



# Microalgae to Energy

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## Research Article

Volume 4 Issue 2

Received Date: March 21, 2020

Published Date: June 01, 2020

DOI: 10.23880/ppej-16000223

## Abstract

Microalgae, an organism which is considered as a potential source of biofuel from the last decade endowed with excellent capability of CO<sub>2</sub> capture and sequestration, water treatment, prolific growth rate and enormous energy content. The Soxhlet extraction of lipids from microalgae (*Chlorella Vulgaris*, *Nannochloropsis* sp., and *Thalassiosira weissflogii*), was carried out with several solvents (methanol, ethanol, isopropanol, acetone, and hexane), pure and mixtures, in order to optimize the extraction process. For the paper, the highest amount of lipid was obtained using a combination of methanol and acetone or methanol alone. The extract liquid fractions were treated with activated carbon to remove the green pigment. Attempts to in situ algae oil transesterification were accomplished using acid (H<sub>2</sub>SO<sub>4</sub>) and base (NaOH and CaO) catalysts. The extend of extraction processes was assessed by infrared spectroscopy.

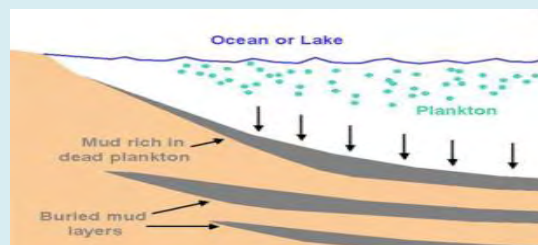
**Keywords:** Renewable energy; Microalgae; Catalysts; Lipid extraction; Catalysis; In situ transesterification

## Introduction

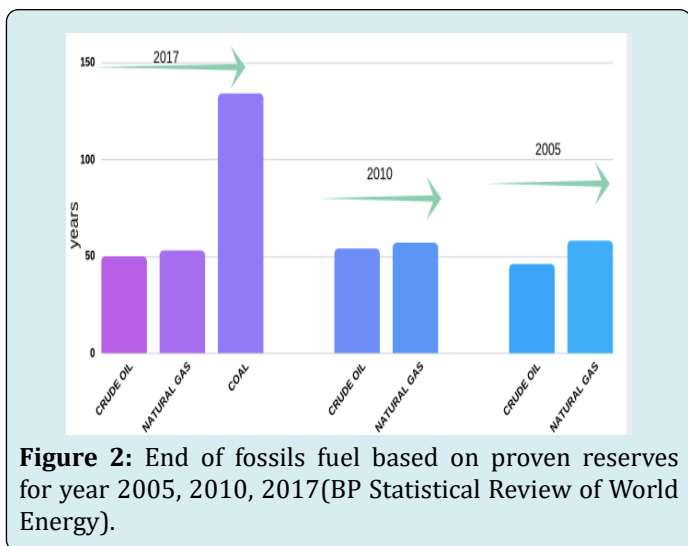
The petroleum reserves of today were simply a legacy of phytoplankton that grew over hundreds of millions of years ago, which were subjected to suitable conditions such as high pressure, temperature, anoxic conditions, and suitable formations and traps. The Present-day descendants of these sources of fossil energy, microalgae, still possess this unique ability to produce the same energy rich compounds that made their ancestors important to the development seen in our modern society. The that chemical engineers and energy technologist can still explore the ability of microorganisms that undergo photosynthesis has gained much interest recently amongst the scientific community [1] (Figure 1).

From the angle of renewability, dirty fuels like coal, oil, and gas are quite good and easy sources of energy and burning them to meet the societal energy demand is also a mature technology. However, this is plagued with a major problem: fossil fuel resources are finite and not renewable. Biomass, on the other hand, grows and is renewable.

Accordingly with data from BP Statistical Review of World Energy, 2018, shows that the proven reserves are currently: Coal - 1,139 billion tonnes, Natural Gas - 187 trillion cubic meters, and Crude Oil - 1,707 billion barrels, and based on this data matching with current consumption, the remaining life for coal would be 134 years, 53 years for Natural Gas, and 50 years for Crude oil, so in short, the proven reserves would soon be out by the years 2169, 2068 for coal and natural gas, respectively, and 2066 for Crude Oil (Figure 2).



**Figure 1:** Process of oil formation from phytoplanktons millions of years ago.



**Figure 2:** End of fossil fuel based on proven reserves for year 2005, 2010, 2017 (BP Statistical Review of World Energy).

## Aim and Objectives

The goal of this paper is to investigate the feasibility of obtaining biofuel from 3<sup>rd</sup> generation biomass, the factors affecting the technical and economic feasibility of obtaining a cost-effective energy (biofuel) from Microalgae, and the factors hindering the commercial viability.

This dissertation was carried out in the context of the project new processes for fuel production for road transportation (PTDC/EMS-ENE/4865/2014).

## Microalgae

Microscopic algae, also called microphytes, are simply unicellular organisms which are ubiquitous, their presence could be seen in any kind of water varying from freshwater to marine systems depending on the species primarily in pigment composition, biochemical constituents and life cycle, size which could vary from a few micrometres ( $\mu\text{m}$ ) to a few hundred micrometres [2].

## Types of Microalgae

In their research Deng [3], observed that microalgae can be found in a large range of places where light and water are present including ocean, lake, soils, ice, and rivers and that Microalgae can be divided into categories depending on their pigmentation, biological structure and metabolism.

## Size Classification

Microalgae are small organisms, which can be divided into 4 size categories as the microplankton (20 to 1000  $\mu\text{m}$ ), the nanoplankton (2 to 100  $\mu\text{m}$ ), the ultraplankton (0.5 to 15  $\mu\text{m}$ ) and the picoplankton (0.2 to 2  $\mu\text{m}$ ) [4]. Their small size allows them to do an effective photosynthesis, converting light energy with  $\text{CO}_2$  dissolved in water to produce lipids, carbon hydrates, proteins, etc. [5].

## Taxonomic Groups

There are 4 main taxonomic groups that microalgae can be classified into which are:

1. Diatoms (Bacillariophyceae),
2. Green algae (Chlorophyceae),
3. Cyanobacteria or blue green algae (Cyanophyceae) and
4. Golden algae (Chrysophyceae).

However, 6 other groups of microalgae exist, which includes the yellow-green (Xanthophyceae), golden algae (Chrysophyceae), red algae (Rhodophyceae), brown algae (Phaeophyceae), dinoflagellates (Dinophyceae), Prasinophyceae, and Eustigmatophyceae [6].

However, unlike the other groups of microalgae, which have lower lipid content and productivity compared with green algae or diatoms, *Botryococcus* sp., *Dunaliella* sp. and *Chlorella* sp. are examples of green algae taxonomic group that are reported to be the most promising species of microalgae for biodiesel production [7] (Table 1).

Microalgae	Known Species	Storage Material	Habitat
Green Algae	8 000	Starch and TAGs	Freshwater
Blue green algae	2 000	Starch and TAGs	Different habitats
Golden Algae	1 000	TAGs and carbohydrates	Freshwater
Diatoms	100 000	Carbohydrates and TAGs	Oceans, fresh and brackish water

**Table 1:** Four most important microalgae groups in terms of abundance [8].

In comparison to higher plants, microalgae do not have roots, stems, or leaves, but they have higher photosynthetic efficiency than plants or trees. Therefore, microalgae biomass cultivation will help in reducing concentration of  $\text{CO}_2$  in the

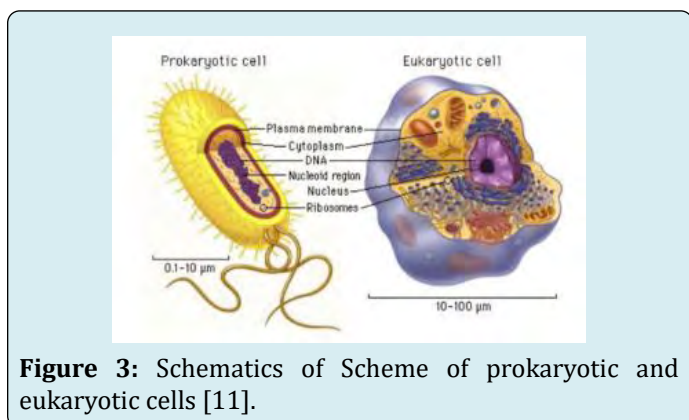
atmosphere at a faster rate than other land-based crops, especially when microalgae are cultivated under optimized environment conditions [9].

Microalgae have received a lot of attention because they grow quiet fast, adapt to their environment easily, and their unique ability to concentrate useful chemicals and capture nutrients in an economical way.

### Cellular Microalgae

Cells can be classified in two main groups: eukaryotes and prokaryotes. The distinguishing factor is the presence of nucleus, and the flotation of the DNA in the cell, and these characteristics are absent in prokaryotes.

Microalgae, generally, are consists of eukaryotic cells. These cells therefore have nucleus and organelles. All microalgae have chloroplasts, the bodies with chlorophyll and other photosynthetic pigments which carry out photosynthesis. In Figure 3 it is shown an example of eukaryotic and prokaryotic algae cells with its different dimensions. Examples of prokaryotic microalgae are Cyanobacteria, and of eukaryotic microalgae are green algae, golden brown algae and diatoms [10].



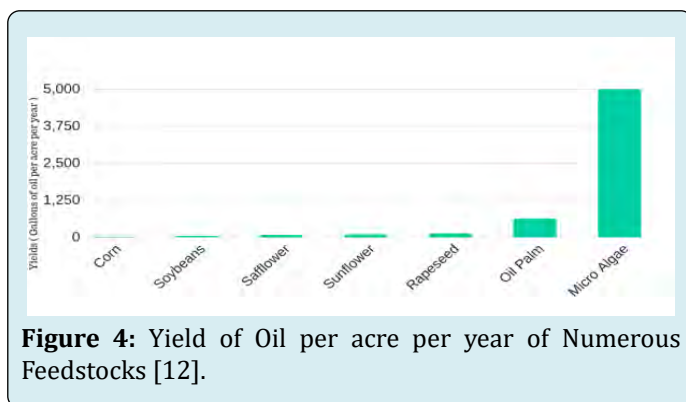
**Figure 3:** Schematics of Scheme of prokaryotic and eukaryotic cells [11].

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Microalgae have received a lot of attention because they grow quiet fast, adapt to their environment easily, and their unique ability to concentrate useful chemicals and capture nutrients in an economical way (Figure 4).

They constitute the most fundamental positions in aquatic ecosystems, and therefore form the basis of food chains. The total biomass they represent is large enough to influence global climate systems. Estimates suggest that some 800,000 species of microalgae exist, yet of these only

50,000 have been documented [13].

