although they may be other unknown causes triggering lower yield in these runs. Transesterification times for these runs are 24 hours (for run 1) and 30 minutes (for runs 2, 3 and 4).

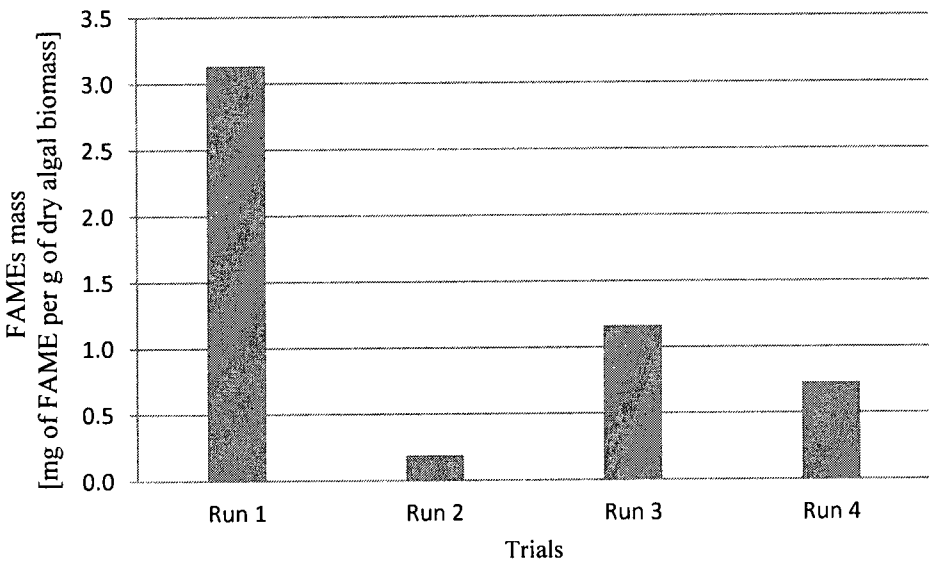


Figure 4.19: FAMEs yield for 4 batches produced through one-step process on a stirrer.

Biodiesel for all four runs of Figure 4.19 was produced using algal biomass from same batch. It is important to note that the reaction time for run 1 was 24 hours, but the remaining trials (run 2, 3 and 4) had a reaction time of 30 minutes. Clearly, the reaction time affects FAMEs yield.

On one hand, the in situ transesterification reduces the biodiesel production time and minimize excessive use of solvent such as n-hexane, chloroform and methanol, which are hazardous and toxic. On the other hand, mixing mechanism using magnetic bar with stirrer may not be sufficient to break Chlorella wall and release algal oil needed to produce biodiesel. Therefore, the in situ process was further explored by using an ultrasonicator to break cells wall, provide thermal energy (heat) and mix well reagents (dry algal biomass and 0.1 N KOH in methanol) engaged into the transesterification reaction

4.7.4.2 One-step transesterification with sonication

It was observed early in the two-step process that increasing the reaction time (transesterification time) results in high Fames yield. This observation was further studied in one-step process by changing the reaction time. In addition, a sonicator was used to enhance the release of lipids from Chlorella Salina dry biomass in order to increase FAMEs yield. Results in Table 4.17 showed FAMEs yield (mg of FAMEs per g of dry algal biomass) produced through in situ transesterification (one-step process) for different reaction time.

Table 4.17: Identified FAMEs produced through transesterification in situ using an ultrasonicator. *Batches with the same letter are replicate performed for reproducibility purpose. For example, A1 and A2 were performed exactly in the same manner; A2 is a replicate of A1*

Batch	Reaction time [minute]	FAMEs yield [mg of FAME per g of dry algal biomass]					Volume of Chloroform used [ml]
		unknown 1	C16	C18	Unknown 2	Total FAMEs	
A1	30	0.068	0.119	0.475	0.076	0.737	15
A2	30	0.111	0.217	0.913	0.070	1.311	15
B1	30	0.118	0.234	1.154	0.101	1.606	
B2	30	0.505	0.602	2.803	0.137	4.047	
B3	30	0.508	1.018	3.243	0.098	4.867	
C1	60	0.055	0.048	0.354	0.039	0.495	10
D1	20	0.146	0.200	0.991	0.031	1.368	
D2	20	0.403	0.509	2.675	0.093	3.679	
D3	20	0.219	0.286	1.504	0.044	2.053	
E1	10	0.153	0.184	0.881	0.038	1.256	
E2	10	0.180	0.185	0.965	0.060	1.390	
E3	10	0.141	0.158	0.798	0.045	1.141	
F1	5	0.146	0.108	0.572	0.0	0.826	
F2	5	0.223	0.172	0.934	0.031	1.360	
G1	1	0.095	0.145	0.388	0.079	0.706	
G2	1	0.071	0.059	0.430	0.056	0.616	

Table 4.17 shows that C18 FAMEs in each run (batch) still is dominant of all FAMEs found in the algal biodiesel. Table 4.17 also shows that sonicating the mixture of algal dry biomass and 0.1 N KOH in methanol increases FAMEs yield compared to FAMEs yield in Table 4.15 for in situ transesterification with mixing (agitation) done using a magnetic stirrer.

Same pattern can be seen in Figure 4.20; FAMEs yield for batches B are higher compared to the yield in Table 4.15 with FAMEs produced through in situ transesterification with

the same reaction time of 30 minutes with different mixing mechanism. Results for batches B were obtained using ultrasonicator as mixing mechanism whereas results in Table 4.15 were obtained using magnetic stirrer as mixing mechanism.

