

PRODUCTION OF BIODEISEL FROM MICROALGAE

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science degree in Biochemistry

By

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Dharshini.G (40070001)**, **Kaviya.NR (40070003)** who carried out the project entitled "**PRODUCTION OF BIODIESEL FEOM MICROALGAE**" under our supervision from December 2022 to May 2023

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ABSTRACT

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 Photo bioreactor 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 photo bioreactors. In situ transesterification of dry algae to fatty acids methyl esters (FAMES) was achieved using ultra sonication. 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.

Key words: microalgae, algal biomass, Biodiesel, Transesterification.

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LIST OF ABBREVIATION

SPV	-	Sulfo phospho vanillin
B&D method	-	Bligh and Dyer method
BBM	-	Bold Basal Medium
SPS	-	Sucrose phosphate synthase
TLC	-	Thin Layer Chromatography
ACS	-	American Chemical Society
CG	-	Gas Chromatography- Mass Spectrometry
FAME	-	Fatty Acid Methyl Esters
TAG	-	Triacylglycerol

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CHAPTER-1

INTRODUCTION

1.1 MICROALGAE

Microalgae are photosynthetic organisms that are found in both freshwater and marine environments. Also, they form an enormous population in the aquatic ecosystem. Microalgae do not have true leaves, stems or roots like other plants. But, they do have chlorophyll pigments that help trap the sun's energy. These microalgae cannot be classified as plants, fungi, animals or bacteria. So they come under a separate kingdom called Protista. Example – Chlorella, Diatoms, Dinoflagellates, etc.

CLASS:

- Cynophyceae
- Bacillariophyceae
- Haptophyceae
- Chrysophyceae
- Xanthophyceae
- Cholorophyceae

1.1.1 Basic characteristics

Microalgae is microscopic and unicellular microorganisms that have been living on the earth through prolonged geological ages. The photosynthetic mechanism of microalgae is similar to that of plants. Owing to its simple structure, it can efficiently obtain water, carbon dioxide, and other nutrients when submerged in water, thus allowing them to convert solar energy into biomass resources. Generally, microalgae are categorized as freshwater and marine microalgae based on their living environment, among which freshwater microalgae are widely distributed in nature, including rivers, lakes, reservoirs, creeks, ponds, marshes, and soils (Singh et al., 2020). Microalgae have been used in numerous fields, such as food, cosmetics, aquaculture, environmental testing, wastewater treatment, and energy. As a bioenergy source, microalgae exhibit high

photosynthetic efficiency and high yields of biomass and lipid with few environmental restrictions. Microalgae can live on non-arable land, such as beaches, saline and alkali soils, and deserts, and it could also grow in wastewater and seawater (Zada et al., 2021). In addition, microalgae exhibit remarkable performance in terms of carbon fixation (Zeng et al., 2021). At a growth rate of 25 g/d, microalgae can fix 12 tons of CO₂ per acre per year. The differences in biomass yield, lipid/fat yield, land use, and biodiesel yield among various crops are presented in Table 1. It indicates that microalgae clearly surpass other crops in all aspects. Therefore, microalgae are the most promising alternatives to replace fossil fuels. The lipid in microalgae or the whole microalgae cell can be utilized to produce gaseous fuels such as hydrogen and biogas, or liquid fuels such as ethanol and liquid hydrocarbon fuels (Sanghamitra et al., 2020). Therefore, the development of microalgae biomass energy at this stage includes biodiesel production from microalgae lipid by transesterification (Vignesh et al., 2021), methane production by anaerobic digestion (Qi et al., 2017), and production of hydrocarbon or crude oil-like substances by gasification and pyrolysis (Su et al., 2022).

Table 1.1 Comparison of microalgae with other feedstock

Lipid Feedstock	Lipid content (%)	Lipid yield	Land use	Biodiesel yield
Corn	44	172	66	152
Sunflower	40	1.070	11	946
Microalgae(low lipid content)	30	58,700	0.2	51,927
Microalgae(medium lipid content)	50	97,800	0.1	86,515
Microalgae(high lipid content)	70	126,970	0.1	121,104

Biodiesel production from lipid in microalgae through transesterification is characterized by the advantages of high growth rate and lipid yield, tolerance to environmental stress, and low competition for land (Ebhodaghe et al., 2022).

Compared with that of traditional diesel, the performance of biodiesel is superior in several aspects:

- (1) Biodiesel is extracted from vegetable and animal oils and fats, and is therefore renewable.
- (2) It contains relatively low sulfur and nitrogen content, thereby reducing SO₂ and nitrogen oxide emissions.
- (3) It also has a high flash point and high combustion performance and efficiency, is not prone to explosion, and is safe to use.
- (4) It also exhibits adequate performance at low temperature and lubrication. These advantages accord it remarkable application prospects in various fields.

1.1.2 Microalgae as a source of biofuels

Microalgae can generate diverse biofuels, which are mainly: biomethane produced by anaerobic digestion (Sialve et al., 2009; Spolaore et al., 2006), biohydrogen by photobiological process (Fedorov et al., 2005; Kapdan & Kargi, 2006), bioethanol by fermentation (Choi et al., 2010; Dexter & Pengcheng, 2009), liquid oil by thermal liquefaction (A. Banerjee et al., 2002; Miao & Wu, 2004; Miao et al. 2004; Sawayama et al., 1995) and biodiesel (M. B. Johnson & Wen, 2009; Koberg et al., 2011; Nagle & Lemke, 1990; H. Xu et al., 2006). Even if industrial scale biofuels from microalgae remain at an early stage, they remain a sustainable solution as a transportation fuel.

1.1.3 Lipid metabolic pathways in microalgae

In microalgae cell, the synthesis of fatty acid usually occurs in the plastids, and the synthesis of triacylglyceride (TAG) would occur in both of chloroplasts and endoplasmic reticulum. In contrast to phospholipids in biological membranes, TAG does not have structural roles in cells and are used to store energy and carbon. In microalgae cells, two main TAG synthesis pathways exist: the Kennedy pathway and the Monoacylglycerol

pathway. In these two pathways, TAG is synthesized through esterification between acetyl-CoA and hydroxyl groups on glycerol. The biosynthesis of fatty acids begins with the carboxylation of acetyl-CoA and acetyl-CoA carboxylase (ACCase)-mediated catalysis to form malonyl-CoA. ACCase is a critical control factor in fatty acid synthesis. In microalgae cells, malonyl-CoA is first transferred to the acyl carrier protein (ACP), and then undergoes a series of acyl chain-elongation reactions. Finally, the C16 or C18 products are catalytically synthesized by multiple subunits of fatty acid synthases. The elongation of fatty acids is terminated by two types of enzymes. First, the acyl groups in the chloroplast acyltransferase are removed by ACP, and the newly synthesized fatty acid is directly transferred from ACP to glycerol-3-phosphate (G-3-P). Second, acyl-ACP thioesterase hydrolyzes acyl-ACP and releases free fatty acids. Free fatty acids are transferred out of the chloroplast to generate glycerides.

In the Kennedy pathway, the first acyl group is esterified to glycerol-3-phosphate (Reddy et al., 2010). Phospholipid synthesis occurs in the second reaction. Before conversion to the TAG, phospholipid is dephosphorylated to diacylglycerol (DAG) by phosphatase enzyme. The Monoacylglycerol pathway starts with 2-monoacylglycerol (sn2-MAG), which is converted to DAG by monoacylglycerol 2 acyltransferase (MGAT). The synthesized DAG is converted to TAG through catalysis by acyl-CoA: DAG acyltransferase, and this is an important step in TAG biosynthesis.