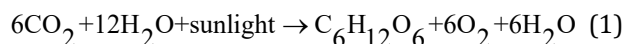


**Figure 4:** Yield of Oil per acre per year of Numerous Feedstocks [12].

Each species is unique, as they can adapt to a particular environment, for example extreme climates, salt levels, pH or light levels [14]. This demonstrates the enormous potential for modification of microalgae, which in turn may lead to an alternative resource pool for today's resource limited world.

### Photosynthesis in Microalgae

A basic understanding of photosynthesis is fundamental for microalgae biotechnology. Just like plants undergo Photosynthesis, it is so interesting to know that microalgae also experience the same redox process (Equation 1), in which carbon dioxide is converted to carbohydrates and oxygen is released as a side product, the water is oxidized (loses electrons) and the CO<sub>2</sub> is reduced (gains electrons).



As the cells have enough energy available for photosynthesis, an accumulation of organic matter inside them takes place. The culture is thus able to reproduce by cellular division and increase the number of cells per volume cells density [15]. On a cellular scale, photosynthesis is also an endothermic reaction with the energy input relying on the pigments inside the cells, meaning that these biomolecules initiate the connection between the incident light and the chemical reactions of this metabolic process.

Depending on the wavelength of the incident light, an amount of electrons from some pigment may get excited, passing through a higher energy level on which they become reactive and may start the photosynthesis reaction [16]. The energy amount required to initiate photosynthesis is the one contained in the photon of wavelength of 680 nm, which simple implies that almost all photons from the Photosynthetically active radiation interval overcomes this value (the energy amount of one photon is inversely proportional to its wavelength, as show in Equation 2 below)

$$E = h \cdot f = \frac{h \cdot c}{\lambda} \quad (2)$$

Where;

$E$  = total energy flux density ( $W/m^2$ )

$h = 6.6256 \times 10^{-34}$  Js = the Planck's constant

$f$  = the frequency in hertz, which depends on  $\lambda$

$\lambda$  = wavelength

Photosynthesis is traditionally split into two stages:

1. The light reactions, and
2. The dark Reactions.

Light Reaction is the primal stage in the oxygenic photosynthesis reaction where water is split in the chlorophyll molecule into ions of  $H^+$  and  $OH^-$  in the grana of chloroplast, in the presence of light resulting in the formation of assimilatory powers such as NADPH<sub>2</sub> (nicotinamide adenine dinucleotide hydrogen phosphate) and ATP (Adenosine triphosphate) [17]. Dark Reaction, the second step of the oxygenic photosynthesis redox reaction, which is independent of light, takes place in the stroma of the chloroplast. It is slower than the light reaction, and in this stage the NADPH and ATP are utilized in the sequential biochemical reduction of carbon dioxide to carbohydrates (Figure 5).

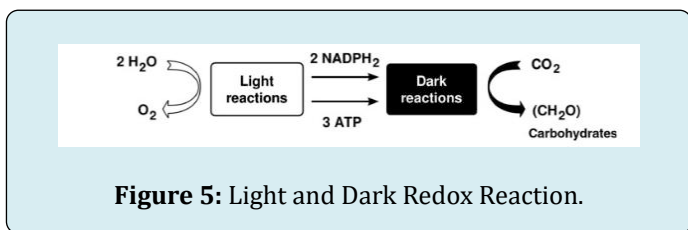


Figure 5: Light and Dark Redox Reaction.

### Biofuels Production from Microalgae

Apart from its biofuel production capabilities, Microalgae can be utilized as sources of many products including chemicals (vitamins, pigments, antioxidants), oils (omega-3 fatty acids), protein, animal feed (for larval bivalves), and biomass for the production of ethanol and methane [18]. Biodiesel production from Microbial oil (MA) is significantly gaining interests due to the following reasons listed below [18]:

1. Rapid growth rate, usually two weeks from inoculation to harvesting,
2. High lipids content as compared to other crops,
3. Problems on utilization of petrodiesel (depleting nature of proven reserves of fossil fuels),
4. Increase in the price of vegetable-based biodiesel and its increasing competition with food,
5. The possibility of using non-arable land for MA cultivation.

Though there is a continuous effort to increase its lipid content and productivity, and one of the solution proffered is the use of genetic engineering (GE) technologies, there is still a controversy between science and environment about the risk posed by MA that are not cultivated for non-consumptive purposes present less risk when exposed to humans, but the effect on the environment is not yet resolved. GE technologies could help increase lipid content, pathogen resistance attributes, and high value of co-products [19].

Nonetheless, algae biofuel conversion methods, such as transesterification, fermentation, and hydro-treatment, are more complex and economically expensive when compared to fossil-derived fuels and even biofuels from other feedstocks [20].

### The Market of Microalgae Biofuel

On a global scale, major oil producing countries have shown great interest in biofuel and renewable energy, leading them to setting specific future goals and targets about the production as well as applications of biofuels. Countries like UAE have planned to run 10 % of its transportation on biofuel by 2020 and USA has proposed to substitute 20 % of its road transport fuel for biofuel by 2022 [21]. By 2021, car manufacturers will have to comply with the 95 g CO<sub>2</sub>/km limit, set by the European Union in order to mitigate CO<sub>2</sub> emissions from their fleet of new sold passenger cars [22]. This means that biofuels, as an alternative fuel, will be one of the viable solutions [23]. The Renewable Energy Directive (RED) has also drafted a legislation to increase the production of advance biofuels from microalgae by the year 2020 (Royal Society of Biology).

### Economics of Microalgae Biofuel

The production cost is determined immensely by cultivation mode, and as at February 2007, the estimated cost of producing a kilogram of microalgal biomass is \$ 2.95 and \$ 3.80 for photo bioreactors and raceways, respectively, with the assumption that carbon dioxide is available at no cost [9]. In 2010, US navy invested 12 million US \$ to Dynamic fuels for biodiesel production from Microalgae. The cost of Biodiesel was around \$ 100/Litres in the first year but dropped to \$ 7/Litres in 2011 [24]. In 2013, Exxon Mobil concluded its R&D program on algal biofuel by stating this type of fuel would not be viable for another 25 years. At the end of that program, the company had invested \$ 600 million into it [25].

In addition to the Cultivation mode, biomass productivity, the purity of lipids extracted, the plant scale, and the capital investment (land, equipment, and construction) also affects the production costs which were about \$ 0.8/L for biodiesel

from vegetable oil and \$ 0.65/L for petrol-diesel, compared to Microalgal biodiesel around \$3/L, as seen in Table 2 according to the US Department of Energy in 2014.

According to the research of Vincent Amanor-Boadu [26], and Sawaengsak W [27], MA biodiesel is still far from

being affordable, though some countries have the policy to give subsidies for biodiesel production, which was around 0.26 US \$ to 0.40 US \$/L, after which biodiesel production from microalgae was still not comparable with vegetable biodiesel and petro-diesel.

Type of cultivation	Process	Conditions	Lipid cost (US \$/L)	Biodiesel cost (US \$/L)
Open pond cultivation	Dewatering; lipid extraction from dry biomass; transesterification	Capacity:1950 ha; Biomass yield: 25 g/m <sup>2</sup> /d; Lipid content: 25 % w/w dry biomass	2.25	2.6
PBR cultivation	Dewatering; lipid extraction from dry biomass; transesterification	Capacity: 1950 ha; Biomass yield: 1.25 kg/m <sup>3</sup> /d, Lipid content: 25 % w/w dry biomass	4.78	5.42

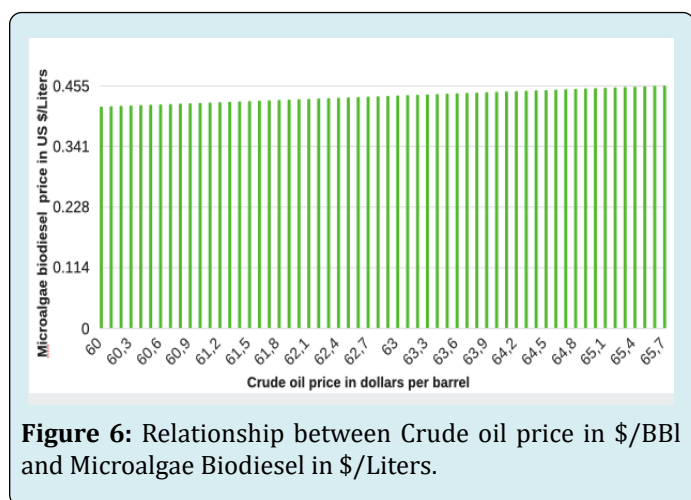
**Table 2:** Cost of biodiesel production from microalgae in US \$/L (adapted from (Chen J [18], (Davis R [28]).

According to (Chisti Y [29]) in “ Biodiesel from Bioalgae“, MA to potentially completely replace petroleum as a source of hydrocarbon feedstock in the petrochemical industry, it needs to fulfil the correlation of Christi Y [29] (Equation 3), which shows the relationship of microalgae diesel price, also Figure 6 shows the output for MA biodiesel for crude oil price between 60 and 65.7 \$/Barrel.

$$C_{\text{microalgae oil}} = 0.00693 * C_{\text{crudeoil}} \quad (3)$$

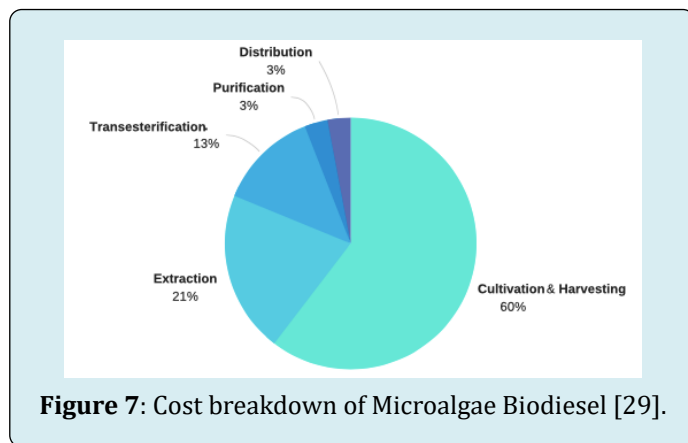
Where:

$C_{\text{microalgae oil}}$  = MA biodiesel price in \$/Liters, and  
 $C_{\text{crude oil}}$  = Crude oil price in \$/Barrel.



By breaking down the total cost needed to produce microalgae biodiesel, Figure 7 shows the percentage of the total cost needed by each segment of production from cultivation and harvesting, extraction, transesterification, purification, and distributions of the total cost with

Cultivation and extraction taking up to 60 %.