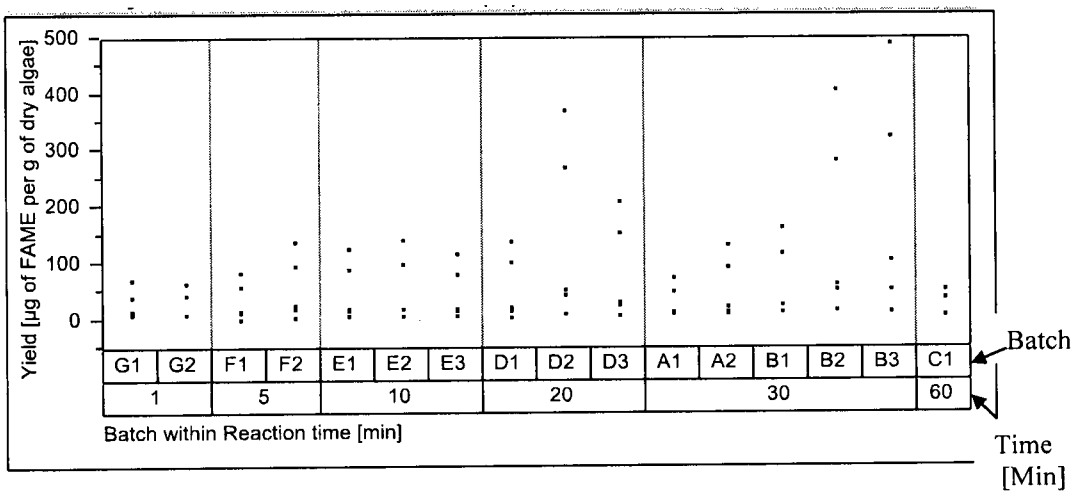


Figure 4.20: JMP output of time effect on FAMEs yields in one-process.
A letter indicates batch name and numbers indicate replicates batches; for example, G1 and G2 indicate batch G, with two replicates (replicate 1 and 2). Dots within column represent a specific FAME yield (µg of FAME per g of dry algae) with no specific identification. A quick comparison of FAMEs yield can be established based on this figure.

4.7.4.3 One-step transesterification with sonication plus chloroform.

Biodiesel was produced through in situ transesterification by adding chloroform ($CHCl_3$) to reagents (algal biomass and methanol). Chloroform was used to enhance the release of algal lipid. Figure 4.20 shows the effect of chloroform on FAMEs yield.

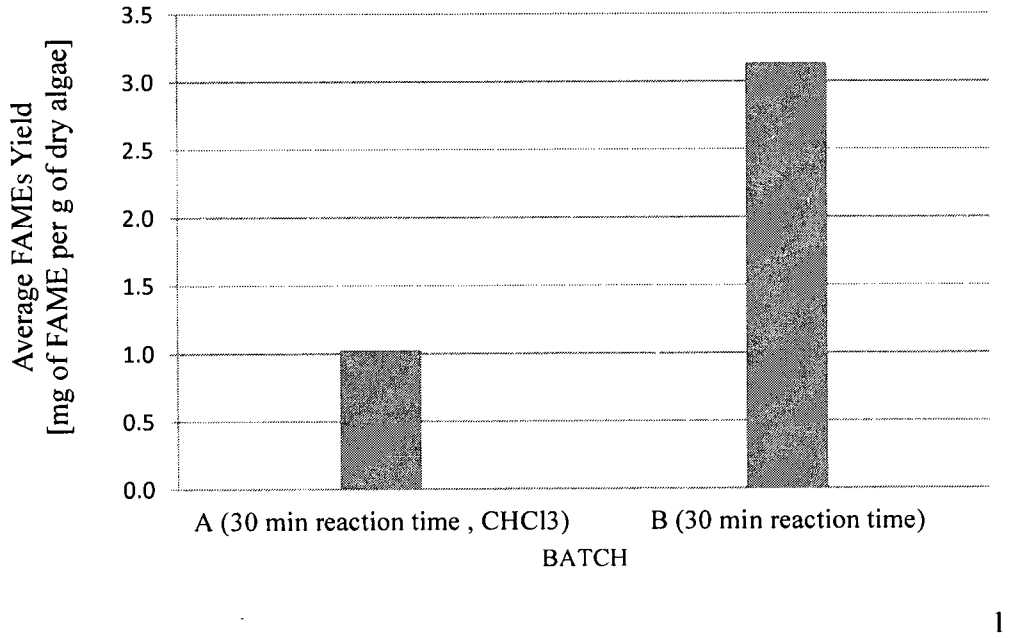


Figure 4.21: Chloroform effect on FAME yields produced in one-step process with an ultrasonicator.
30 minutes of transesterification. Data from Table 4.1.7

Clearly, in situ transesterification with chloroform has low FAMEs yield compared to the FAMEs yield in the sample transesterified without $CHCl_3$. It can be inferred that Chloroform does enhance the increase of FAME yield. Thus, the ultrasonication process is sufficient in breaking Chlorella wall and releasing algal lipid needed for transesterification reaction.

4.7.4.4 Reaction Time effect on FAMEs yield in one-step process

The effect of reaction time on the FAME yield of one-step process using ultrasonication was studied. Six different runs were made with reaction time of 1, 5, 10, 20, 30 and 60 minutes. The total FAME yields are shown in Figure 4.22.