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 34 double bonded carbon. Table 1.2 gives a list of some saturated and unsaturated FAs found in algal cells

Table 1.2: Saturated and unsaturated FAs found in Algal cells

FA name	Formula	Categories
Oleic acid	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	Unsaturated
Linoleic acid	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	Unsaturated
Palmitic	$\text{CH}_3-(\text{CH}_2)_{14}-\text{COOH}$	Saturated
Cupric acid	$\text{CH}_3-(\text{CH}_2)_8-\text{COOH}$	Saturated

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. Lipids are either polar or nonpolar. The polar lipids have a hydrophilic end, which make them soluble in water. It is important to understand the metabolism of fatty acids in algal cells during their growth cycle.

CHAPTER-2

REVIEW OF LITRATURE

Xanthophyceae algae characteristics and cultivation. Move over, we provide information about the use as fertilizers and growth stimulants, the definition of bio fertilizer and bio stimulant, and how they help the plant to grow and to produce a higher yield. Algae are one of the largest groups in the eukaryotic domain; it consists of a vast group organism that differ in shape and size. However open systems have several disadvantages including, the uncontrolled environment in and around it, and this result in lower biomass productivity. In this literature review, we discuss algae characteristics and cultivation they may be single cellular such as chlorella or multi cellular xanthophyceae (Singh 2012)

Open ponds have higher rates of contamination by other microorganisms such as bacteria fungi and other algae species. PBRs are a stable environment for the growth of many organisms including xanthophyceae algae. Different types of xanthophyceae such as vertical tubular, bubble column, flat panel, airlift, stirred tank and hybrid type. Algal culture systems can be illuminated by artificial light, solar light or by both. Naturally illuminated algal culture systems with large illumination surface areas include open ponds (Ugwu, 2008)

Xanthophyceae microalgae potential to integrate a bio refinery using waste to grow is discussed. Lipid productivity and fatty acid profile are related to cultivation conditions (G.F.Ferreira 2018).

Xanthophyceae species now placed in the formerly included in the chlorophyceae. Luther created the group Heterokontae for green algae with unequal flagella. Xanthophyceae can be achieved together with lipid accumulation in microalgae. Addition of CO₂ during cultivation can improve lipid productions. Waste water use can affect product quality and requires further investigation (Luther 1899)

Algal cells are aquatic organism. They grow well in water rich in nutrient in the presence of CO₂ 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).

The term bio stimulant refers to any substances that enhance plant growth and development when applied in small concentrations, they work as metabolic enhancers that regulate the uptake of essential nutrients xanthophyceae are morphology simple microalgae present in aquatic habitats (Khan et al., 2009, van oaten et al., 2017)

The role of “xanthophyceae in Aquatic Food Webs” by John H. Ryther (Limnology and Oceanography 1969) – this classic review provides a comprehensive overview of the importance of algae as primary Producers in aquatic ecosystems, and how they are consumed by other organisms up the food chain. This reviews covers the use algae in xanthophyceae, including their potential as source of biofuels

M. Gilbert and Joann M. Burkholder (2002) – this reviews focuses on the causes environmental and biodiesel produce toxins. As the need for new and more effective microalgae increases, sources of biodiversity are being explored in an effort to provide lead structures for biodisel discovery. Microalgae have been identified as a potential source of biologically active biodiesel although data to support this is limited.

“Algal Diversity and Biogeography” by Michael D. Guidry (2015) – this reviews provides an overview of the diversity of algae , including their taxonomy, evaluation and distribution in different marine and pond water environments Biochemical composition and productivity of xanthopyceae algae for biofuel production (1986)

Dr. Jean-Philippe stayer from the French National Institute for Agricultural Research- microalgae currently leading a project to developed a sustainable and cost-effective biofuel production system using Xanthopyceae algae. Xanthopyceae provides an analysis of the potential environmental impacts of biodisel production from microalgae High production yields of microalgae have called forth interest of economic and scientific production of biodiesel is environmentally interesting and which transformation steps need further adjustment and optimization (2002)

Species of xanthophyceae also have been found to increase in warmer water by Patrick (1971), Squires et al (1979) and Klarer and Hickman (1975). Although generalizations concerning dominance at high taxonomic levels are fairly well described there is much variation at the species level. Lowe (1974) lists thermal preference for many freshwater periphytic diatom species, based on field distribution.

Xanthophyceae changes in taxonomic composition of periphyton with temperature are reflected by species richness and diversity have been found to increase with temperature up to 25-30 degree Celsius. Then decrease above 30 degree Celsius. As species rich diatom floras are replaced by fewer species of yellow-green algae (Patrick-1969; Kullberg 1971; Vinson and Rushforth, 1987)

Small xanthophyceae temperature increased diatom diversity in the winter when ambient temperature was near the lower tolerance range for diatoms diversity decreased as xanthophyceae. Species similarly, Squires (et al 1979 and Wilde and Tilly 1981) found that the effect of artificial heating or xanthophyceae on the seasonal temperature.

Microalgae are considered as a promising material to capture CO₂ because of extraordinary biomass productivity and capability of production of various value-added products. Currently, two main obstacles exist in the way of industrialization of microalgae, including low efficiency in photosynthetic CO₂ fixation and the need for carbon-intensive microalgae process. Among all photosynthetic CCU technologies, microalgae have attracted more attention due to their numerous advantages over other plants. In practice, in contrast with the edible crops, microalgae do not suffer from an ethical problem because arable sites are not necessary for cultivation. Additionally, proliferation of microalgae takes place all year round, so target products could be produced during all seasons. Furthermore and most importantly, they have incomparable productivities with other types of photosynthetic activities. High productivity rate of microalgae ensures quick generation of desired products and more importantly cause higher capacity and rate of CO₂ fixation (Choi et al., 2019).

Microalgae have large bioengineering prospects to be used as in CO₂ fixation and even to reduce carbon dioxide. Because of their great efficacy and effectiveness, they might be a clean and sustainable energy alternative to plant species in the future. Some microalgae have the potential to be used in CO₂ industrial processes. Respiration has been the only natural strategy that allows the utilization of biomass by using sunlight as both the source of power and CO₂. The unicellular photoheterotrophic microalgal species are much more effective than dominant plants using light. The benefits of cultivating microalgae are identified in competition with terrestrial plants (Lam et al., 2012; Pires et al., 2012).

The wastewater treatment process carried out with microalgae represents a versatile possibility as these microorganisms have a high capacity for fixing carbon dioxide from the air and removing nutrients dissolved in the water (Shahid et al., 2020). In addition, at the end of the wastewater treatment process with microalgae, the generated biomass can be transformed into third-generation biofuels, giving rise to biodiesel, ethanol, bio kerosene, bio hydrogen, biogas (methane), and chemical intermediates for the petrochemical sector (Dasan et al., 2019).

Among the three types of feed stocks, microalgae-based biofuel is considered as the promising one and has attracted more and more attention in the past years, owing to the characteristics such as fast growing rate, high-efficiency photosynthesis, and high lipid content for some species (Peng et al. 2016).

Autotrophic microalgae consume carbon dioxide and produce carbohydrates or hydrogen, protein, and lipids, which can be further utilized as feedstock of biofuels, including biodiesel, biogas, bio hydrogen, bioethanol, butanol, bio-oil, char, and even power (Cheng et al. 2015; Francavilla et al. 2015; Im et al. 2015; Khandelwal et al. 2018; Kim et al. 2017; Lee et al. 2015; Song et al. 2017; Yun et al. 2016; Zhao et al. 2014)

Microalgae-based biodiesel is generally obtained through two steps of firstly extract lipid from algal cells, and followed by transesterification of lipid fraction using alcohol in the presence of catalysts (Rodinova et al. 2017).

Biomass for power (e.g., electricity) and heat can be achieved by combustion direct-firing in a boiler, where high-pressure steam is produced and introduced into a steam turbine, and flows over a series of turbine blades to make the turbine and electric generator rotate and there-fore the electricity is produced (ClimateTechWiki 2006).

The choice of the standard is important, since saturated fatty acids do not form a chromogen and are not detected. In addition, the reactivity of polyunsaturated fatty acids decreases with increasing unsaturation (Knight et al., 1972).