Till date no affordable Microalgae biodiesel is produced on a commercial scale, but it is worth investing in the studies of MA as fossil fuels are depleting and price of vegetable oils are gradually increasing.

### Microalgae Diesel Price Parity

The cost reduction required for Microalgae diesel to gain parity with petro-diesel can be achieved through:

1. The biorefinery approach, which involves simultaneous biodiesel production with other co-products just like in the oil and gas refinery where petro-oil is removed with other fractions of crude oil.
2. In addition, advances in engineering of photobioreactors with highly efficient dewatering and extraction technology and studying and improving the technology that combines extraction and transesterification;
3. Improving capabilities of microalgae through genetic engineering, by isolating high productive microalgae

strain with lipid content;

4. MA cultivation in rural area, this strategy will bring life to the rural areas both economically and socially, curbing rural urban migration. In reality around 68 % of the Total biodiesel production cost is by the capital expenditure (land, construction, and instrumentation), some researchers also suggested the that use of desert land can reduced the capital expenditure by 4 %, for which the land takes 15 % of the investment [29].

### Cultivation of Algae Biomass

The breeding of algae feedstock is a relatively easy task which can be done with little or no aid, using waste water while absorbing CO<sub>2</sub> from the atmosphere, but adequate knowledge about algae culturing is required before venturing

into commercial cultivation of MA. Table 3 highlights the generalized condition for the cultivation of algae, which include parameters like temperature, salinity, light intensity, photoperiod and pH.

The cultivation methods are classified based on the interaction between the culture and the environment into the:

1. Open systems (where the culture is directly exposed to the environment), and
2. Closed systems (where the culture is wholly enclosed within the culture vessel called the photo-bioreactor).

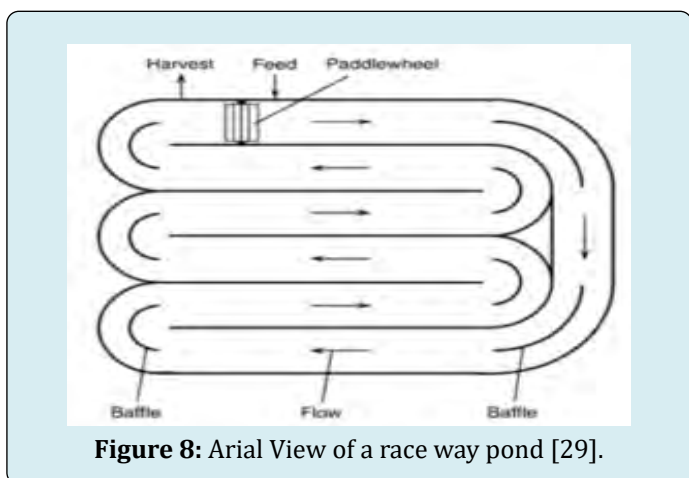
Open race way ponds, a type of the open system and photo-bioreactor (PBR) are the most applied systems [30].

Parameters	Range	Optima
Temperature (°C)	16–27	18–24
Light intensity (lux)	1000–10,000 (depends on volume and density)	2500–5000
Salinity (g/l)	12–40	20–24
Photoperiod (light: dark, hours)	12:12:00,18:06	16:8 (min) 20:0 (max)
pH	7–9	8.2–8.7

**Table 3:** Generalized conditions for algae cultivation.

### Open Race Way Ponds

The open system methods also include the use of lakes and lagoons, but the most popular is the pond, which are further classified based on natural water bodies, e.g. shallow ponds, circular ponds and raceway ponds [31]. Raceway ponds have been used since the 1950s and it is considered a mature technology. The culture in the ponds must be circulated at about 20–30 cm/s to keep the algae suspended as well as to provide relatively even illumination to the algae and prevent thermal stratification [32] (Figure 8).



**Figure 8:** Aerial View of a race way pond [29].

The advantages of using these systems are [33]:

1. Mature technology,
2. Simple technology
3. Low energy consumption,
4. Low capital costs,
5. High Net energy ratio (NER = 8.34).

However, despite the advantages listed above, the system has some weaknesses limiting it, which are [33]:

1. High contamination level,
2. High water use (high water footprint)
3. Climatic dependence (annual average temperature > 15 °C),
4. Large land demand,
5. Dilute biomass, and
6. Low productivity, of around 11g/m<sup>2</sup>/day.

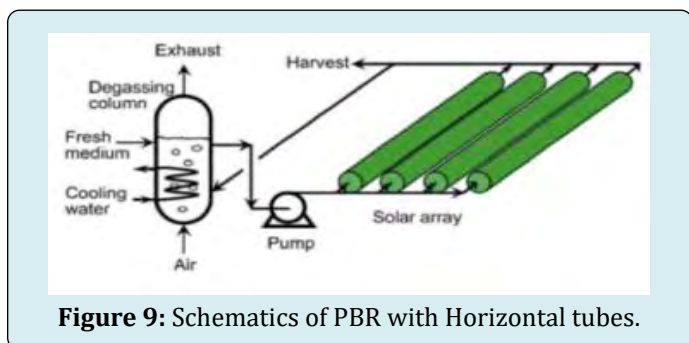
### Photo-Bioreactor (PBR)

Unlike the ponds, a PBR is a closed system equipment/container where MA can grow and the growth conditions can be controlled. PBRs facilitate better control of culture environment such as carbon dioxide supply, water supply, optimal temperature, efficient exposure to light, culture density, pH levels, gas supply rate, mixing regime, etc.

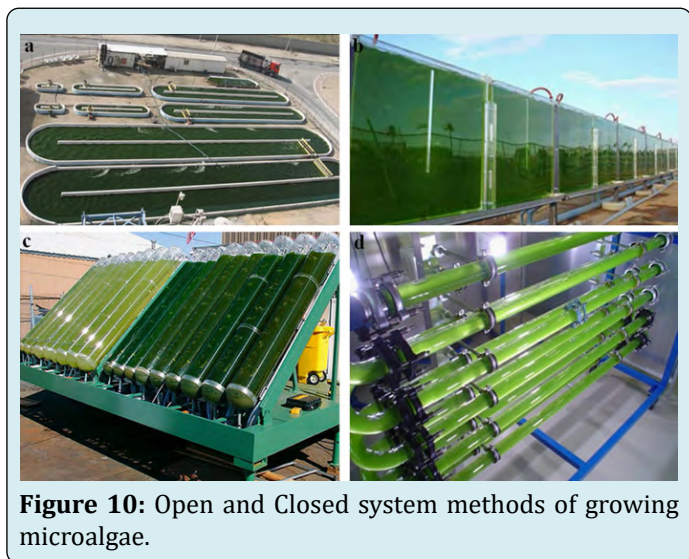
[18]. There are different forms of PBR, which are mainly tubular and flat panel reactors with Table 4 showing the characteristics of an existing PRB. Other options are bags, coils or domes. The algae in closed systems, depending on their construction, are set into motion by induced CO<sub>2</sub> or pumps, as seen in the schematics of a PBR in Figures 9 & 10 below. According to Darzins A, et al. most of the start-ups in the algae biofuel sector focus on photobioreactors (PBR) for the reasons described below:

1. Significantly higher productivity, usually 27 g/m<sup>2</sup>/day;
2. Lower risk of contamination and low water consumption;
3. Low space requirement and excellent purity of the biomass (food grade);
4. High reproducibility of results and constant and predictable production volume;
5. Cost-efficient harvest due to higher algae concentration;
6. The possibility of an easy change of the cultivated algae;
7. Low weather dependency, and the possibility of a 24 h production with artificial lighting.

But high capital, high operating cost, high energy requirements, and the fact that is a technology in the research phase are the main setback of the PBR [34].



**Figure 9:** Schematics of PBR with Horizontal tubes.



**Figure 10:** Open and Closed system methods of growing microalgae.

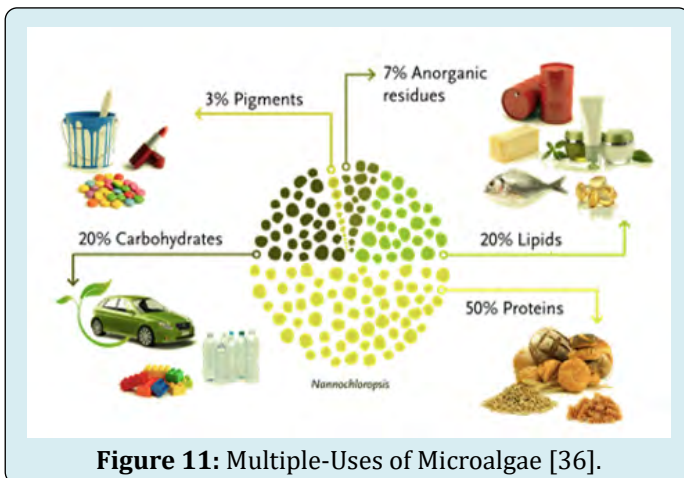
Characteristics	PBR
Number of tubes	24
Number of collectors	4
External diameter	63 mm
Internal diameter	56 mm
Material	PMMA
Vertical distance between axes	98 mm
Horizontal distance between axes	185 mm
Length (m)	18 m
Orientation	E-W
Maximum temperature	30 °C
pH (set point)	8
Tank volume	350 L
Total volume	1.4 m <sup>3</sup>

**Table 4:** Example of a PBR configuration for a startup.

### Application of Microalgae

Microalgae can be used to produce a wide range of metabolites such as proteins, lipids, carbohydrates, carotenoids or vitamins for health, food and feed additives, cosmetics and for energy production as seen in Figure 11 [35].

The Figure 12 shows that the value of added products is more economically viable, with around 10-100 euro per kilogramme. Furthermore, Microalgae has been reported being used for photobioremediation of toxic waters, treatment of sewage systems, and even cleaning of flue gases produced in power plants, thereby reducing the cost of treatment, the microalgae is fed with the gases as a source of food [36].



**Figure 11:** Multiple-Uses of Microalgae [36].

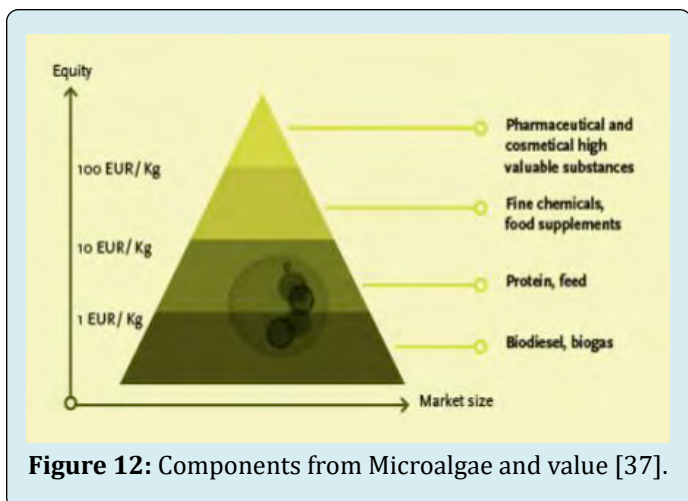


Figure 12: Components from Microalgae and value [37].