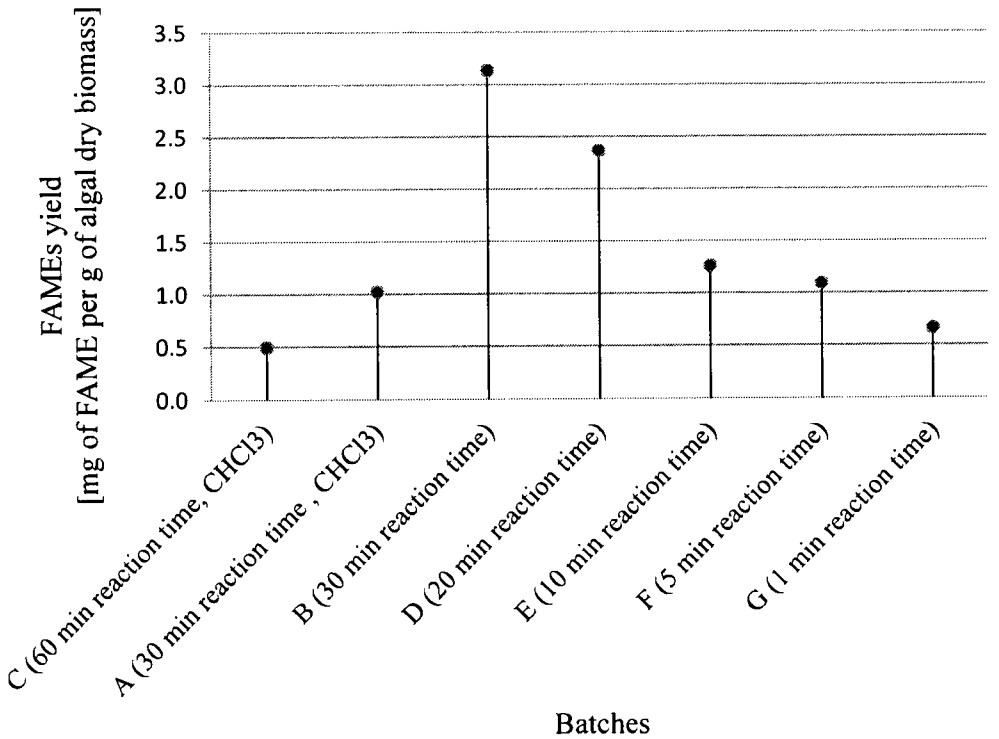


Figure 4.22: Effect of time on average total FAMEs yield, in situ transesterification.

In Figure 4.22, batch C (60 minutes of transesterification adding chloroform, in situ process with ultrasonicator) has the lowest yield of FAME produced in this investigation. Thus, lengthening the reaction time decreases FAME yield in one-step transesterification using ultrasonicator. The decrease in yield FAMEs can be due to solvent evaporation, for as the sonication proceeds, sonicator probe provides heat (thermal energy) to the reagents increasing the temperature up to 50 °C.

Ferrentino (2007) found that lengthening the transesterification time in one-step process decrease FAMEs yields, which agrees with observation made in this investigation.

Figure 4.22 also shows that FAMEs (biodiesel) can be produced through one-step process even within 1 minute of reaction time. In addition, a high FAMEs yield can be obtained

through in situ transesterification in 30 minutes or half hour (batch B) without adding $CHCl_3$. On the other hand, batch D performed in only 20 minutes through one-step process using ultrasonication shows high yields of FAMEs similar to the yields obtained with 30 minutes of transesterification in the same conditions. Therefore, 20 minutes transesterification time is the ideal for this investigation.

Thus, in situ transesterification shows high yield of FAMEs (biodiesel) for a reaction time of 20 minutes without the use of chloroform or n-hexane in lipid extraction, compared to the yields obtained in two-step (conventional) transesterification in 24 hours. The composition of FAMEs produced using in situ process is shown in Figure 4.23. These results agree with the composition of FAMEs found using the two-step process. Clearly, C18s is the majority of total FAMEs found in biodiesel from Chlorella Salina.

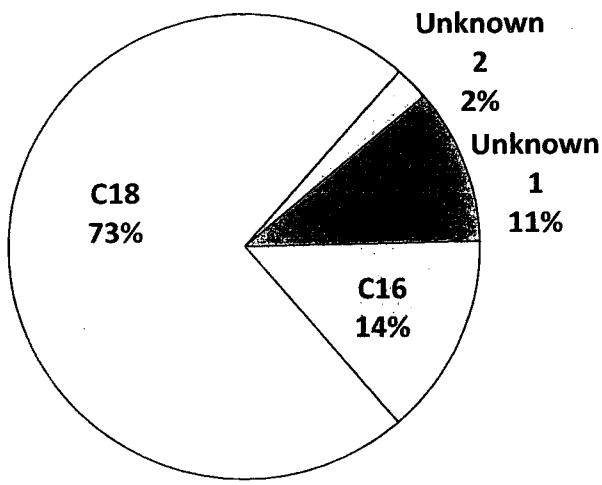


Figure 4.23: Composition of FAMEs produced using one-step process.

4.7.4.5 Analysis of B100

Neat B100 was diluted in chloroform at 1:100 in chloroform (1 volume of B1000: 100 volume of chloroform). 1 µl of diluted sample was injected in the HP GC and the output results are as follow.

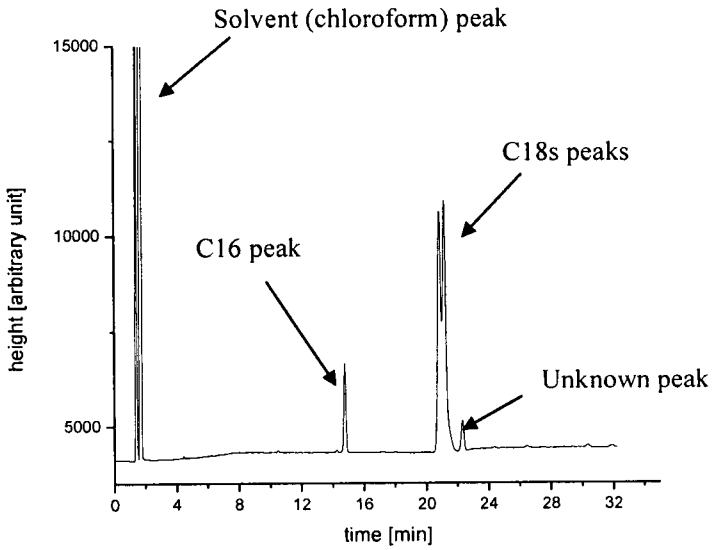


Figure 4.23: FAMEs in B100 from WVO

Table 4.18: FAMEs compositions in B100 produced from WVO

Peak	Area	Concentration [mg/ml]
C16	370.78193	0.0285
C18s	3359.64409	0.443
Unknown peak	189.29302	0.012

Results in this work (for total FAMEs per batch) varied from 0.4 mg to 4.9 mg per g of dry algae biomass.