The SPV method has been modified for diverse applications such as the determination of total lipids in serum, food and ecological samples (Desvilettes et al., 1997; Haskins et al., 2010; Knight et al., 1972; Nakamatsu et al., 2004; Turlo et al., 2010; Visavadiya et al., 2009).

To date, studies were carried out using different ratio combinations of organic solvents, such as chloroform and methanol as a preliminary step (Cheng et al., 2011; Inouye et al., 2006)

The SPV reaction is performed in two steps, initial reaction of the lipids with concentrated sulfuric acid at high temperature followed by a second reaction of the derived products with vanillin in the presence of phosphoric acid. Consensus understanding is that a positive SPV reaction requires the presence of double bonds or free hydroxyl groups within the lipid analytes (Johnson et al., 1977; Knight et al., 1972).

Studies to explore the microalgae as feedstock for the production of liquid fuels had been started for the mid-1980s. In order to solve the energy crisis, the extraction of lipids from diatoms was attempted by some German scientists during the period of World War-II (Cohen et al., 1995).

CHAPTER-3

AIM AND OBJECTIVE

3.1 AIM:

To extract lipid, estimation of lipids and biodiesel production From the Yellow-Green Algae.

3.2 OBJECTIVE:

- Bligh and Dyer method-lipid extraction from xanthophyceae.
- Total Lipid estimation by colorimetric method.
- Production of biodiesel from microalgae.

CHAPTER-4

METHODS AND MATERIALS

4.1 CULTIVATION OF MICROALGAE

Algae culture is a form of aquaculture involving the farming of species of algae. The majority of algae that are intentionally cultivated fall into the category of microalgae (also referred to as phytoplankton, microphysics, or planktonic algae). Microalgae are capable to grow rapidly. Their high photosynthesis efficiency coupled with the ability to accumulate a large amount of bio products within their cells make them a suitable candidate to serve as industrial raw material. Besides, cultivation of microalgae does not require fertile land, a large quantity of freshwater, and herbicides and pesticide when compared to the other crops and thus will not be competing for resources. Furthermore, cultivation of microalgae can even be performed using wastewater such as domestic sewage water and palm oil milling effluents which can assist in bioremediation of wastewater. Apart from wastewater treatment, cultivation of microalgae can also help with reduction of atmospheric carbon dioxide through photosynthesis, effectively contributing to the efforts of tackling greenhouse effect and global warming. Despite the benefits of microalgae cultivation, its developments are still plagued with various problems. For example, the low biomass production and the small size of cells when they are cultured in liquid medium render the harvesting process of microalgae very costly.

One of the ways to work around the shortcomings of usage of microalgae in the industry is by increasing their growth rate to compensate for their low cell density and difficulties in harvesting. Numerous equipment and technologies have been improved across the years to ramp up the production of microalgae. Although microalgae can be easily cultured in a highly controlled laboratory condition, however, it is still harder to ensure the high productivity of microalgae in large-scale production. An ideal microalgae culturing system should possess the characteristics,

Including:

- (1) Adequate light source,
- (2) Effective transfer of material across liquid-gas barrier,
- (3) Simple operation procedure,
- (4) Minimal contamination rate,
- (5) Cheap overall building and production cost,
- (6) High land efficiency

. In general, microalgae culturing system can be broadly classified into two categories, which are the open pond and photo bioreactor. Each system has its pros and cons.

Algae cultivation consumes energy and water, requires land, and can generate N_2O emissions. Because the magnitude and relevance of these environmental impacts depend on each process location, design, and operation, assessing and benchmarking the environmental performance of algal biotechnologies requires careful and specific quantifications in context. Therefore, and rather than attempting to provide typical values of impacts, this chapter presents a practical methodology to quantify energy expenditure, water consumption, land use and greenhouse gases emissions during full-scale algae cultivation. As a case study, the environmental impacts of algae cultivation in closed photo bioreactors and open raceway ponds are estimated at five locations representative of five distinct climate.

4.1.1 Open ponds system

Proportioned Microalgae can be cultivated in open ponds system such as lakes, lagoons, ponds and artificial ponds or containers. Microalgae cannot be grown under control in

open ponds system. One of the major advantages of open ponds is that they are easier to construct and operate than most closed systems. However, major defects in open ponds include poor light utilization by the cells, evaporative losses, diffusion of CO₂ to the atmosphere, and requirement of large areas of land. Microalgae growth is effected by all pollutants in open ponds system. However, it is not possible to control the productivity and the quantity of the microalgae. In addition, microalgae can only be grown during certain period of the year when all the conditions required for their growth are available. Also, another one of the major problems of microalgae cultivation in open ponds system is the difficulty to harvest them.

4.1.2 Closed pond system

Microalgae can be grown in closed ponds such as photo bioreactor and other types. One of the major features of closed pond such as all the conditions can be controlled like percentage of carbon dioxide, light utilization and not required for large areas of land. However, one of the major limitations in closed ponds is difficult to construct, operate and very costly.

4.1.3 Cultivation conditions of microalgae

Microalgae was cultivated in fed-batch system. The medium and fermentation conditions were the same as described by Nashua et al.

Conditions were the same as described microalgae was cultivated in a fed-batch system. Stock cultures were propagated and maintained on synthetic BBM medium, at the following composition (per liter): NaNO₃, 0.25 g; KH₂PO₄, 0.175g; H₃BO₃, 0.115 g; K₂HPO₄, 0.075 g; MgSO₄·7H₂O, 0.075 g; KOH, 0.031 g; CaCl₂·2H₂O, 0.025 g; NaCl, 0.025 g; Na₂EDTA, 0.01 g; FeSO₄·7H₂O, 0.0049 g; MnCl₂·4H₂O, 1.81 mg; NaMoO₄·5H₂O, 0.390 mg; ZnSO₄·7H₂O, 0.222 mg; CuSO₄·5H₂O, 0.079 mg; CoCl₂·6H₂O 0.0404 mg, pH 6.8. The incubation conditions used were 26 °C, photon flux density of 15μmolm⁻² s⁻¹ and a photoperiod of 12 h.

Cultures of microalgae were harvested at the end of the cultivation, centrifuged at 2,000 g for 10 min and the retained pellet was lyophilized. Fresh cells were used for colorimetric

assay using micro plates and the results were compared with the total lipid measured using the macro-gravimetric method.

4.2 IDENTIFICATION OF MICROALGAE

Algae are classified into four major groups (palmer 1962): Blue-greens, yellow greens, diatoms and Flagellates. By knowing the major group of your algae, verification and identification to the genus and species level with the use of a microscope and classification key becomes easier. Microalgae have some shapes such as round, elliptical, rectangular, trapezoidal, rod, cylinder, and star and form combinations. Microalgae are found in the form of uni non-motile cells (such as Chlorella and Selenastum), uni motile cells (Chlamydomonas), a non-motile colonies (Scenedesmus), and motile colonies. In the lab, where the identification of algae is validated using a compound microscope. Since there exists many diverse species within each genus of algae and many looking very similar macroscopically, verification of algae must be done using a microscope. There are two common types of microscopes used in laboratories when studying algae: the compound light microscope (commonly known as a light microscope) and the stereo microscope (commonly known as a dissecting microscope). A light microscope is used to visualize objects flattened onto glass slides in great detail.

Algae Identification, Analysis, and Lab Services

- Phytoplankton Analysis
- Periphyton Analysis.
- Algal Toxin Screening & Analysis.
- Photo microscopy.
- Zooplankton Analysis.
- Chl- α Analysis.
- Diatom Slide Preparation.
- Field Sampling.