### Microalgae and Human Food

The first human use of microalgae for food dates back 2000 years, when the Chinese used “Nostoc” to escape starvation during famine. However, microalgal biotechnology only started receiving attention from the middle of the last century [38]. In the mid 20<sup>th</sup> century, microalgae gained attention as being a good supplement and/or fortification in diets for malnourished children and adults, as a single cell protein, but nowadays it is marketed in different forms of

tablets, capsules and liquids [39].

Aside just being a source of food, the nutritional value of microalgae in human food is one of the most complete source, being so rich in carbohydrate, protein, enzymes, and fibres, also having in abundance are vitamin A, C, B1, B<sub>2</sub>, B<sub>6</sub>, niacin, iodine, potassium, iron, magnesium and calcium [35]. Research from Pangestuti R, et al. [40] shows that the pigments found in microalgae such as chlorophyll,  $\beta$ -carotene, and lutein are beneficial to human health, possessing cancer prevention activity and functioning as antioxidants and anti-inflammatories [40]. However, vitamins cell content fluctuates with environmental factors, the harvesting treatment and the biomass drying methods (Brown [41]), (Borowitzka, [42]).

The work of (Alice Ferreira [43]) proved the effectiveness of *Scenedesmus obliquus* to effectively treat the brewery effluents (liquid and gaseous), with a production of valuable biomass useful for bioenergy production, such as an energy carrier BioH<sub>2</sub> (67.1 mL H<sub>2</sub> g<sup>-1</sup> (in terms of VS)), or the three products bio-oil (64 %), bio-char (30 %) and bio-gas (6 %) from a single pyrolysis process. This work proved that it is possible to integrate several sustainable processes, resulting in a closed loop system that allowed an efficient nutrient recycle (wastes) and the full use of the energy [43] (Table 5).

Microalgae	Major Producers	Products	World production (t/year)
Spirulina (Arthrospira)	Hainan Simai Pharmacy Co. (China)	powders, extracts	3000
	Earthrise Nutritionals (California, USA)	tablets, powders, extracts tablets, powders, beverages, extracts	
	Cyanotech Corp. (Hawaii, USA) Myanmar Spirulina factory (Myanmar)	tablets, chips, pasta and liquid extract	
Chlorella	Taiwan Chlorella Manufacturing Co. (Taiwan) Klötze (Germany)	tablets, powders, nectar, noodles	2000
Dunaliella salina	Cognis Nutrition and Health (Australia)	powders b-carotene	1200
Aphanizomenon flos-aquae	Blue Green Foods (USA) Vision (USA)	capsules, crystals powder, capsules, crystals	500

Table 5: Microalgae for food table, (Adapted from (Spolaore [39]) (Pulz [44]), and (Hallmann [45])).

### Oil Extraction Methods

Microalgae biomass varies from other biomass feedstocks used in the production of biodiesel in the chemistry of cell wall, plasma membrane, the presence of a large amount of water and the small size of the cells. In order to make biodiesel, the extraction of the lipids is necessary, however, the extraction of lipids from microalgae at laboratory scale has been attempted by both mechanical and chemical extraction, with solvent extraction being the most workable

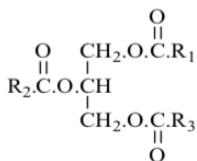
approach so far Hidalgo P [46].

### Microalgae Lipid

Lipids are classified into two types, broadly as neutral or polar lipids. A neutral lipid has no overall polarity. It is located inside the cells in the form of triacylglycerides, monoglyceride-and diglycerides or free fatty acids. They are more soluble in non-polar solvents, such as hexane and chloroform. Neutral lipids are energy storage products. The



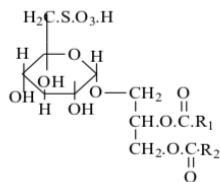
general structure of triacylglycerides is shown in Figure 13 below.



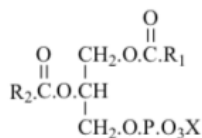
**Figure 13:** Triglyceride structure.

Polar lipids could be either phospholipids or glycolipids which contains polar groups, such as choline, ethanolamine, serine, water, glycerol, and phosphatidylglycerol in phospholipids. Glycolipids are other form of polar lipid but are less polar than phospholipids. They are simple sugar-containing lipids. They include monogalactosyl diglyceride, digalactosyl diglyceride and sulpholipids. They are located in the cell walls. They are, as might be expected, more soluble in polar solvents, such as methanol and water. They give cell walls their structural rigidity [33].

Their various structures are listed below in Figures 14 & 15, where R1, R2, R3 in Figure 13-15 denote fatty acid molecules which could be involved in transesterification process as commonly observed in triglycerides.



**Figure 14:** Sulpholipid structure.



**Figure 15:** Phospholipids general structure.

Neutral lipids (TAGs) are usually regarded as energy storage products, whereas the Polar lipids like Phospho lipids and Glycol lipids are structural lipids, contained mainly in the cell membranes. The type of species and the time of harvesting of MA determines the lipid composition. MA harvested during the exponential growth phase usually contains more polar lipids (GLs and PLs) than those harvested in the late stationary phase of growth (usual for batch cultures), which contain more neutral lipids (TAGs).

The lipid fractionation procedure and the selection of solvent, or solvent system, will depend on the particular classes of lipids present. Overall, neutral lipids are recovered by non-polar solvents, whereas polar lipids are recovered by polar ones (Borowitzka MA [47]).

### Physical or Mechanical Methods

**Expression/Expeller Press:** Dried Algae retains its oil content, which then can be „pressed“ out with an oil press. Since different strains of algae vary widely in their physical attributes, various press configurations (screw, expeller, piston, etc) work better for specific algae types. Many commercial manufacturers of vegetable oil use a combination of mechanical pressing and chemical solvents in extracting oil [48].

**Ultrasonic-Assisted Extraction:** Ultrasonic extraction, a branch of sonochemistry, can greatly accelerate extraction processes like in the case of Ferreira AF, et al. [19], where ultrasound assisted method with solvents extraction was carried on three species of MA. Using an ultrasonic reactor, ultrasonic waves are used to create cavitation bubbles in a solvent material. When these bubbles collapse near the cell walls, it creates shock waves and liquid jets that causes those cells walls to break and release their contents into the solvent.

### Chemical Extraction Methods

MA oil can be extracted using chemicals such as, Benzene and ether. Oil can also be separated by hexane extraction, which is quite used in the food industry and is relatively inexpensive. The downside to using solvents for oil extraction are included in the dangers involved in working with the chemicals. Care must be taken to avoid over exposure to vapours and direct contact with the skin, either of which can be highly dangerous. Benzene is classified as a carcinogen. Chemical solvents also present the problem of being an explosion hazard [49].

### Chemical Methods are:

**Hexane Solvent Method:** Hexane solvent extraction can be used in isolation or it can be used along with the oil press/expeller method. After the oil has been extracted using an expeller, the remaining pulp can be mixed with cyclohexane to extract the remaining oil content. The oil dissolves in the cyclohexane, and the pulp is filtered out from the solution. The oil and cyclohexane are separated by means of distillation. These two stages (cold press & hexane solvent) together will be able to derive more than 95 % of the total oil present in the algae [49].

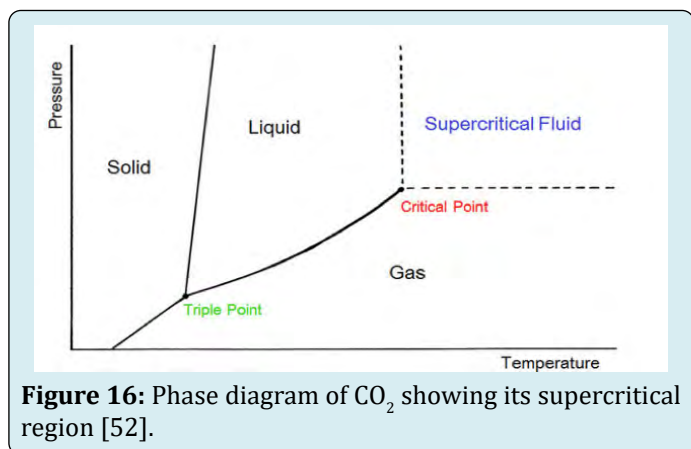
**Soxhlet Extraction:** Soxhlet extraction is an extraction method that uses chemical solvents. Oils from the algae are extracted through repeated washing, or percolation,

with an organic solvent such as hexane or petroleum ether, under reflux in a special glassware. Pardo-Cárdenas Y, et al. reported that a wide variety of organic solvents are often used to extract oil from microalgae, with hexane and ethanol as the most used solvents. However, ethanol is a polar solvent and its selectivity to lipids is relatively low compared to other solvents, so in extractions with ethanol, other microalgae components may also appear, such as sugars, pigments or amino-acids. Also, in his research using a hexane-ethanol mixture, results showed that approximately 80 % of fatty acids present in biomass can be extracted.

Hexane is cheaper than other non-polar solvents such as cyclohexane; it is easy-to-recover after extraction and is selective to neutral lipids [49]. Veera concluded that solvent mixtures containing mixture of a polar and a non-polar solvent extracts a greater amount of lipids. For example, a combination of chloroform (non-polar), methanol (polar) and water, known as the Bligh & Dyer method, has been used for lipid extraction from a wide range of biological samples.

**Supercritical Fluid Extraction:** Although supercritical fluid has liquid-like density, it exhibits gas-like diffusivity, surface tension and viscosity. Its gas-like viscosity results in high mass transfer. Its low surface tension and viscosity lead to greater penetration into porous solids. Because of its liquid-like density, a supercritical fluid's solvent strength is comparable to that of a liquid [50].

In supercritical CO<sub>2</sub> extraction, CO<sub>2</sub> is liquefied under pressure and heated to the point that it has the properties of both a liquid and a gas, more specifically, it behaves as a supercritical fluid above its critical temperature ( $T_c$ : 32.1 °C), which is near ambient temperature, making it an attractive solvent for temperature-sensitive materials. CO<sub>2</sub>'s critical pressure is 73.8 bar ( $P_c$ : 1.070 psi), as shown in its phase diagram (Figure 16) [51].