Based on Table 4.18 the total FAME concentration in B100 is 0.483 mg/ml of B100. Assuming a B100 density of 0.85 g/ml (or 850 mg/ml) we obtain 0.57 mg lipid/mg B-100, so the B-100 has 57% lipids that we detect on our GC.

CHAPTER V

CONCLUSIONS

This investigation was conducted to design and construct a Tubular Photobioreactor that can be used to culture a suitable microalgae strain rich in lipid. This lipid can be converted into biodiesel through in situ transesterification termed as one-step process. The following can be concluded from this work:

1. Chlorella termed Salina was selected from 8 algae strains screened for fast growth and high lipid content.
2. A tubular photobioreactor (TPBR) was designed and constructed. It was run for more than 6 months without any operation problems. This TPBR was characterized as followed. The compressed air containing CO_2 was supplied to the reactor at a pressure ranging between 5.4 psi and 5.8 psi with a liquid flow rate of 285.7 ml/s or 0.075 gal/s. The broth culture had an average viscosity of 0.88 cp. It was flowing in the tubing at 0.6 m/s in a turbulent regime (Re = 12,955).
3. Chlorella Salina was successfully cultured in this TPBR using a modified fresh medium, which had double concentration of NaCl (at 0.2 M) and KNO_3 (at10.4 mM). A healthy culture of Chlorella Salina grown in this reactor reached a broth concentration of $60*10^6$ cells per milliliter of fluid culture (broth). All cultures performed in this work started with a diluted broth at 0.1 g of dry algal biomass per

liter of broth culture and reached an average of 1.1 g of dry algal biomass per liter of broth in two weeks of culture period. The yield in algal biomass was 1.0 g of dry algal biomass per liter of broth culture.

4. The maximum growth rate of 0.4/day in average was determined using Huesemann et al. (2009) approach as kinetic model.
5. We were able to harvest cultures performed in this TPBR by using flocculent technique to precipitate algal cells followed by centrifugation technique and lyophilization. The dry algal (Chlorella Salina) biomass could contain up to 20 % of lipid (20 g lipid per 100 g of dry biomass) able to be converted into biodiesel through transesterification process.
6. We were also able to convert Chlorella Salina lipid into biodiesel (FAMEs) through in situ transesterification or one-step process. The one-step transesterification was performed using ultrasonication technique (sonication) to produce up to 3.679 mg of FAMEs per g of dry algal biomass within 20 minutes compared to the two-step transesterification which takes hours even days to produce the same amount of FAMEs. The in situ transesterification or one-step process is fast, easy to perform and eliminate excessive use of solvent especially n-hexane and chloroform..
7. Transesterified samples were analyzed in HP 5890 Series II GC equipped with RESTEK capillary column. Data captured by an HP integrator connected to the HP GC were transferred to a laptop equipped with OriginPro version 8.1. We successfully analyzed data in OriginPro version 8.1 and found that C18 FAMEs were the most dominant in biodiesel produced from Chlorella Salina biomass. The biodiesel contained also C16 and 4 unidentified FAMEs. Two of these unknown FAMEs were

quantified and the two remaining were not quantified due to their low concentration (being out of calibration range). The two unidentified FAMEs with low concentrations were considered as traces.

CHAPTER VI

RECOMMENDATIONS FOR SUCCESSFUL CULTURE AND PRODUCTION OF BIODIESEL

The following recommendations are made in order to perform successful culture using the tubular photobioreactor (TPBR) constructed in this work and produce biodiesel through in situ transesterification:

1. To double the concentration of NaCl and KNO_3 to 0.2 M and 10.4 mM respectively in fresh medium used to culture Chlorella Salina.
2. To clean the TPBR with bleach followed by several rinse with tape water and reversed osmotic (RO) water respectively to remove any algae stuck on the tubing wall and avoid contamination of the next batch culture.
3. To inoculate the culture at a ratio of 1:10 (1 volume of inoculum for 9 volume of medium) to maximize algal biomass yield. Starting with much diluted broth culture tends to lead to culture collapse.
4. To dry transesterified samples with an appropriate hydrophobic substance such as sodium sulfate anhydrous (Na_2SO_4) to avoid FAMEs alteration. This process is done prior sample analysis into GC.
5. To avoid long reaction times for in situ transesterification. They tend to decrease FAMEs yield.

CHAPTER VII

FUTURE WORK RECOMMENDATION

The following investigations need to be studied in details in order to addressed questions or some difficulties encountered all along the present work. However, most of these questions and/or difficulties were not among the main goals of the present work.

1. An investigation of culture collapse or failure mode needs to be conducted. This work will allow us to understand causes of cultures collapse and suggest eventually ways to prevent its occurrence.
2. More work is needed to study light effect on cultures performed in this TPBR and find optimum light intensity for maximum algal biomass and algal lipid yields.
3. A study needs to be conducted to determine all limiting factors on algae cultivation in this TPBR and define kinetic models that will characterize algae growth in this TPBR. It is important to understand the kinetic of algal cells in this TPBR, which will give us a complete understanding of the best harvesting time (BHT). Kinetic models are required for both cells growth and lipid content. Some algae strains grow fast but produce low lipid or vice versa. If the kinetic models for both cell growth and lipid production are known, their common

denominator will define the BHT. It may help in selecting an improved algae strain for biodiesel production.

4. More work is needed to study in depth the lipid metabolism. Not only this study will allow us to understand a formation of lipid within algal cells, but it will also give us an insight on the paths of lipid production within algal cell. This is an important work, which can serve as basis to modify genes sequences of algal cells for high lipid production.
5. The algae harvesting process requires more study to eliminate the use of flocculent and lyophilization (freeze-drying). The lyophilization process is suitable for laboratory work, but it may increase the production cost of biodiesel at industrial scale. It is imperative to find different way to harvest algal broth and dry the algae pellet in a time and cost effective ways.
6. More investigation is needed to study a scale up of the TPBR by increasing the sizes (length and diameter) of tubing. It is important to assess the effect of the tubing sizes on algae growth, which will allow us to know if this type of reactor can be used at industrial level.
7. A profound investigation is needed to refine the biodiesel produced from microalgae and compare the final product with B100 from WVO or soybean. The present work was conducted on crude algal oil extracted from crude algal biomass that may contain impurities. The elimination of impurities should improve the quality of the algal biodiesel.