4.2.1 Yellow green algae (xanthophyceae)

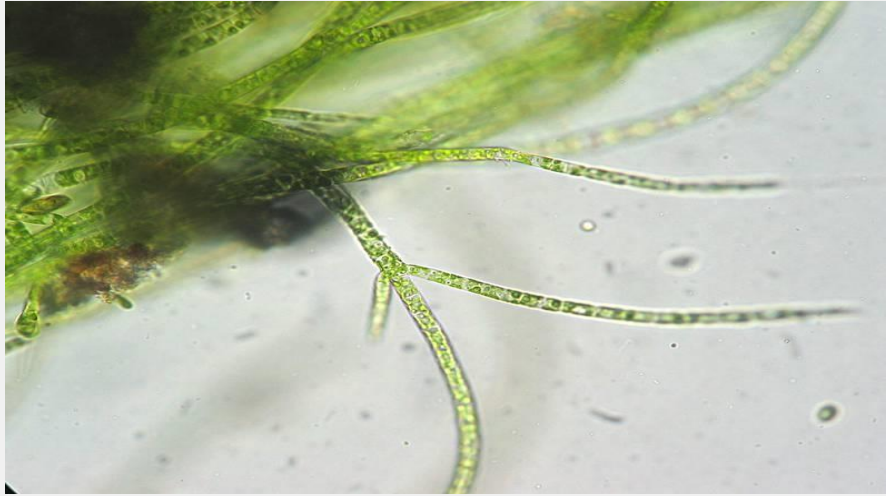


Figure 4.1 Xanthophyceae (yellow green algae) Microscope view

- Type: yellow-green algae
- Scientific name: xanthophyceae
- Kingdom: chromate
- Subphylum :gyrostat
- Scientist : Luther ,pastier
- Class :Vaucheria

Yellow-green algae or the Xanthophyceae (xanthophytes) are an important group of heterokont algae. Most live in fresh water, but some are found in marine. Yellow-green algae, (class Xanthophyceae), class of approximately 600 species of algae in the division Chromophyta, most of which inhabit fresh water. The yellow-green algae are photosynthetic species of organisms belonging to the Xanthophyta Phylum, which is one of the phyla pertaining to the Chromite Group in the Protista Kingdom. Xanthophyta encompasses 650 living species so far identified. Xanthophyta live mostly in freshwater, although some species live in marine water, tree trunks, and damp soils. Some species are unicellular organisms equipped with two unequal flagella that live as free-swimming individuals, but most species are filamentous. Filamentous species may be either siphons or coenocyte. Coenocytes are organized as a single-cell multinucleated phallus that form

long filaments without septa (internal division walls) except in the specialized structures of some species. Siphonous species have multiple tubular cells containing several nuclei.

Xanthophyta synthesize chlorophyll a and smaller amounts of chlorophyll c, instead of the chlorophyll b of plants; and the cellular structure usually have multiple chloroplasts without nucleomorphs. The plastids have four membranes and their yellow-green color is due to the presence of beta-carotene and xanthine, such as vaucheriaxanthin, diatoxanthin, diadinoxanthin, and heretoxanthin, but not fucoxanthin, the brown pigment present in other Chromista. Because of the presence of significant amounts of chlorophyll a, Xanthophyceae species are easily mistaken for green algae. They store polysaccharide under the form of chrysolaminarin and carbohydrates as oil droplets.

One example of a relatively common Xanthophyta is the class Vacherie that gathers approximately 70 species, whose structure consists of several tubular filaments, sharing its nuclei and chloroplasts without septa. They live mainly in freshwater, although some species are found in seawater spreading along the bottom like a carpet. Other Xanthophyceae Classes are Tribonema, whose structure consists of unbranched filaments; Botrydiopsis, such as the species Botrydium with several thalli, each thallus formed by a large aerial vesicle and rhizoidal filaments, found in damp soil; Olisthodiscus, such as the species Ophiocytium with cylindrical and elongated multinucleated cells and multiple chloroplasts

4.3 LIPID EXTRACTION FROM MICROALGAE

The main interest in algae cultivation currently is to convert the algal lipids to biodiesel. Biodiesel is an alternative diesel fuel that offers several advantages to the environment; it is biodegradable and nontoxicity as well as has high lubricity, and low SO_x and low CO emissions. After the algal biomass is dehydrated, the biomass then proceeds to lipids extraction process. Unlike terrestrial oil-bearing crops, extraction of lipids from algal biomass is relatively difficult because of the presence of thick cell wall that prevents the release of interlipids. Hence, the use of mechanical press that is effective in extracting oil from terrestrial oil-bearing crops is generally not applicable to algal biomass. Ideally, the algal lipid extraction technology should display a high level of specificity and selectivity solely toward algal lipids (e.g., acyl glycerol) to avoid the co-

extraction of other compounds such as protein, carbohydrates, ketones, carotenes that cannot be directly converted to biodiesel.

Apparently, using chemical solvent to extract algal lipids seems to be the most suitable method, since it is widely practiced in laboratory-scale research. This is because chemical solvent has high selectivity for the algal lipids and the algal lipids are soluble in the chemical solvent. This allow even inter lipid to be extracted by diffusion across algal cell wall. Chemical solvents such as *n*-hexane, methanol, ethanol and mixed polar/nonpolar chemical solvents (e.g., methanol/chloroform and hexane/isopropanol) are effective for extraction of the algal lipids, but the extraction efficiency is highly dependent on microalgae strains. In the interest of reducing the energy consumption, lipid extraction from wet algal biomass that could eliminate the energy used for algal biomass dewatering process was encouraged. Thereby, a novel extraction method was developed by that employed switchable polarity solvents (SPS) to extract lipid from diluted algal cultures. The lipid recovery efficiency was around 70%-80%, in which the recovery was performed by adding the carbon dioxide into the extracting SPS.

Nevertheless, before chemical solvent extraction can be implemented on the commercial scale, several issues must be addressed:

- (1) Large quantity of chemical solvent is required for effective lipids extraction,
- (2) Solvent toxicity and safety must be considered,
- (3) Additional energy input will be needed for solvent recovery.
- (4) Additional costs will be incurred for wastewater treatment.

Other advanced technologies to improve algal lipids extraction efficiency such as autoclaving, supercritical CO₂ and ultra-sonication, are still under investigation and further optimization is urgently required before extending the technologies at the commercial scale.

The most common methods used for lipid extraction are modified Folch and Bligh and Dyer methods as shown in Figure 5. The harvested microalgae bio-mass was added with methanol and chloroform (2:1 v/v) for lipid extraction. Then, lipid extracts were washed with 0.9% saline followed by vortex and separation phase either using thin layer chromatography (TLC) or chromatography followed this method for total lipid

quantification and after centrifugation separation, lipids were visualized by thin layer chromatography (TLC).

Similarly, had measured lipid content in *Nannochloropsis ovulate* using the Bligh and Dyer extraction method followed by high performance thin layer chromatography. Cultivated under nitrogen starvation conditions produced triglycerides in the range of 31% to 43% due to a shift in cell metabolism used GC-MS to analyze lipid content.

The Folch and Bligh and Dyer methods were used for total lipid recovery and extraction was used; it was widely performed by researchers due to the simple procedures, time efficiency, energy savings and suitability for a large sample size.