**Figure 16:** Phase diagram of CO<sub>2</sub> showing its supercritical region [52].

Supercritical CO<sub>2</sub> is becoming an important commercial and industrial solvent due to its role in chemical extraction in

addition to its low toxicity and environmental impact. Typical methods of extracting oil from MA has some drawbacks [53].

1. MA biomass is required to be in form of a powder prior to traditional solvent extraction process for high efficiency extraction of lipids which is quite energy intensive;
2. Large volumes of solvents are required, which are costly;

Contamination of the MA extract with pigments, such as chlorophyll, requires further purification. To avoid these issues, wet lipid extraction is considered as a feasible technique [53].

### Biodiesel Production Processes

Microalgae biodiesel production essentially involves two main steps:

1. Extraction of oils from the biomass, including cell disruption by mechanical and chemical methods [54];
2. Conversion (transesterification) of oils (fatty acids) to biodiesel (alkyl esters).

To date, biodiesel production from algae biomass is generally performed by one of the following methods:

1. The conventional route, a two-step protocol in which algae oil is extracted and then converted to biodiesel using a catalyst, such as an acid, a base, or an enzyme.
2. Direct single step *in situ* production of biodiesel from algae biomass using an acid catalyst at atmospheric pressure and ambient temperature.

In situ Transesterification	Extraction- Transesterification
Heating value is high	Heating value is low
Yield is high	Yield is low
Simple processes in operation	Process is complex
Due to absence of extraction and dewatering the production cost is low	Production cost is low
Avoid the potential lipid loss	Lipid loss during process
Reduce the waste water pollutants	Waste water pollutes environment
Less time-consuming process	Time consumption is high

**Table 6:** Differences between the use of in situ and conventional route [15].

The current microalgae-based biodiesel is mainly produced by the conventional route: extraction of the lipids from the MA biomass followed by its conversion to FAMES and glycerol. However, such method consumes time, is energy intensive, and has high water footprint, in short, the

method is really costly, and difficult to be implemented in algae's crushing steps because of the rigid cell walls (Table 6).

### Transesterification

Biodiesel is commonly produced by the transesterification of vegetable oil, animal fat, or algal feedstock, and the exciting news is that MA could have a lot of oil as shown from Table 7 [55]. Transesterification is the reaction between an ester and an alcohol which produces a new alcohol and a new ester. In transesterification, a simple alcohol, such as methanol, is used with a catalyst.

Microalgae	Oil content (% dry wt)
Botryococcus braunii	25–75
Chlorella sp.	28–32
Cryptocodinium cohnii	20
Cylindrotheca sp.	16–37
Dunaliella primolecta	23
Isochrysis sp.	25–33
Monallanthus salina	>20
Nannochloris sp.	20–35
Nannochloropsis sp.	31–68
Neochloris oleoabundans	35–54
Nitzschia sp.	45–47
Phaeodactylum tricornutum	20–30
Schizochytrium sp.	50–77
Tetraselmis sueica	15–23

**Table 7:** Microalgae species and their oil content [29].

In transesterification reaction, an excess of alcohol leads to a shift in the reaction equilibrium towards product formation. Alcohols used in the transesterification reaction could be short chained, long chained, and cyclic. At the end of the reaction the excess alcohol and glycerol, co-produced with FAME, can be separated. Methanol and ethanol are commonly utilized due to low cost, low solubility in the reaction medium, and obvious availability, however ethanol is not so commonly used because of its low reactivity towards transesterification, it is used sometimes because it can be produced from biomass so the process becomes more sustainable.

Biodiesel from MA seems to be the only renewable biofuel that has the potential to completely displace petroleum-derived transport fuels without adversely affecting supply of food and other crop products. Most productive oil crops, such as oil palm, do not come close to MA in being able to

sustainably provide the necessary amounts of biodiesel. Similarly, bioethanol from sugarcane is no match for MA biodiesel [9].

Chemically, transesterification involves taking a triglyceride molecule or a complex fatty acid, removing the glycerol, and creating an alkyl ester. Transesterification requires 3 moles of alcohol for each mole of triglyceride to produce 1 mol of glycerol and 3 mole of methyl esters (Figure 17) to achieve equilibrium. Industrial processes use 6 moles of methanol for each mole of triglyceride. This large excess of methanol ensures that the reaction is driven in the direction of methyl esters, i.e. towards biodiesel. Yield of methyl esters exceeds 98 % on a weight basis [29].



**Figure 17:** Generalized transesterification reaction [56].

### Catalysis

Berzelius introduced the term “catalysis” early 1836 in order to explain various decomposition and transformation reactions. He assumed that special powers that can influence the affinity of chemical substances were inherent in catalyst, and Williams Ostwald, in 1895, defined a catalyst as follows: “a catalyst accelerates a chemical reaction without affecting the position of the equilibrium.” Ostwald saw catalysis as a ubiquitous phenomenon that was to be explained in terms of the laws of physical chemistry. Furthermore, the catalyst is not consumed in a chemical reaction and its amount added to the reaction is much lower compared to the quantity of reagents [57].

The suitability of a catalyst for an industrial process depends mainly on the following three properties:

1. Activity (reaction speed measure);
2. Selectivity (preference for one direction of transformation);
3. Stability (lifetime of the catalyst during its operation).

The judgment of the importance level is generally difficult to answer since the demands made on the catalyst are different for each process. Today the sustainable use of energy and raw materials is of much importance, and it is preferable to optimize existing processes than to develop new ones.

For various reasons, the target quantities should be given the following order of priority:

Selectivity > Stability > Activity [57].

Generally, catalysts used for biodiesel production are categorized into three groups which are:

1. Alkalies,
2. Acids and,
3. Enzymes.

The use of alkali and acid catalysts are much more prevalent in biodiesel production in comparison to the use of enzyme catalysts [12]. Furthermore, acid and basic catalysts were categorized into homogeneous and heterogeneous catalysts. The avoidance of soap formation, and simple purification process by the use of enzyme catalysts has

made them much more attractive for biodiesel production. Nonetheless, due to the longer reaction time, and a higher cost, their viability commercially can be questioned. When biocatalysts are used the main advantage is that no purification is necessary. On the other point, alkali and acid catalysts are more commonly used in transesterification because of their availability [58].

Content of 55 % wt. Moreover, Johnson and Wen [59] reported conversions over 50 % wt. using the microalgae *Schizochytrium limacinum* with a shorter reaction time (40 min) due to the addition of chloroform in the reaction (Table 8).

Catalysis	Advantages	Disadvantages
Homogeneous alkaline	High reaction rate and high conversion yield (>90 %)	Formation of soaps in the presence of FFA (>1 %) and water (>0.06 %)
	Low cost and widely available	Necessity of a stage for removing the catalyst
		Wastewater production from biodiesel washing for glycerol and remaining catalyst removal
Heterogeneous alkaline	Higher reaction rate than acid catalysts	Sensitive to FFA content in oil with soap formation
	Easy separation of catalyst from product	Leaching of catalyst during the reaction
	Possibility to reuse and regenerate the catalyst	
Homogeneous acid	Not affected by water content and FFA in feedstock	Low reaction rate (4000 times lower than alkaline catalyzed transesterification).
	Simultaneous esterification and transesterification	Necessity for catalyst neutralization
		Necessity of a stage for catalyst removal
Heterogeneous acid	Not affected by water content and FFA in feedstock	Leaching of catalyst during the reaction
	Simultaneous esterification and transesterification	High reaction temperature, high alcohol/oil molar ratio and high reaction time are required
	Easy separation of catalyst from product.	
	Possibility to reuse and regenerate the catalyst	

**Table 8:** Summary of advantages and disadvantages of catalysis in microalgae biodiesel formation [6].

## Standards

For microalgae to be sold commercially, it must meet the European (EN 14214) and American standard (ASTM D6751-10), though they are similar, they differ on some requirements. It is example of these differences the case of EN 14214, which stipulates that, polyunsaturated ( $\geq 4$  bonds) ester content and linoleic ester content must be less than 1 % (mol/mol) and 12 % (mol/mol), respectively [60].

The restrictions are important for biodiesel production from microalgae as the ester composition is often rich in

polyunsaturated content and over these levels. Also, EN 14214 includes some parameters not present in ASTM D6751-10, like esters content, density, iodine value, and fatty acid contamination [61].

EN 14214 is more prohibitive for biodiesel from microalgae, as the boundary of cetane number is higher (51 vs 47), the maximum viscosity is lower (5 vs 6 mm<sup>2</sup>/s) and the oxidative stability must be higher (6 vs 3 h). Consequently, the ASTM D6751 standard seems to be more adapted to biodiesel from microalgae as seen in Table 9 [59]. Xu & Miao in their journal, stated that despite the fact

that many studies produced biodiesel from microalgae, few studies ensure that the latter satisfies the official standards. In another study, Xu & Miao satisfied to meet the Grade S15 ASTM standard of flash point (closed cup) (115 °C), viscosity (5.2 mm<sup>2</sup>/s, 40 °C) and acid number (0.374 mg KOH/g). However, based on the present knowledge, methanol content

(EN 14110), sulphated ash (D874), cloud point (D2500), cold soak filterability, P content (D4951), distillation temperature (D1160), sodium and potassium (EN 14538), oxidative stability (EN 15751) have not been measured for microalgae biodiesel, as microalgae biodiesel is relatively new.

Properties	Microalgal oil Biodiesel	Biodiesel fuel	ASTM biodiesel standard
Density (kg L <sup>-1</sup> )	0.864	0.838	0.86–0.9
Viscosity (mm <sup>2</sup> s <sup>-1</sup> , cSt at 40°C)	5.2	1.9–4.1	3.5–5.0
Flash point (°C)	115	75	Min 100
Solidifying point (°C)	-12	-50 to 10	-
Cold filter plugging point (°C)	-11	-30 (Max -6.7)	Summer max 0; winter <15
Acid value (mg KOH g <sup>-1</sup> )	0.374	Max 0.5	Max 0.5
Heating value (MJ kg <sup>-1</sup> )	41	40–45	-
H/C ratio	1.81	1.81	-

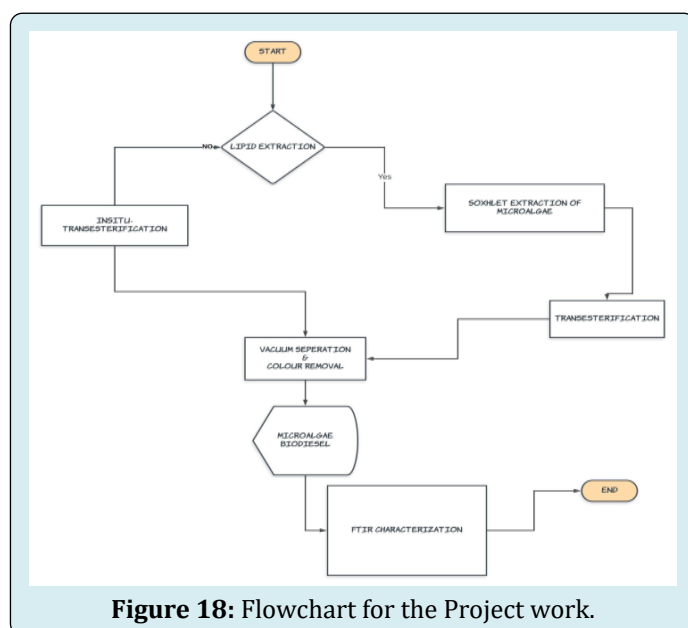
**Table 9:** Properties of biodiesel from microalgal oil, biodiesel fuel & ASTM biodiesel standard.