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APPENDICES

APPENDIX I

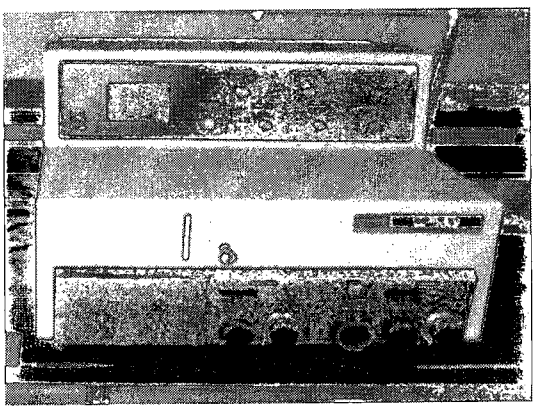
SPECTROFLUOROMETER SETUP

Model: Varian SF-330 Spectrofluorometer

Read mode: 0.25	Tile Constant: 1
Sensitivity: x100	Zero Adjust: 0
Selector: x1	Variable: 0
Excitation: 525 nm	Slit 1 (left): 3 nm
Slit 2 (right): 20 nm	Emission: 575 nm

This spectrofluorometer was run as follow:

Turn the power on and wait for about 5 minutes for stability. The needle should be on green region.

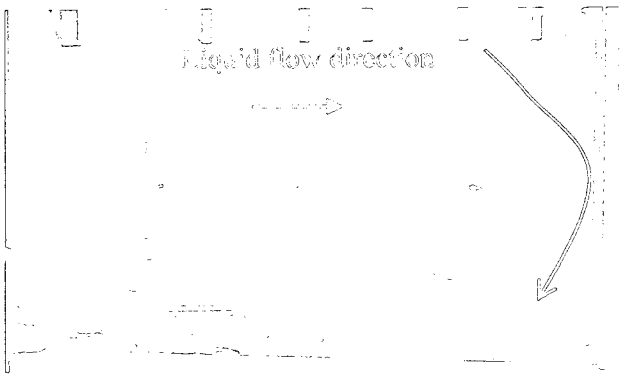


Spectrofluorometer Varian SF-330

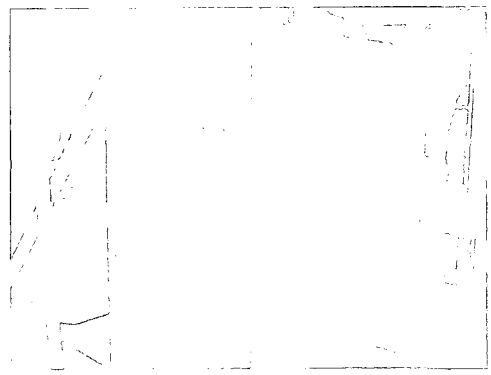
APPENDIX II

PICTURES OF TUBULAR PHOTOBIOREACTOR CONSTRUCTED IN THIS WORK

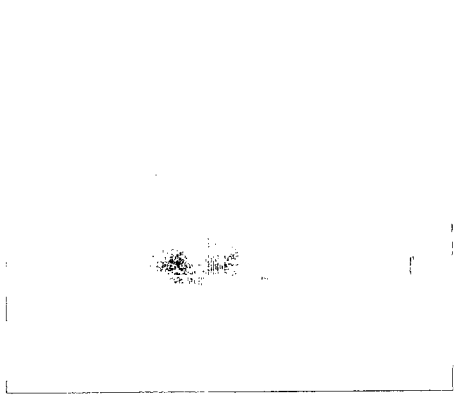
1. Construction phase



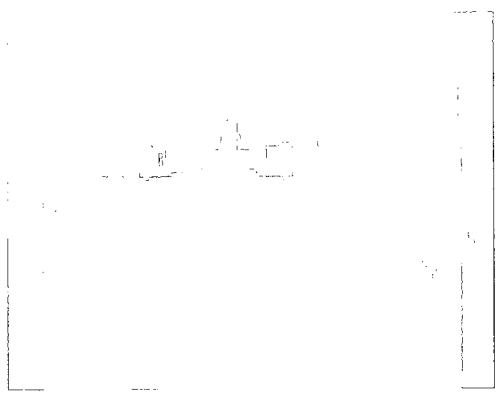
Tubing setup



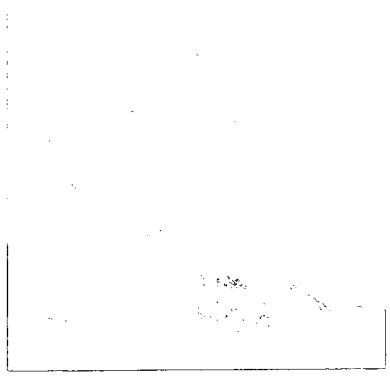
Fluorescent lamps setup



Pump used to propel liquid

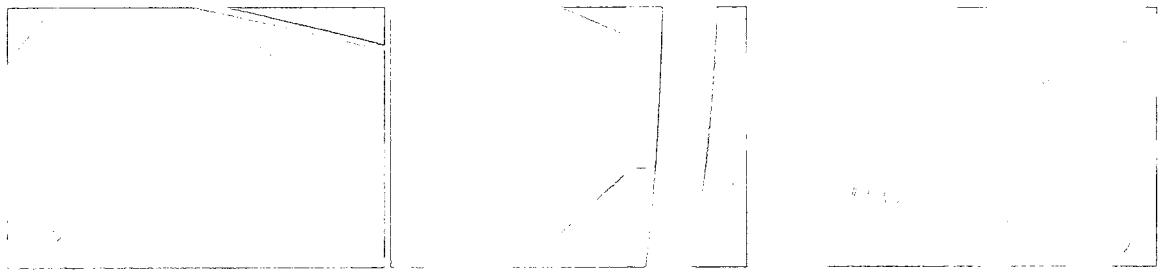


Mazzei nozzle and bypass line

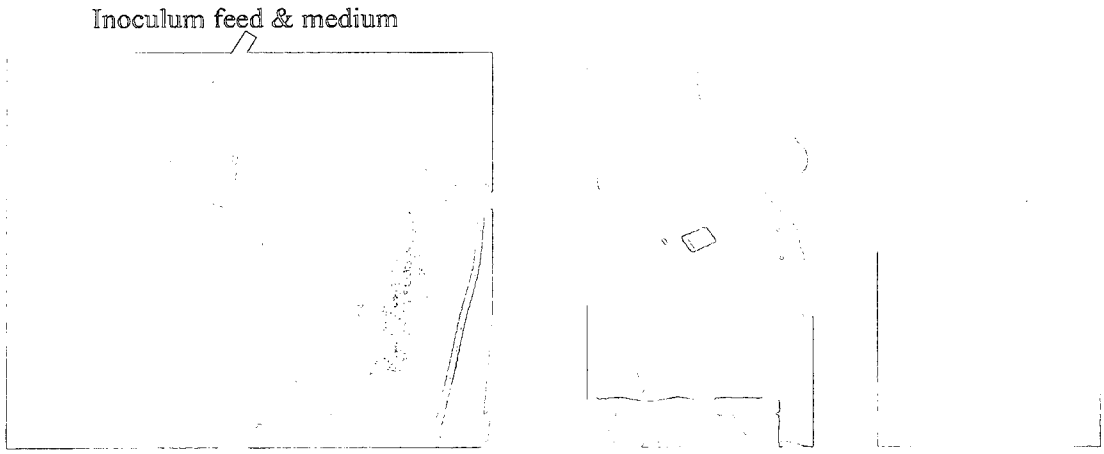


Air pressure gauge

2. Culture phase

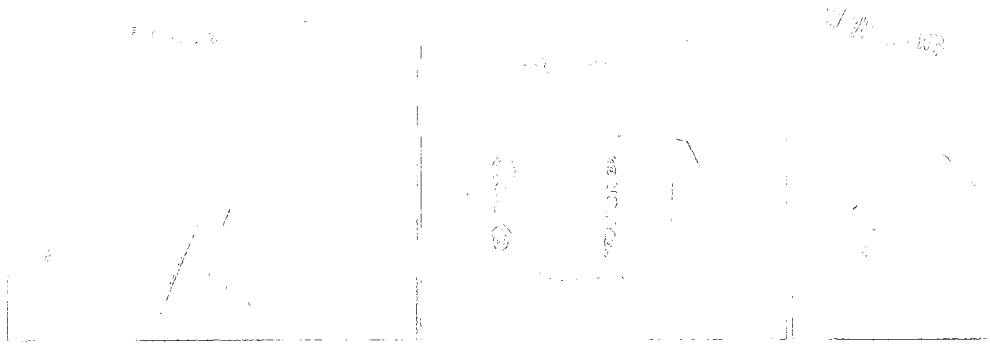


First day of inoculation | A week of culture period | Two weeks of culture period.