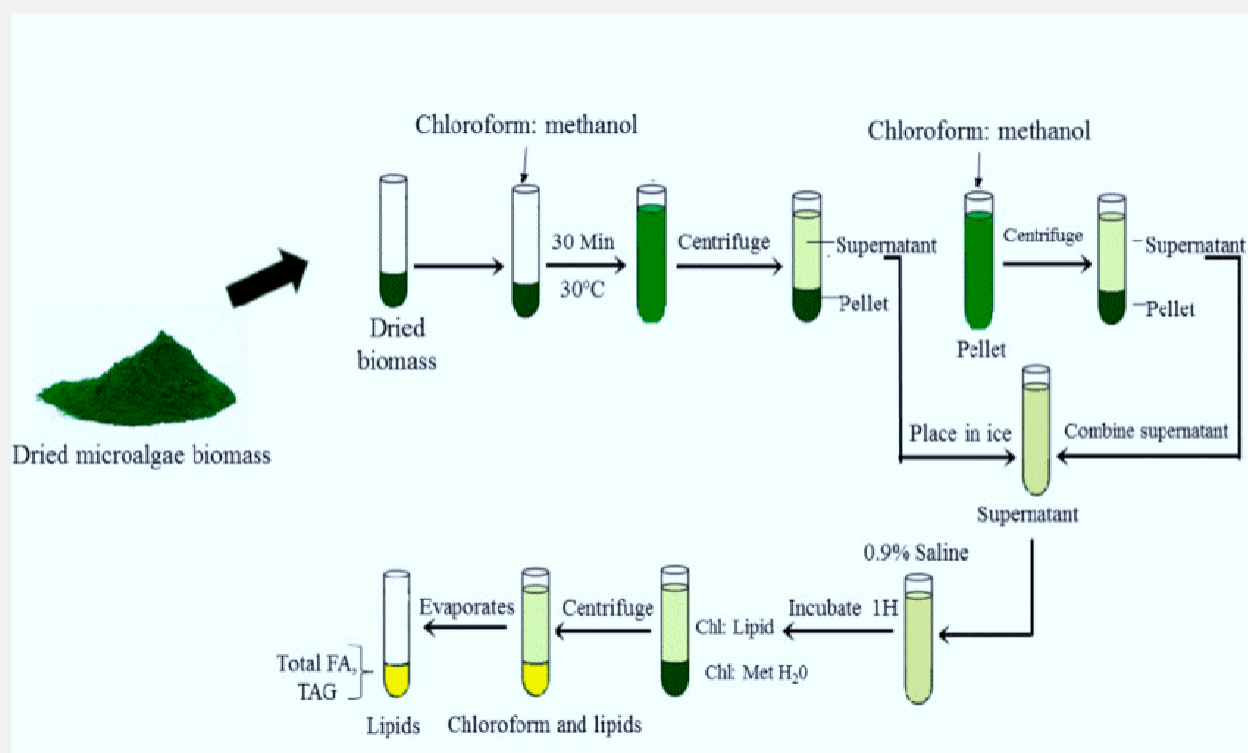


Figure 4.2 lipid extraction

Another promising method for lipid extraction was an osmotic shock that degrades microalgae cell structures to increase lipid yield efficiency.

Studied the effect of osmotic shock on *Dunaliella* saline and *Chaetoceros* Mueller for biogas (methane) generation. The study reported that Mueller lipid recovery efficiency

for methane production was higher than *D. Salina* with 72% and 21%, respectively. Microalgae biomass can be used for bio methane generation through anaerobic digestion or the biomass gasification and methanation process. Biomass produced in limited nitrogen culture media was more stable with low inhibitory substances such as ammonia which caused acidosis. Microalgae with high lipids content will yield greater volumes of bio methane than microalgae rich in carbohydrates and protein. Reported that high bio methane productivity was achieved under nitrogen-limited biomass conditions.

Similarly, osmotic shock enhanced the lipid recovery by two-fold in wet biomass of *Chlamydomonas Reinhardt*. In this technique, microalgae biomass was mixed with hexane, chloroform and methanol solvent in the Teflon-sealed tubes for 10 min for better lipid recovery since hexane and methanol required low heat for evaporation and rupture of microalgae cells. It concluded that an osmotic shock is a promising technique for microalgae lipid extraction recovery from wet biomass.

4.3.1 Qualitative test for lipids

Lipids

Lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, which are related more by their physical than by their chemical properties. Lipids are a class of compounds distinguished by their insolubility in water and solubility in non-polar solvents. Lipids are important in biological systems because they form the cell membrane, a mechanical barrier that divides a cell from the external environment. Non polar lipids, such as triglycerides, are used for energy storage and fuel. Polar lipids, which can form a barrier with an external water environment, are used in membranes. Polar lipids include glycerophospholipids and sphingolipids. Fatty acids are important components of all of these lipids.

Lipids have the common property of being relatively insoluble in water and soluble in nonpolar solvents such as ether and chloroform. They are important dietary constituents

not only because of their high energy value but also because of the fat-soluble vitamins and the essential fatty acids contained in the fat of natural foods.

4.3.2 Solubility test

Principle

This test is used to know the solubility of lipids in some solvents, according to polarity feature lipids are insoluble in polar solvents because lipids are non-polar compounds, thus lipids are soluble in non- polar solvents like chloroform, benzene and boiling alcohol.

Procedure

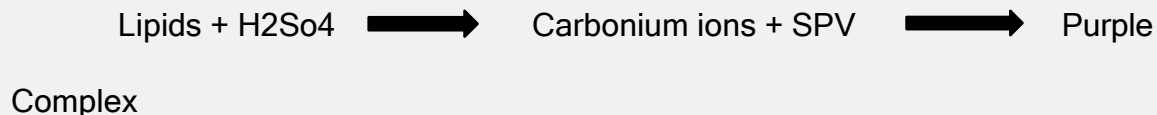
- In two clean dry test tubes add 1 ml of olive oil for each tube.
- Add for the first one 1 ml of chloroform.
- Add for the second tube 1 ml of distilled water.
- Shake both tubes vigorously for 2 minutes.
- Allow the tubes to stand and note the formation of homogenous solution with chloroform indicating that the lipid is dissolved and the formation of two layers with water indicating that the lipid is insoluble in water.

4.4 LIPID ESTIMATION

A colorimetric sulfo-phospho-vanillin (SPV) method was used to validate a high throughput method for total lipids analysis in fresh microorganisms. This method uses a small amount of biological material, does not require lot of sample manipulation, can be automated, is reproducible and easy to implement. A well micro plate SPV assay allows one to determine quickly total lipids in lyophilized cells of microalgae. The new assay method possesses many advantages compared to the others described in the literature: requires a small amount sample, less time and less labor and does not require organic reagents like chloroform in the reaction.

Keywords: Colorimetric method; microalgae; Lipid.

4.4.1 PRINCIPLE



4.4.2 MATERIALS AND METHOD

Sulfo-Phospho-Vanillin (SPV) Method

Reagents ACS grade concentrated sulfuric acid and orthophosphoric acid (85%). Vanillin (98%), Commercial olive oil. Phosphovanillin (PV) reagent was prepared by using six milligrams of vanillin dissolved in 100 mL of hot water and further diluted to 500 mL with 85% phosphoric acid. Oleic acid was used as standard and diluted properly with concentrated sulfuric acid to reach 1 mg.mL⁻¹.

Sample Preparation

- Phosphovanillin (PV) reagent was prepared by using six milligrams of vanillin dissolved in 100 mL of hot water and further diluted to 500 mL with 85% phosphoric acid.
- A stock solution of lipid (1000 mg/L) was prepared by dissolving 25 mg of lipid in 25 mL of methanol.
- Then, 10 working standard solutions were prepared from the stock solution to obtain the concentrations of 2, 6, 10, 25, 40, 50, 75, 100, 300, and 350 mg/L.

4.4.3 PROCEDURE

About 3g of the total lipid extract evaporated to dryness, 10 ml of phosphovanillin reagent added. 2 ml Standard solution were added. Blank consist of 3 ml of distilled water .To, all the test tubes, 2ml of sulfuric acid add to all the tubes and the contents were mixed well and kept in ice bath. After 20 min the color developed was read at 540nm using a colorimeter.