### Used Materials

1. The lipid extraction, transesterification, insitu-transesterification, and Purification of the microalgae species was carried out with the aid of the soxhlet extractor, density scale, petri-dishes, heaters, vacuum separator, filter paper, stop watch, and round bottom conical flasks, silica, activated carbon using various analytical reagents, on *Chlorella vulgaris*, *Nannochloropsis oceanica*, and the 25g of *Thalassiosira* spp supplied by Algae for future in Lisbon (Figures 18 & 19). For the extraction of the lipids from Microalgae, the effectiveness of various reagent was experimented either by using only a single solvent or by mixing the solvents in various proportions, the procedure below was followed (Table 10);
2. Firstly, the weight of the thimble is measure after drying for few second in the dryer;
3. Then a known weight of the MA is dried, measured and transferred into the thimble which is made of cellulose,
4. After which the thimble is placed in the soxhlet extractor and, connected to water source for the condenser to cool the vapour,
5. A known volume of the reagent is placed in the conical flask, and after double checking all is set, Temperature is set to a little above the boiling point of the reagent(s) in the flask, then 3 hours was set for extraction,

6. After extraction, the thimble is dried in the over oven, and measured to compare to the initial weight before extraction,

Based on the current weight the lipid extracted is calculated using a program in Matlab, after which the extracted lipids content was calculated.



**Figure 18:** Flowchart for the Project work.

No	Microalgae Specie	Solvent	Co-Solvent	Sample Weight(g)	Ratio of Solvent/Co-Solvent
E1	Chlorella vulgaris	n-hexane	isopropanol	10	2:01
E2	Nannochloropsis sp.	n-hexane	isopropanol	10	2:01
E3	Chlorella vulgaris	n-hexane	isopropanol	10	3:01
E4	Chlorella vulgaris	n-hexane	isopropanol	10	3:01
E5	Nannochloropsis sp.	n-hexane	isopropanol	10	3:01
E6	Chlorella vulgaris	Isopropanol		10	1
E7	Nannochloropsis spp.	Methanol		10	1
E8	Thalassiosira spp.	Acetone	methanol/isopropanol	2.5	2:01
E9	Chlorella vulgaris	Methanol		10	1
E10	Chlorella vulgaris	Propanol	Acetone/cyclohexane	6	4:02:01
E11	Nannochloropsis spp.	Distilled water		10	

**Table 10:** Performed experiments using different chemical solvents.

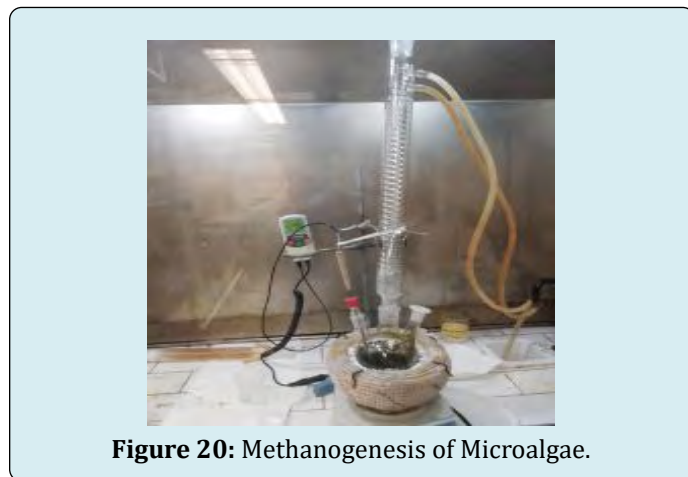


**Figure 19:** The stages of Microalgae Lipids extraction.

### Transesterification Process

The two methods of insitu-transesterification and extract-transesterification were conducted on the three species of MA, the ratio of methanol to biomass ranged from 5:1 to 40:1, with changing temperature, time, varying different

catalyst type, and finally alcohol content in the process, basically using methanol and ethanol as tabulated in Table 11. After extraction of the Lipids from the dry biomass, the extracted liquid was divided into two for transesterification, using methanol in excess with the aid of acidic/basic catalyst with the resulting fluid characterized with FTIR (Figure 20).



**Figure 20:** Methanogenesis of Microalgae.

Microalgae Type	Sample Number	Solvent	Cosolvent	Sample Weight (G)	Catalyst	Methanol/Biomass
Chlorella	TX1	Methanol	acetone	25	H <sub>2</sub> SO <sub>4</sub>	2.2
Chlorella	T1	Methanol	acetone	5	H <sub>2</sub> SO <sub>4</sub>	12
Thalassiosira sp	T2	methanol	acetone	5	H <sub>2</sub> SO <sub>4</sub>	12
Chlorella v.	T3	methanol	acetone	5	H <sub>2</sub> SO <sub>4</sub>	16
Nannochloropsis	T4	methanol	acetone	5	H <sub>2</sub> SO <sub>4</sub>	12
Thalassiosira sp	T5	methanol	acetone	5	NaOH	12
Chlorella v.	T6	ethanol	acetone	5	H <sub>2</sub> SO <sub>4</sub>	12
Thalassiosira sp	T7	methanol	acetone	5	NaOH	12
Nannochloropsis	T8	methanol	acetone	5	CaO	16
Chlorella v.	T14p	methanol		5	Na <sub>2</sub> CO <sub>3</sub>	40

**Table 11:** Insitu-transesterification of microalgae under various condition.

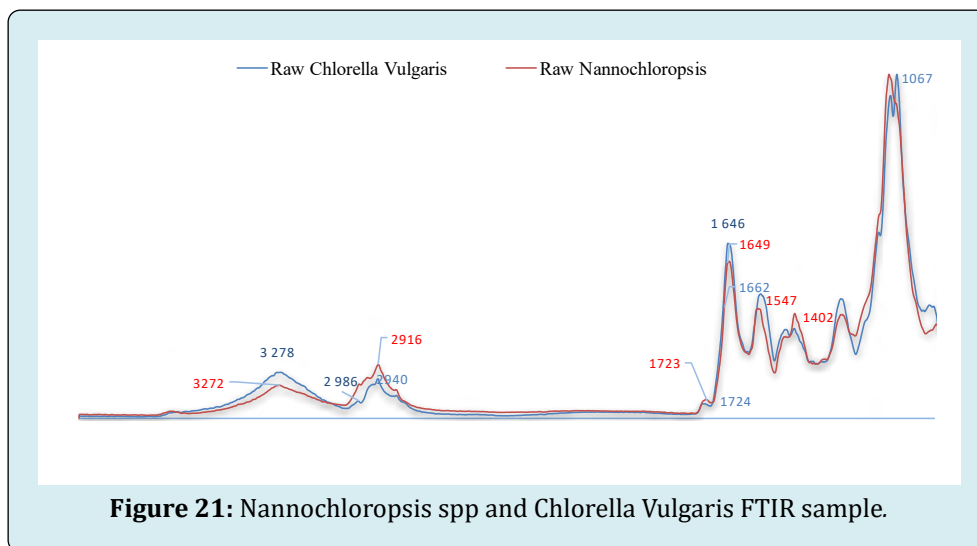
For insitu-transesterification the procedure are as follows;

1. A methanol of 60-80ml is measure into the round bottom flask, with or without a co- solvent which was acetone for proper extraction of the lipids,
2. Then the acid or basic catalyst is introduced into the flash, and it is stirred for 30 mins, after which the biomass is introduced and heated to around 65 degrees.
3. Then the mixture is separated with a vacuum pump through filtration
4. The resulting fluid is thereby characterized and decolourized with activated carbon
5. The residue is dried and weighted to see how much extraction was carried out.

## Results

The FTIR spectrum shows the way the macromolecular pool are distributed. There are three main regions that relate to macromolecular pools, according to the lipid band (around  $1740\text{ cm}^{-1}$ ), the amide I and amide II bands representing proteins (around  $1660$  and around  $1540\text{ cm}^{-1}$ ) and the carbohydrate region ( $1200 - 900\text{ cm}^{-1}$ ).

The spectra of fresh samples of *Chlorella vulgaris*, and *Nannochloropsis* spp. were characterized as shown in the Figure 21 with each peak assigned a functional group, and the peak value of *Nannochloropsis* spp. in bracket.



The spectra belonging to protein are characterized by two strong features at  $1646(1649)\text{ cm}^{-1}$  for amide I, and  $1545(1547)\text{ cm}^{-1}$  for (amide II). These bands were due primarily, to C=O stretching vibration and a combination of N-H bending and C-N stretching vibrations in amide complexes, respectively. Lipid spectra were characterized by two sets of strong vibrations, the C-H at  $2916\text{ cm}^{-1}$ , and the C=O mode of the side chain from ester carbonyl group at  $1742\text{ cm}^{-1}$ , carbohydrate absorption bands due to C-O-C of polysaccharides at  $1146\text{ cm}^{-1}$ ,  $1067\text{ cm}^{-1}$ ,  $1049\text{ cm}^{-1}$  respectively.