Main tank | Collected algae broth at the end of 12 days culture period | Dry algal biomass

3. Light measurement using Cooke light meter



APPENDIX III

GC HP PEAK96 SETTING

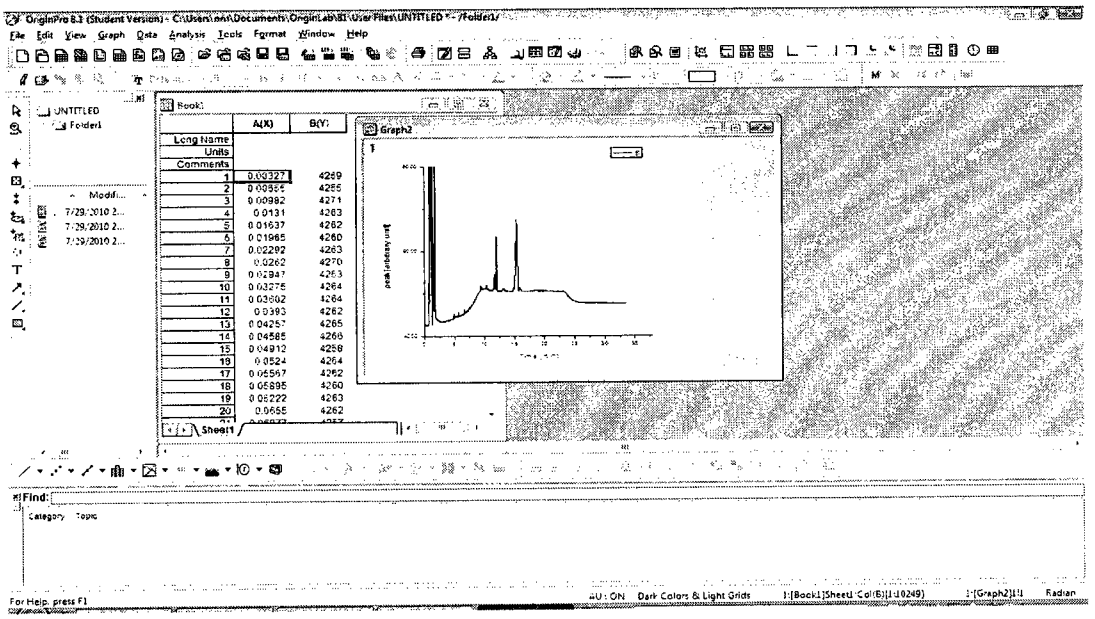
Peak96 Data Transfer Procedure

1. Run Peak 96 on computer that is connected to running HP 3396 Integrator.
2. Set integrator setting to save signal file to storage device M, bunched data
3. Perform GC run per HP 5890 manual instructions, controlling start of run from GC keypad
4. After run has ended and signal file is closed (will be displayed on integrator printout), use Peak 96 to transfer file
5. Go to Utilities: Transfer : Integrator-to-PC, hit enter
6. File options to transfer will include "Signal.BNC," select this and hit enter
7. After file has transferred (1-2 minutes), go to Utilities: Files: Rename, hit enter
8. From the list of files, select "Signal.BNC" and rename- making sure to add the ".BNC" extension
9. Next go to Utilities: Files: Export
10. Select file/files to export and export them to the default (Export1) directory
11. In windows, files can be transferred through e-mail by attaching exported files