4.5 PRODUCTION OF BIODESEL

Biodiesel has gained much attention in recent years due to its eco-friendly nature, non-toxic characteristics, biodegradability and lower net carbon cycle compared to conventional diesel fuels. In the current study, potential algal specie xanthophyta were collected from different pond. In the first step, oil from algae specie was extracted using n-Hexane and Di-ethyl Ether as solvents, while in the second stage; extracted oil was converted into biodiesel via transestrification reaction. The effects of solvent to oil ratio, size of algal biomass and contact time were studied on the percentage yield of oil extracted. The maximum extracted oil was 0.09 fraction of biomass, by using a blend of both solvents at solvent to biomass ratio of 3.5, algal biomass size of 0.4 mm and contact time of 24 hours. While in transestrification reaction, effects of molar ratio, temperature, reaction time and amount of catalyst (Sodium Hydroxide) were evaluated on the amount of biodiesel produced. Almost 95% conversion of extracted oil into biodiesel was achieved after 25 minutes of contact time at 60 ° C with catalyst amount of 0.5% weight of oil and oil to methanol ratio of 8.

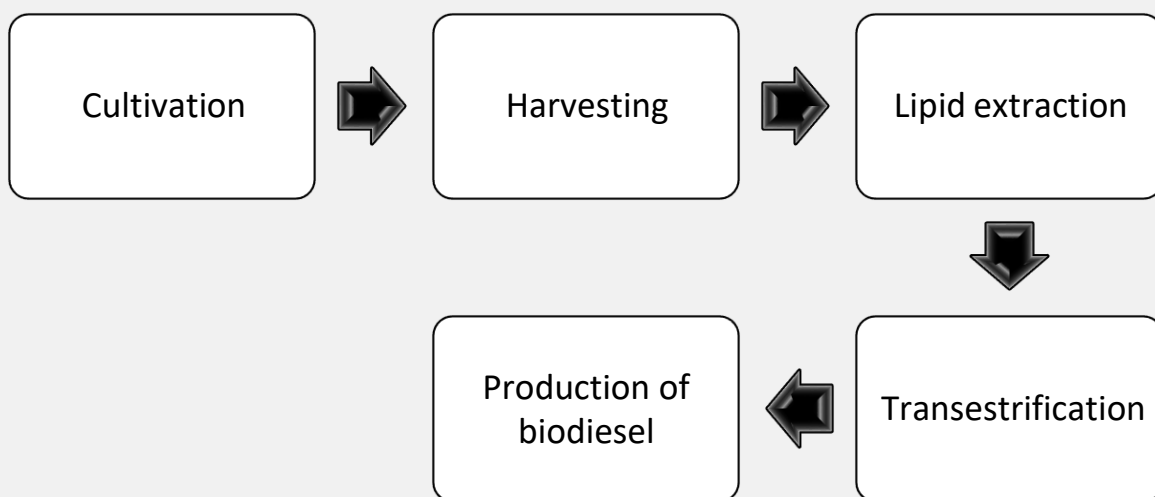


Figure4.3Steps of biodiesel production

4.5.1 MATERIALS

- 200 ml of algal oil
- 50 ml of ethyl alcohol
- 1gm of sodium hydroxide

4.5.2 PROCEDURE

- First dissolve a gram of NaOH in 50 ml of ethyl alcohol. Make sure the alcohol solid particle are dissolved.
- Heat the oil until it reached 60°C(7to8 mints)
- Turn off heat
- Add the sodium hydroxide ethyl alcohol mixture in the oil.
- Stir the mixture continuously for 30 minutes (change in colors).
- After stirring, let the mixture leave for 24 hours.
- After 24 hours decant to separate biodiesel from glycerol.
- By product was biodiesel.

4.5.3 Direct transesterification

Direct transesterification is a process that blends the microalgae with an alcohol and a catalyst without prior extraction. Number of acid catalysts have been investigated for heterotrophic microalgae biomass including hydrochloric (HCl) or sulphuric acid (H₂SO₄) but acetyl chloride (CH₃COCl) remains the catalyst producing the higher FAME yield of 56% (g FAME /g dry weight). A less polar solvent, like hexane or chloroform, can be added to increase the yield of biodiesel production. Direct Tran's esterification using a heterogeneous catalyst could be more effective coupled with microwaves heating. As an example, using microwave with direct Trans esterification of *Nannochloropsis* in presence of a heterogeneous catalyst (SrO), reported an increase in the FAME yield from 7 to 37%

(g FAME /g dry weight). However, direct transesterification requires a dry biomass, increasing the cost of harvesting.

4.5.4 Transesterification

The direct use of crude vegetable oils in diesel engines is envisage able, but could lead to numerous technical problems. For example, their characteristics (high viscosity, high density, difficulty to vaporize in cold conditions) cause deposits in the combustion chamber, with a risk of fouling and an increase in most emissions. These drawbacks can be mitigated, but not without some modifications of the diesel engine. To overcome all these inconveniences, the transformation of microalgae lipids in corresponding esters is essential.

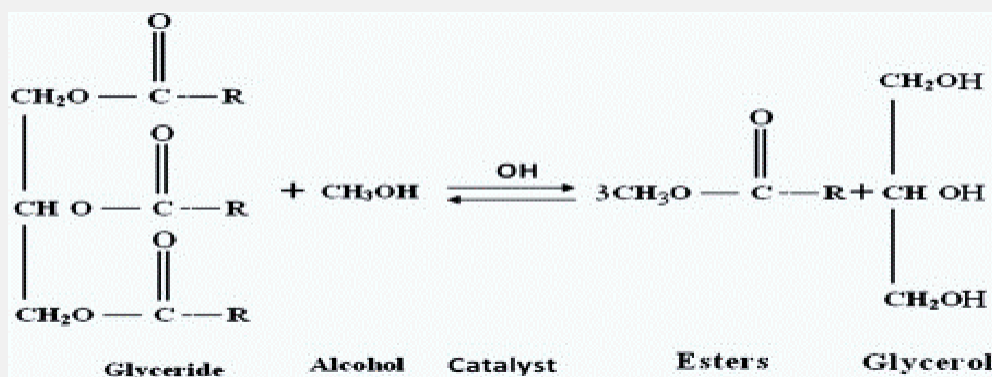


Figure 4.4 Transestrification process

4.5.5 Reaction

In the transesterification process, a catalyst and an alcohol are added to a blend of microalgae lipids. The reaction reduces the molecular weight, the viscosity and increases the volatility of microalgae lipids. Different parameters can influence the yield of transesterification like the ratio of alcohol-oil, catalyst types and concentration, reaction time, temperature and agitation rate.

4.5.6 Microalgae lipid content

The yield of the Trans esterification reaction depends on the nature of lipids. For example, monoglycerides like palmitic acid (C16:0) produced a FAME yield of 93% (g

FAME /g lipid) while triglycerides like triolein had a yield of 88% (g FAME /g lipid). Furthermore, phospholipids and glycolipids gave a lower yield of 54 to 65% (g FAME /g lipid) and 47 to 56% (g FAME /g lipid), respectively (Nagle & Lemke, 1990). The nature of lipids is an important data for biodiesel production because some microalgae can contain up to 93% (g /g lipid) of phospholipids and glycolipids (Williams & Laurens, 2010). Moreover, some microalgae can also contain lipids such as unsaponifiable lipids carotenoids and other elements (chlorophyll) which are considered as by-products.

3.4.3 Alcohol

Methanol is the most commonly used alcohol because of its low price. However, other alcohols such as ethanol or butanol can also be employed (Chisti, 2007). In traditional alkalibased catalyst transesterification of vegetable oil, the most used methanol to oil molar ratio for Tran's esterification is 6:1 even if the stoichiometric value is 3:1 for triglycerides. For microalgae lipids transesterification, the optimal methanol to oil ratio is higher. For example, performing a direct transesterification during 8h at 25°C, Ehimen et al. (2010) obtained a decrease in the specific gravity (SG) of the biodiesel from 0.8887 to 0.8849 when the molar ratio methanol to oil was increased from 105:1 to 524:1.