### Lipid Content Calculation

After chemical extraction with various solvents, the lipid content was calculated with some algorithms inputted in a matlab form (Table 12) (Figure 22).

$$\text{Oilpercent} = \left( \frac{\text{Bex} - \text{Aex}}{\text{Bex}} \right) * 100 \quad (4)$$

where;

Bex = Fresh sample before extraction

Aex = Fresh sample after extraction

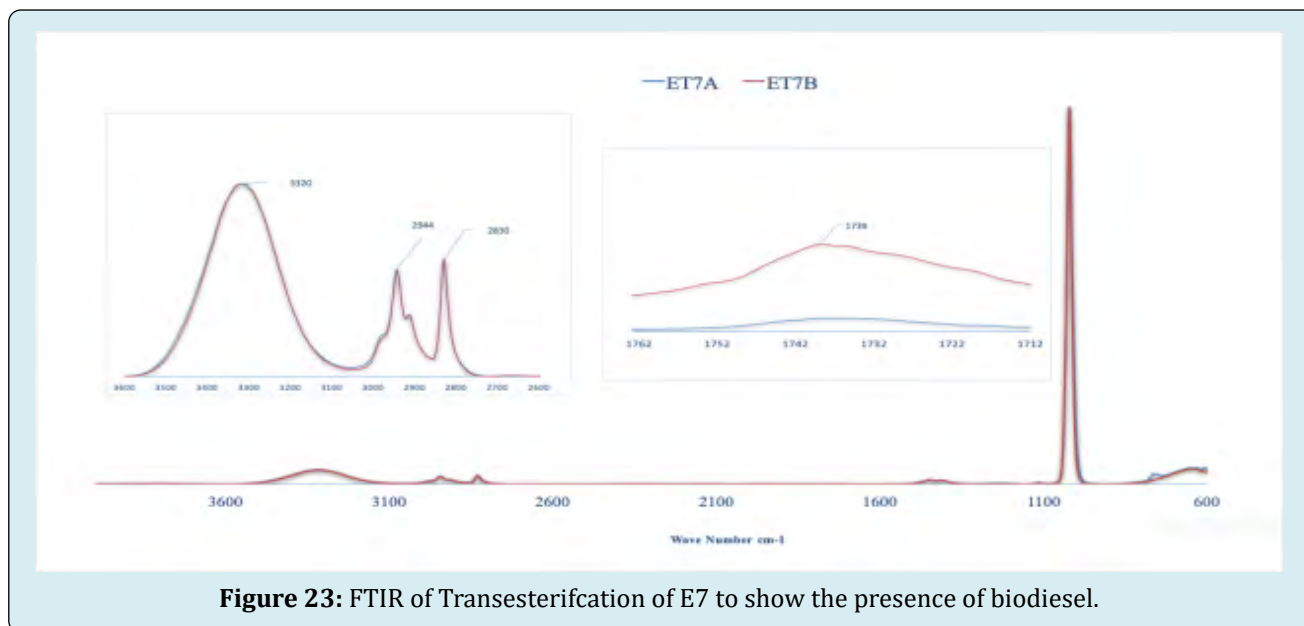


No	Microalgae Specie	Solvent	Co-Solvent	Lipid Content+Pigment (%)
E1	Chlorella vulgaris	n-hexane	Isopropanol	4
E2	Nannochloropsis spp.	n-hexane	Isopropanol	15.5
E3	Chlorella vulgaris	n-hexane	Isopropanol	8.75
E4	Chlorella vulgaris	n-hexane	Isopropanol	8.79
E5	Nannochloropsis spp.	n-hexane	Isopropanol	18.36
E6	Chlorella vulgaris	Isopropanol		11.8
E7	Nannochloropsis spp.	Methanol		24.3
E8	Thalassiosira spp.	Acetone	methanol/isopropanol	33.4
E9	Chlorella vulgaris	Methanol		11
E10	Chlorella vulgaris	Isopropanol	Acetone/cyclohexane	22

**Table 12:** The Result from the chemical extraction

After the soxhlet extraction of 10g of Nannochloropsis spp. with 400ml of methanol (E7) for 4 hours, after which the liquid extract was divided into 150 ml of 2 parts, then transesterification was carried out with 0.54g and 0.1ml

of sodium hydroxide (NaOH), and sulphuric acid ( $H_2SO_4$ ) for 1hour and 700 rpm respectively on the 2 parts, giving rise to ET7A being acid catalyzed, and ET7B being base catalyzed (Figure 23).



**Figure 23:** FTIR of Transesterification of E7 to show the presence of biodiesel.

The peak of  $1739\text{ cm}^{-1}$  is due to the presence of Fatty Acids due to the vibrations of C=O stretching of esters in ET7A, and ET7B, however ET7B showed higher intensity, which means more products were obtained in relative to ET7A.

### Insitu Transesterification

Instead of the method of extraction of the lipids and

further transesterification which is energy consuming, and takes much time to successfully achieve, therefore an insitu-transesterification method was used with both the process of extraction and transesterification taking place at the same time using different solvents, co-solvents and catalysts.

The Table 13 below shows the insitu-transesterification of three specie of microalgae with methanol, ethanol, and acid and basic catalysts.



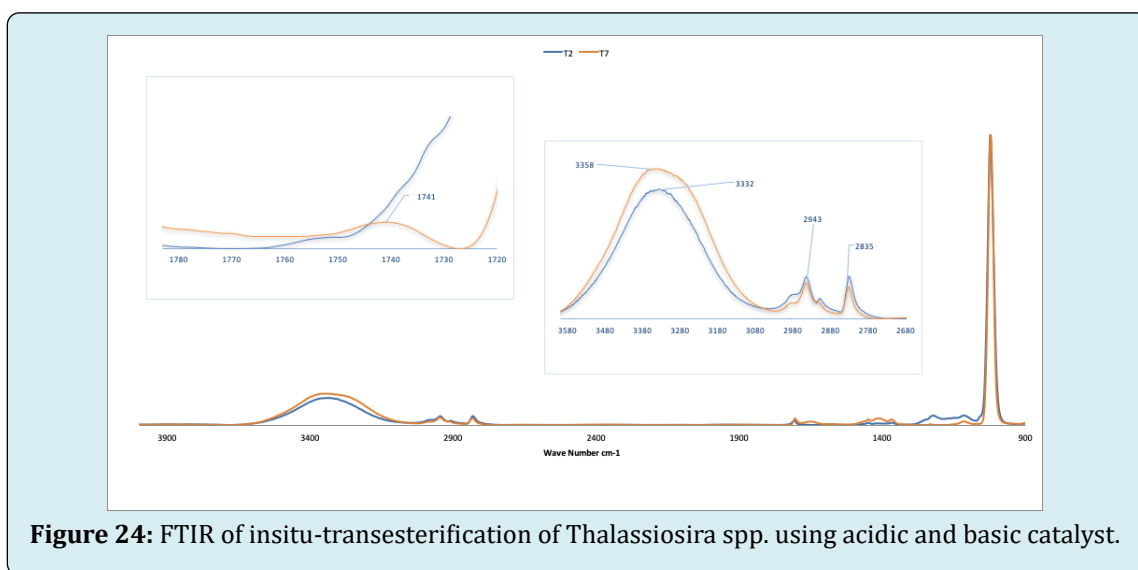
Microalgae Type	Sample Number	Solvent	Cosolvent	Catalyst	Lipids Content (%)
Chlorella vulgaris	T1	methanol	Acetone	H <sub>2</sub> SO <sub>4</sub>	22
Thalassiosira spp.	T2	methanol	Acetone	H <sub>2</sub> SO <sub>4</sub>	47
Chlorella vulgaris	T3	methanol	Acetone	H <sub>2</sub> SO <sub>4</sub>	27
Nannochloropsis spp.	T4	methanol	Acetone	H <sub>2</sub> SO <sub>4</sub>	38
Thalassiosira spp.	T5	methanol	Acetone	NaOH	22
Chlorella vulgaris	T6	ethanol	Acetone	H <sub>2</sub> SO <sub>4</sub>	16
Thalassiosira spp.	T7	methanol	Acetone	NaOH	35
Nannochloropsis spp.	T8	methanol	0	CaO	20
Chlorella vulgaris	T14	methanol	0	Na <sub>2</sub> CO <sub>3</sub>	

**Table 13:** Lipids content from Insitu-transesterification.

The table above shows the insitu-transesterification of three specie of microalgae with methanol, ethanol, and acid and basic catalysts.

In the Figure 24 below the diatom Thalassiosira spp. microalgae of 5g is used in an insitu-transesterification process with 60ml of methanol, 20ml of acetone, and 0.5ml of H<sub>2</sub>SO<sub>4</sub> for 4hours in the T2 sample, and the catalyst is

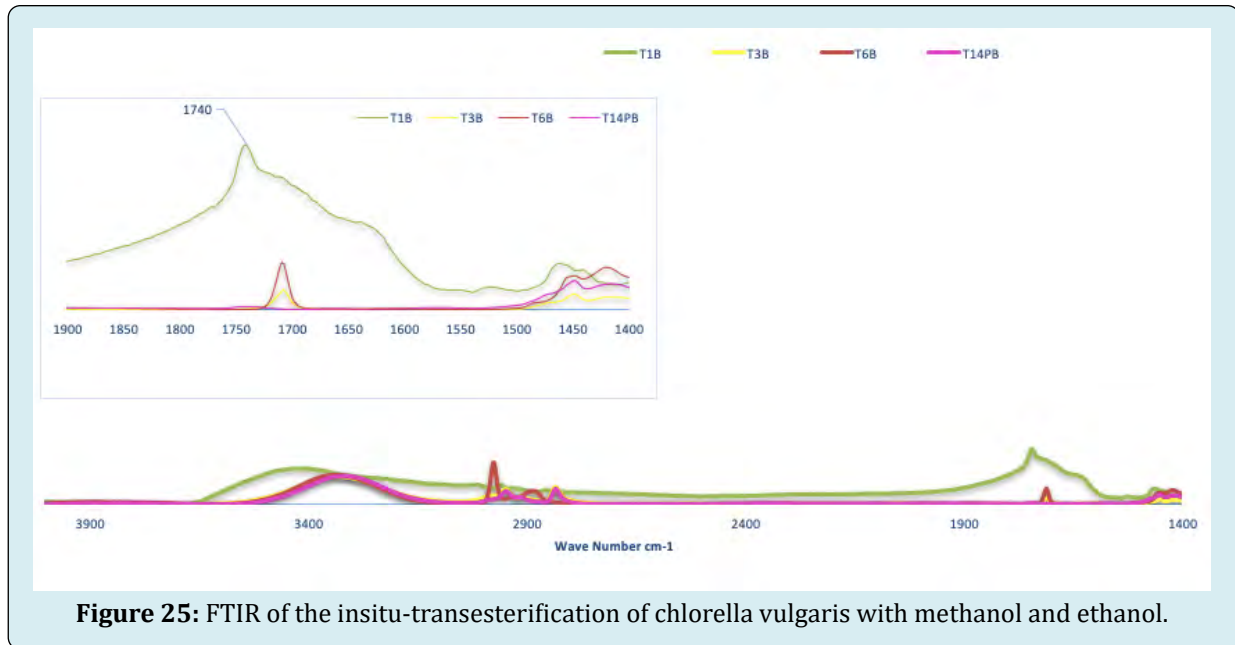
replaced with 0.54g of NaOH in T7. A peak of 1741cm<sup>-1</sup> in the sample T7 shows the presence of Fatty Acids esters due to the vibration of the C=O stretching of the esters, and T7 was visually sighted to contain white substances possibly soap, also the peaks 2943 and 2835 cm<sup>-1</sup> is due to Lipid – carbohydrate Mainly due to the asymmetrical vibration of CH<sub>2</sub> stretching.