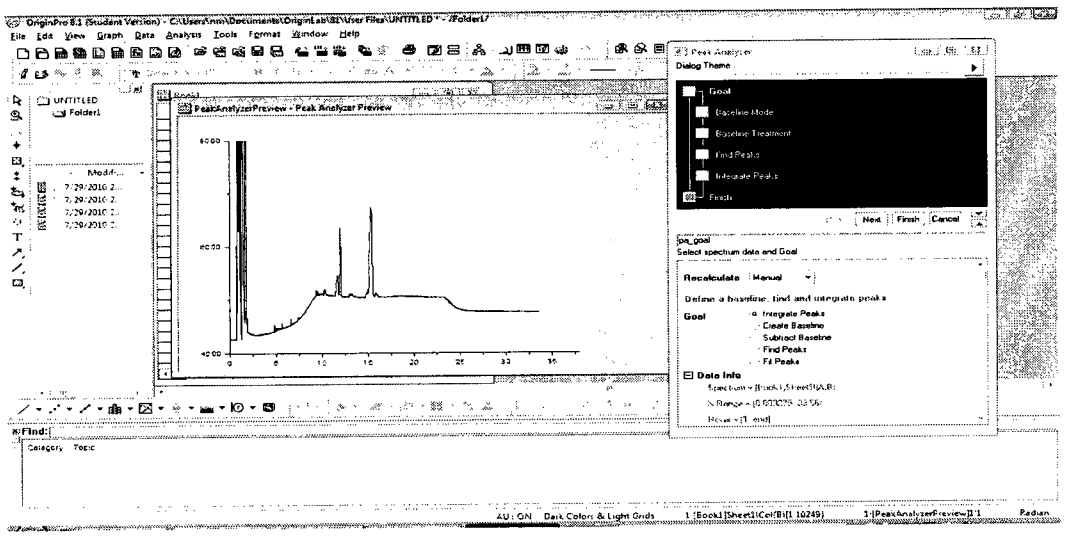
APPENDIX IV

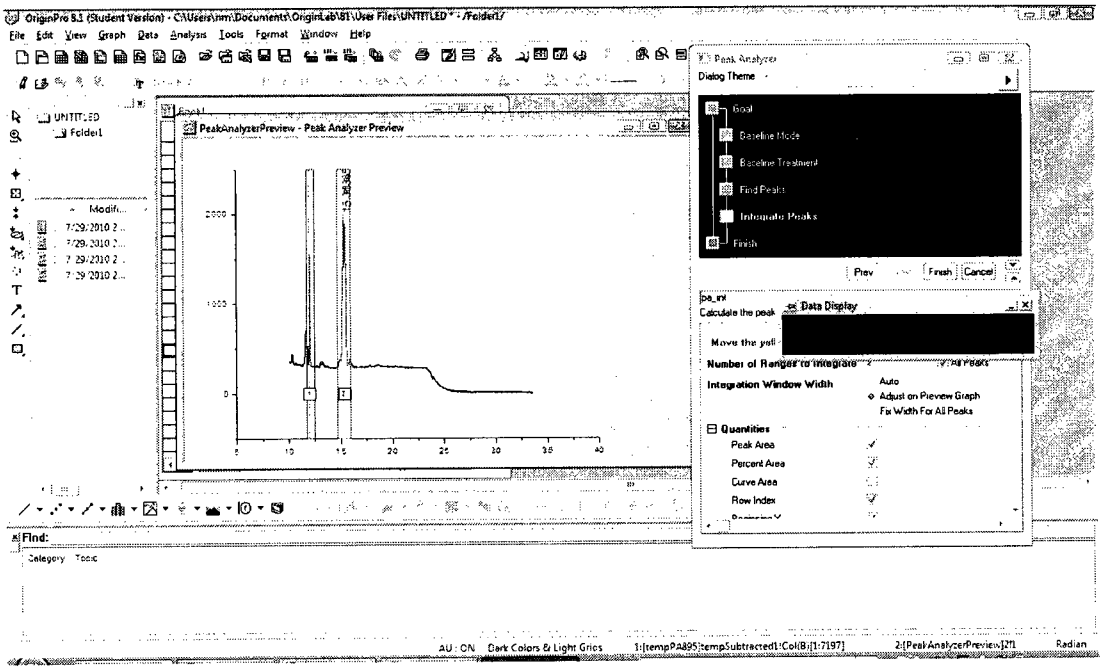
EXAMPLE OF ORIGINPRO OUTPUT

1. Plotting the GC data in OriginPro 8.1

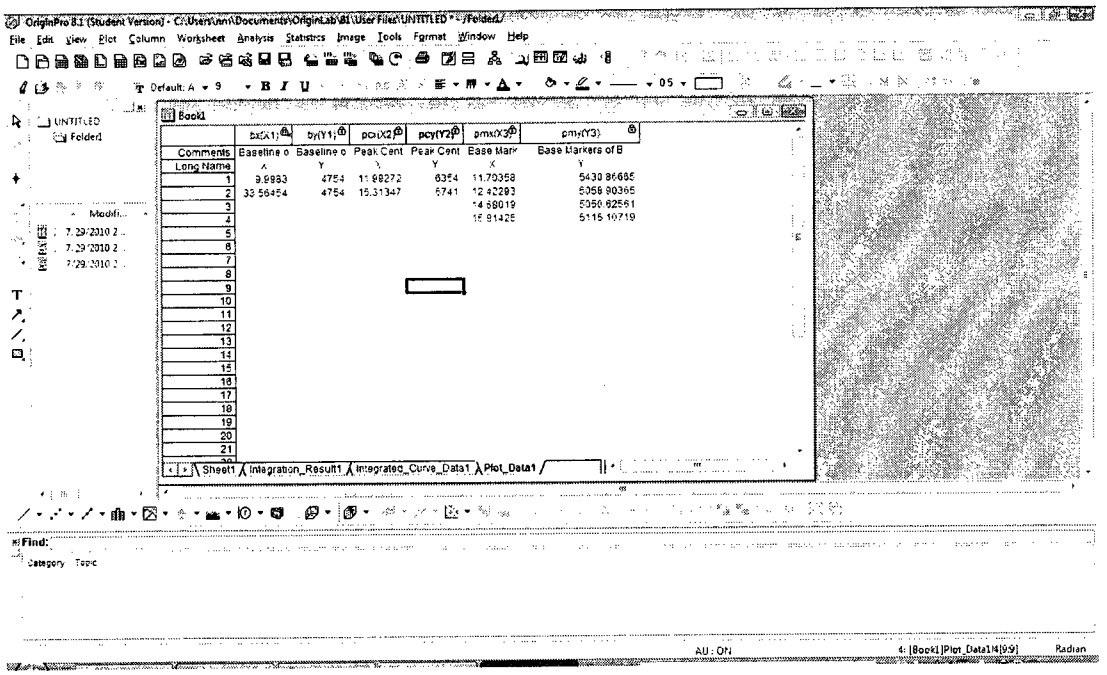


2. Manual integration of FAMEs peaks

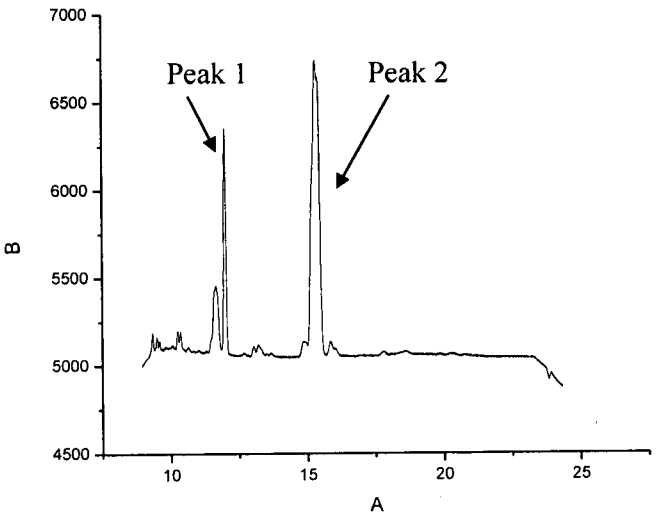




3. Data display



4. Chromatograph



5. Table of Data output

	Peak 1	Peak 2
Integrated area	**373.92912**	**943.34248**
Area integrated P (%)	7.04617	17.77598
Row index	609	1623
Beginning X	11.70453	14.68142
Ending X	12.42173	15.91278
RWHM Center	0.10699	0.3684
Peak Center	11.99272	15.31347
Height	1600	1987

APPENDIX V

RESULTLS OF LIPID CONTENT USING RED NILE METHOD

1. Fluorescent readings for Chlorella sp. and Salina

Chlorella sp	Chlorella Salina
-0.023	-0.021
-0.015	-0.02
-0.014	-0.016
-0.014	-0.019
-0.09	-0.017
-0.011	-0.016
-0.012	-0.017
-0.015	-0.011
-0.013	-0.017

The lipid content results for the two strains of Chlorella are not conclusive, for a negative signal does give indications of good binding between lipid and the Red Nile.

APPENDIX VI

EXTENDED ABSTRACT

PRODUCTION OF BIODIESEL FROM MICROALGAE

By

NKONGOLO MULUMBA

University of New Hampshire, August, 2010

Biodiesel production from microalgae is a promising technique, with advantages of high biomass yield with high lipid content. Challenges include effective techniques to grow microalgae and harvest the grown microalgae, extraction of the algal oil and its transesterification to biodiesel. The goals of the present research are to design a photobioreactor (PBR) able to grow and maintain a healthy algal culture, and then produce biodiesel from the harvested algal biomass through an integrated process called in situ transesterification.