4.5.7 Catalyst

Catalysts used for transesterification of microalgae lipids are mainly homogenous or heterogeneous. Another method of Tran's esterification using methanol in the supercritical state (without catalyst) has been developed, but the cost of this technology renders its use impossible to date (Tan & Lee, 2011). Homogenous alkaline catalysts used for trans esterification of vegetable oils mainly include sodium or potassium hydroxide (NaOH or KOH) and sodium or potassium meth oxide (CH₃ONa or CH₃OK)) while homogenous acid-catalysts includes H₂SO₄, HCL and euphonic acids (R-SO₃H) (Helwani et al., 2009). In industrial vegetable oil biodiesel, homogenous alkali-catalysed Tran's esterification is commonly used because homogenous acid-catalyzed Tran's esterification is around 4000 times slower and these catalysts (NaOH or KOH) are relatively less expensive (Helwani et al., 2009). However, acidic catalyst is preferable for homogenous trans esterification of microalgae lipids because the biodiesel yield obtained by Nagle & Lemke (1990) was more than 50 times higher using HCL than NaOH (68 and 1.3% (g FAME/g lipids), respectively) as a catalyst for the same conditions of trans

esterification (0.1h, 70°C). The most used acid catalysts are CH₃COCl or H₂SO₄ (Miao & Wu, 2006). Using 4 concentrations of H₂SO₄ (0.56, 1.13, 1.35 or 2.25 mole/L) and a temperature of 90°C, Miao & Wu (2006) found that adding 2.25 mole/L catalyst gave lowest SG (0.863) but a slightly lowest yield of biodiesel (38%, g biodiesel/g lipid). Different homogenous acid-alkaline (H₂SO₄-CH₃OK, KOH-HCl) catalysts can also be used to Trans esterify lipids from microalgae (Halim et al., 2010; J. Lee et al., 2010). Using a 1st Trans esterification step of 2 hours with H₂SO₄ at 50°C and a 2nd step of 2 hours with CH₃OK at 55°C, Halim et al. (2010) obtained a maximum biodiesel yield of 44% (g FAME/g lipid). The purpose to add an alkaline catalyst is mainly to increase the FAME yield (Trans methylate acylglycerols and phospholipids) (Halim et al., 2010). Heterogeneous catalyst used for Trans esterification of triglycerides can be acid, alkali or enzymatic (immobilized enzymes) (Haldane et al., 2009). Trans esterification studies testing heterogeneous catalysts were conducted with *Nannochloropsis* microalgae using alkaline earth oxide base catalyst such as strontium oxide (SrO) (Oberg et al., 2011), calcium oxide (CaO) or magnesium oxide (MgO) (Umdur et al., 2009). As example, performing a direct transesterification, Koberg et al. (2011) reported biodiesel yields up to 99.9% (g biodiesel/g lipid) for a time of reaction of 2 min at 60°C for ratio SrO: microalgae of 30% (g catalyst/g microalgae). Few studies used enzymatic catalysts to Trans esterify lipids from microalgae. Generally, the most common used enzyme is lipase. A biodiesel yield of 98% (g FAME/g lipid) is reported for heterotrophic microalgae *Chlorella protothecoides* lipids transesterified using lipase (*Candida* sp. 99-125) immobilized on macro porous resins at a concentration of 30% (g/g lipid, 12000 U/g lipid) at 38°C during 12h (Li et al., 2007; Xing et al., 2008). However, enzymatic biodiesel production is often seen expensive due to the cost of the catalyst from 236 to 836 \$US/tonbiodiesel (Setoff et al., 2010). In comparison, the price of acid catalysts like H₂SO₄ (98%,w/w) is as low as 100 \$US/ton H₂SO₄ (Tao & Aden, 2009) for a price of catalyst for biodiesel production from 1.4 to 5.3 \$US/ton biodiesel (1 and 5% (w/w) catalyst, respectively) (Loreto et al., 2005).

4.5.8 Reaction time, temperature and stirring

Increasing the reaction time has a positive effect on the SG of the biodiesel produced. For example, performing a direct Tran's esterification of xanthophyceae microalgae at 30°C with H₂SO₄ as catalyst, Ehimen et al. (2010) found that the SG decreased from 0.914 to 0.884 when the reaction time was increased from 0.25 to 12h. The temperature seems to have less effect on the microalgae biodiesel production than reaction time except for high temperatures. For example, Miao & Wu (2006) used H₂SO₄ catalyst (2.25 mole/L) and found similar biodiesel yield of 56 and 58% (g biodiesel/g lipid) at temperatures of 30 and 50°C, respectively. At 90°C, the biodiesel yield dropped of about 38% (g biodiesel/g lipid). Stirring can have a positive effect on the biodiesel quality. For example, Hymen et al. (2010) observed a decrease of the SG from 0.9032 (stirring at 500 rpm) to 0.8831 (no stirring).

4.5.9 Purification of biodiesel and by-products

Microalgae biodiesel and by-products must be separated for increasing the biodiesel production. The main separation processes used hot water (50°C) (Li et al., 2007), organic solvents such as hexane (Halim et al., 2010; Wiltshire et al., 2000) and water-organic solvent for a liquid-liquid separation (Couth et al., 2010; Lewis et al., 2000; Satori et al., 2010). When using a non-polar co-solvent for Tran's esterification of lipids, only water is added to separate biodiesel from the by-products (M. B. Johnson & Wen, 2009). To our present knowledge, there is no study on the purification of biodiesel from microalgae. Based on 1st generation biodiesel (Leung et al., 2010), three main means of purification on biodiesel could be applied to microalgae biodiesel purification: "1-water washing 2-dry washing 3-membrane extraction." Based on vegetable oil biodiesel production (Berrios & Skelton, 2008) and microalgae composition, the main by-products could be unreacted lipids, water, alcohol, chlorophyll, metals and glycerol. Among the by-products obtained from the biodiesel production, glycerol is the most interesting. Glycerol worldwide consumption remains relatively constant, in recent years, with a consumption of 600 knot/year. Twenty-six percent of glycerol consumption was associated with pharmaceutical, cosmetic and soap industries (Bodily, 2003). From 2004 to 2011, massive biofuel production created a problem of overproduced glycerol and the price of

crude glycerol (80% pure) decreased from 110 to 7.5 \$US/ton (The Jacobsen, 2011; Aydan & Gonzalez, 2007). Gained glycerol can be transformed into added-value products using many paths including chemical, thermochemical or biological conversion. Among the chemical added-value products, glycerol can be oxidized or reduced to many compounds like propylene glycol, propionic acid, acrylic acid, propanol, isopropanol, ally alcohol and caroling but only some of these products are interesting in terms of market or profitability (D. T. Johnson & Taconic, 2007). Glycerol can be also converted into Fischer-Tropic fuel at low temperature (225-300°C) by catalytic processes (Soars et al., 2006) or transformed into hydrogen (H₂) by catalytic (generally nickel, platinum or ruthenium) or non-catalytic reforming (Vaidya & Rodrigues, 2009). Biological conversion of glycerol includes fermentation into alcohols (ethanol, butane, 1, 3- propanediol) and other products like H₂, format, propionic or succinic acid (Aydan & Gonzalez, 2007). Anaerobic digestion of by-products is another possible way to make biodiesel from microalgae cost effective if the lipid content of the microalgae does not exceed 40% (g lipid/g dry weight) (Salve et al., 2009). For example, Hymen et al. (2010) used anaerobic digestion of microalgae residues issued from a direct Trans esterification with a constant loading rate of 5 kg volatile solids (VS)/m³, temperatures and carbon-to-nitrogen (C/N) mass ratio varying from 25 to 40°C ant 5.4 to 24, respectively. For a temperature of 40°C and a C/N mass ratio of 8.53, a maximum methane (CH₄) concentration of 69% (v/v) with a specific CH₄ yield of 0.308 m³ CH₄/kg VS was obtained.

4.6 CONFIRMATION TEST FOR BIODIESEL

4.6.1 Wash soap test

- Apparatus: Beaker, syringe, timer and spectrometer.
- Chemicals: Biodiesel, water.
- The above method is used to the initial soap and catalyst content.
- Combine 1 part biodiesel and 1 part water in a beaker.
- Shake vigorously.