**Figure 24:** FTIR of insitu-transesterification of Thalassiosira spp. using acidic and basic catalyst.

In the Figure 25 below the microalgae chlorella T1B is catalyzed by 0.5 ml of sulphuric acid, T3B is catalyzed by 1ml of sulphuric acid and the solvent is replaced by ethanol in T6B, and finally the Catalyst is replaced with Na<sub>2</sub>CO<sub>3</sub> in T14PB. A peak of 1740 cm<sup>-1</sup> in the sample T1B shows the

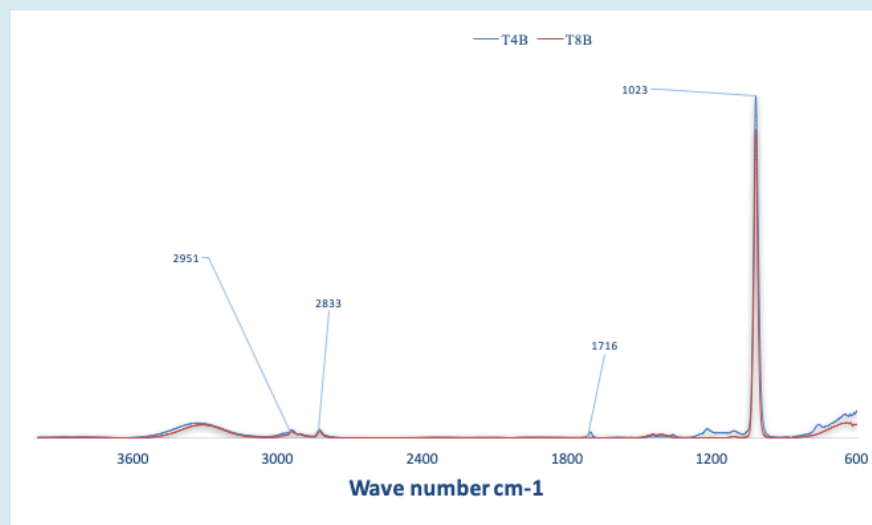
presence of Fatty Acids esters due to the vibration of the C=O stretching of the esters, and T14PB was visually sighted to contain white substances possibly soap, also the peaks 2943 and 2835 cm<sup>-1</sup> is due to Lipid – carbohydrate Mainly due to the asymmetrical vibration of CH<sub>2</sub> stretching.



**Figure 25:** FTIR of the insitu-transesterification of chlorella vulgaris with methanol and ethanol.

Also, the Figure 26 shows the FTIR spectra from the product of insitu-transesterification of *Nannochloropsis* spp. microalgae, the sample T4B being a product of transesterification with methanol, acetone and acid as catalysts, and T8B a product of using just methanol, no acetone and calcium Oxide (CaO) calcinated at 900 degrees

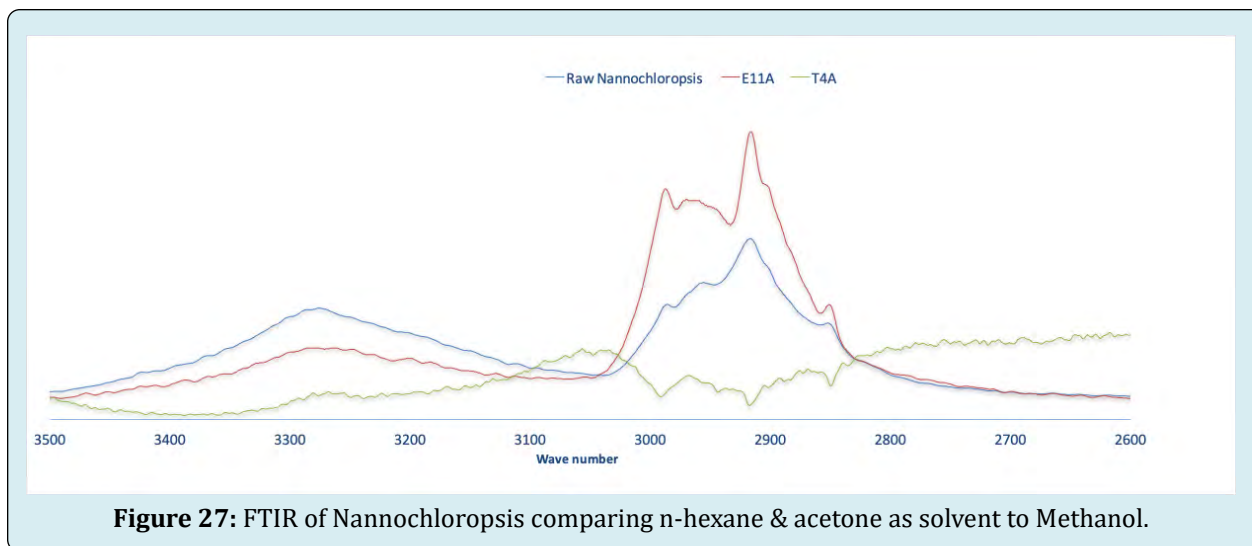
celcius. The peak of 1716 in the sample T4B shows the presence of Fatty Acids esters due to the vibration of the C=O stretching of the esters, also the peaks 2951 and 2833  $\text{cm}^{-1}$  is due to Lipid - carbohydrate. Mainly due to the asymmetrical vibration of  $\text{CH}_2$  stretching.



**Figure 26:** FTIR spectra of the product of the insitu-transesterification of *Nannochloropsis* spp. with acidic and basic catalysts.

Figure 27, shows the levelling of the wave length between 2850 to 3200  $\text{cm}^{-1}$  of *Nannochloropsis* sp. microalgae, in the quest to remove the lipids, it shows methanol is a good solvent for the task.

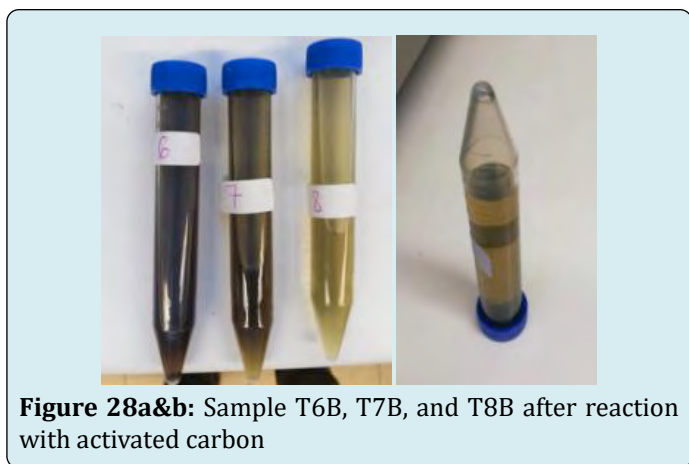
Comparing the raw sample of the microalgae used, it evident that the band belonging to Lipid is reduced to prominent level.



### Decolorization of the Biodiesel

The microalgae biodiesel produced from acid catalysis was quite darker, compared to the Biodiesel from basic catalysis which maintained the original green colour of the pigments contained in the microalgae sample. To meet the standard colour of colourless to yellow, activated charcoal of 0.3 g was added, and the mixture allowed to react for around 20 mins which after, the method of filtration was used to separate the mixture Figure 28 a&b.

A change of colour was obvious and, the activated charcoal retrieved was seen to be still active for reuse.



### Conclusion

The result proved that the best solvent for Lipid extraction proved to be acetone or combination of acetone and methanol based on 3 species of microalgae which are Nannochloropsis spp., Thalassiosira spp., and Chlorella vulgaris. Currently, esterification and transesterification are

the most commonly applied technology in the production of biofuel. For the paper, Insitu-transesterification as method of production of biodiesel, was used because it is becoming rather attractive and highly efficient in terms of bio-fuel production. The main draw-back of this process is that it consumes lots of solvents, water and it is highly energy intensive (about 30ml/seconds).

Biodiesel is a non-toxic, renewable, biodegradable, and more environmental friendly fuel which appears to be an alternative fuel to petro-diesel, but in the future other thermochemical methods, like hydrothermal liquefaction and pyrolysis would be needed to transform the residue left after making biodiesel into other combustible products. Although, the industrial scale of production of biofuels from microalgae is at its early stage, it still represents a sustainable solution for transportation fuel that could serve as a replacement to the petro-diesel, since there is a possibility to produce microalgal biodiesel to satisfy the fast-paced rise in the energy demand within the restraints of land and water resources.

The biorefinery approach of biodiesel can lead to a more economical viable fuel which could replace petroleum based fuels or compete with other renewable energy technologies such as wind, solar, geothermal and other forms. Microalgae biodiesel is not yet economically viable despite the high potential in terms of productivity and sustainability. Most algae based biofuel concepts still require significant investment in research and development to make the process really become commercially viable.

The possibility of Microalgal farming should be incorporated with flue gas CO<sub>2</sub> mitigation and wastewater treatment to reduce the operating cost, thereby ensuring

a cost-effective farming. Microalgae have the ability of decarbonization of flue gases as proven by the cultivation of these organisms to utilize the nitrogen and phosphorus as nutrients from waste water sources as a form of wastewater bioremediation. Moreover, microalgal biodiesel can reduce the emission of NO<sub>x</sub>. Microalgae produce valuable co-products or byproducts such as H<sub>2</sub>, ethanol, biopolymers, proteins, cosmetic products, carbohydrates, fertilizer, animal feed, biomass residue etc.

Microalgae cultivation does not require fertilizer, herbicides and pesticides, also from the combustion point of view the heating value of Microalgal biodiesel is more than that of other terrestrial plants, which is around 41MJ/kg as compared to the 37MJ/kg of soybeans or rapeseed. Changyan Yang also concluded that pyrolysis temperature of MA is mainly at 400–550 °C, which is slightly lower than the direct pyrolysis of lignocellulosic biomass (commonly 450–650 °C).

The research work of Yulin Hua showed that methanol was identified as the most effective reaction medium for the production of bio-crude oil from low-lipid microalgae. Afterwards, the effects of other reaction conditions (residence time, biomass/solvent mass ratio, and reaction temperature) on the liquefaction in methanol were examined. Liquefaction experiments at 225 °C for 60 min with 1:5 biomass/solvent mass ratio resulted in the highest bio-crude oil yield of 85.5 wt%, along with a higher heating value (HHV) of 30.6 MJ/kg.

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