A microalgae strain was selected from 8 different species screened for growth rate and lipid content. This strain was cultured in a modified nutrient medium in which the concentrations of sodium chloride and potassium nitrate were doubled to expedite the production of algal biomass rich in lipid. Techniques were developed to monitor growth and harvest algae, obtain dry algae biomass, extract and quantify the microalgae lipids, and biodiesel production. A simple kinetic model for the algae growth was developed, which indicated that the microalgae doubling time is about 2.5 days.

A Tubular Photobioreactor (TPBR) was designed and constructed to study microalgae growth. Water and air flow studies were done to determine the average residence time and the stable operating regions of the TPBR. The selected microalgae strain was cultivated in this TPBR to assess its functionality. Cultures were monitored over time by measuring the pH and the concentrations of nitrate/nitrite and algal biomass. The TPBR produced 1g of dry algal biomass per liter of medium within 12 days, with lipid content up to 20%. Healthy algal culture grew well in the TPBR reaching about $60x10^6$ cells/ml of culture medium. The TPBR achieved a ten times increase in algae concentration, which is higher than those reported for open ponds and helical PBR. Lyophilized algal biomass was successfully converted into fatty acids methyl esters (FAMEs) or biodiesel through in situ transesterification or one-step process using methanol, alkaline catalyst (potassium hydroxide) and an ultrasonicator. The resulted FAMEs were analyzed in a gas chromatograph equipped with a Restek FAME column. It was found that C18 methyl esters were the most dominant FAMEs in biodiesel produced from the selected microalgae. Approximately 3.679 mg of FAMEs per g of dry algal biomass were produced in 20 minutes. The in situ transesterification reduces production time by eliminating the algae oil extraction process and the long transesterification time compared to the conventional transesterification which produced 1.2 – 2.3 mg of FAMEs per g of dry algal biomass in one day.