- The biodiesel phase should be on top and appear bright clear and the water phase at the bottom appears clear after settling.
- Check the clarity of both phases with a spectrometer and record it.
- Add a small known amount of soap to the biodiesel.
- Repeat the above steps three times for error purpose.

4.6.2 Characteristics of biodiesel

The physicochemical properties of biodiesel are nearly similar to diesel fuel. The most important properties for biodiesel are certain number, heat of combustion, viscosity.

1. The certain number is an indicator of quality of ignition of a fuel which increases with the number of carbon and decreases with the number of unsaturated carbon bounds (Hart Energy Consulting, 2007). Consequently, a higher unsaturated biodiesel like microalgae biodiesel would have a lower cetane number.

2. The heat of combustion shows if a biodiesel is suitable to burn in a diesel engine. The heat of combustion increases with the length of the carbon chain. Using lipids extracted from heterotrophic microalgae in the presence of H₂SO₄ in methanol obtained a biodiesel with a heat of combustion of 35.4 MJ/L which is in the range of diesel fuel (36-38 MJ/L).

3. As the cetane number, the viscosity increases with the number of carbon and decreases with the degree of unsaturation. A higher kinematic viscosity would create engine problems like engine deposits. Transesterification favors a decrease in the viscosity of the oil at values usually between 4 to 6 mm²/s.

4. Oxidation of biodiesel could happen when the FAME are in contact with oxygen and are transformed into hydrogen peroxides, aldehydes, acids and other oxygenates, which might form deposits. Oxidation of the biodiesel increases as a function of the degree of unsaturation (Hart Energy Consulting, 2007). Oxidation stability of microalgae lipids is therefore a real problem that can be overcome by adding antioxidants if the biodiesel blend is stored more than a few months (National Renewable Energy Laboratory, 2009)

5. Cold flow properties are important parameters for biodiesel production for northern countries like Canada and could be measured by cloud and pour points. The decrease of temperature could lead to the formation of visible crystals ($d \geq 0.5 \mu\text{m}$) in the biodiesel at a limit called cloud point. Cloud point temperature decreases with the mole fraction of unsaturated compounds and slightly increases with the length of the carbon chain. Pour point is defined as the temperature at which biodiesel does not flow anymore. Usually, cloud and pour points increase as a function of the molar ratio of biodiesel in diesel fuel from 0 to 100% (National Renewable Energy Laboratory, 2009). A higher level of polyunsaturated compounds in microalgae biodiesel could be a benefit in terms of cold properties (cold and pour points) for a blend microalgae biodiesel/petro diesel in cold climates.

6. The definition of lubricity for a fuel is “the ability to reduce friction between solid surfaces in relative motion”. The lubricity of diesel fuel is influenced by the viscosity, the acidity, the water content and the sulphur compounds. Even with additives, the measured friction (no unit reported) of biodiesel is lower than the one of petro diesel.

4.6.3 Advantages of biodiesel from algae lipid

Algae oil/algae biofuel: 1) provides rapid growth rates, 2) grows practically anywhere, a high per-acre yield (7-31 times greater than the next best crop—palm oil), 3) algae biofuel is non-toxic, 4) algae biofuel is highly bio-degradable, 5) high levels of polyunsaturates in algae biodiesel is suitable for cold weather climates, 6) can reduce carbon emissions based on where it's grown, 7) algae biomass can be produced at high volumes and this biomass can yield a higher percentage of oil than other sources, 8) algae oil has limited market competition, 9) algae can be cultivated on land, fresh water, or seawater, and 10) innovations of algae production allow it to become more productive while consuming resources that would otherwise be considered waste, 11) have a higher photon conversion efficiency, 12) can be harvested batch-wise nearly all-year-round, providing a reliable and continuous supply of oil, 13) and can utilize salt and waste water streams, thereby greatly reducing freshwater use (Schenk et al., 2008).

Algae have the potential of growing in places away from the farmlands and forests, thus minimizing the damages to the ecosystem and food chain supply. Algae can also be

grown in sewage and next to power-plant smokestacks, where they can digest the pollutants and deliver the oil (Vijayaraghavan & Hemanathan, 2009).

4.6.4 Disadvantages of biodiesel from algae lipid

Biodiesel from algae lipid: 1) produces unstable biodiesel with many polyunsaturates, 2) biodiesel performs poorly compared to its mainstream alternative, and 3) it is relative new technology. Other limitations include the low concentrations of free CO₂ that are required for peak algal growth and that algal grazers are a significant, but somewhat ignored problem (Schenk et al., 2008).

Challenges in Agriculture:

- 1) Too much direct sunlight can kill algae,
- 2) Temperature must be constant,
- 3) Overcrowding will inhibit algae growth,
- 4) The “waste” oxygen they produce must be continually removed from the water,
- 5) Open algal ponds are subject to evaporation and rainfall, which causes salinity and pH imbalances,
- 6) and local species of algae often overgrow the desired strain. The difficulties in efficient biodiesel production from algae are not from the extraction of the oil, but in finding an algal strain with a high lipid content and fast growth rate that is not too difficult to harvest, and a cost-effective cultivation system (type of photobioreactor) that is best suited to that strain (Demirbas & Demirbas, 2010). New materials and designs for cultivation in closed bioreactors and the use of cutting-edge metabolic engineering and screening/selection techniques are thought to provide the biggest promises (Schenk et al., 200)

CHAPTER-5

RESULT AND DISCUSSION

5.1 RESULT

5.1.1 Cultivation of microalgae



Figure 5.1 Collection of pond water and cultivation of microalgae

5.1.2 Identification of microalgae



Figure 5.2 Microscope view (xanthophyceae)

5.1.3 Lipid extraction from microalgae

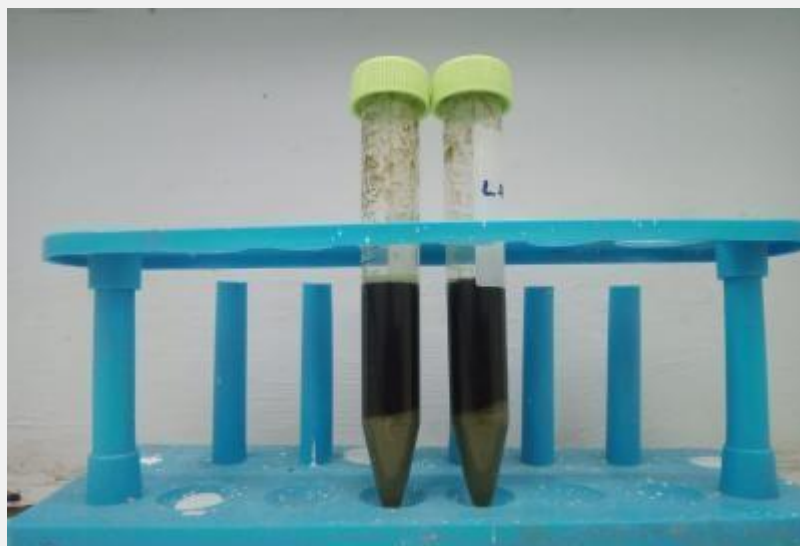


Figure 5.3 lipid extraction from microalgae

5.1.4 Qualitative test of lipids



Lipids in water



Lipids in cold ethanol



Lipids in chloroform

Figure 5.4 Solubility test of lipids

5.1.4.1 Result and conclusion

- The solubility test gives us an idea about the nature of the sample i.e., polar or non-polar
- Fats are soluble in organic solvents like chloroform, ethanol, etc. It is insoluble in water.
- So, if the given sample forms an oily layer above the surface of the water then fat is present.
- Partially soluble in ethanol and fully soluble in chloroform then presence of fat is confirmed.
- The based on the property of solubility of lipids in organic solvents and solvents and insolubility in water

5.1.5 Estimation of total lipids by colorimetric method

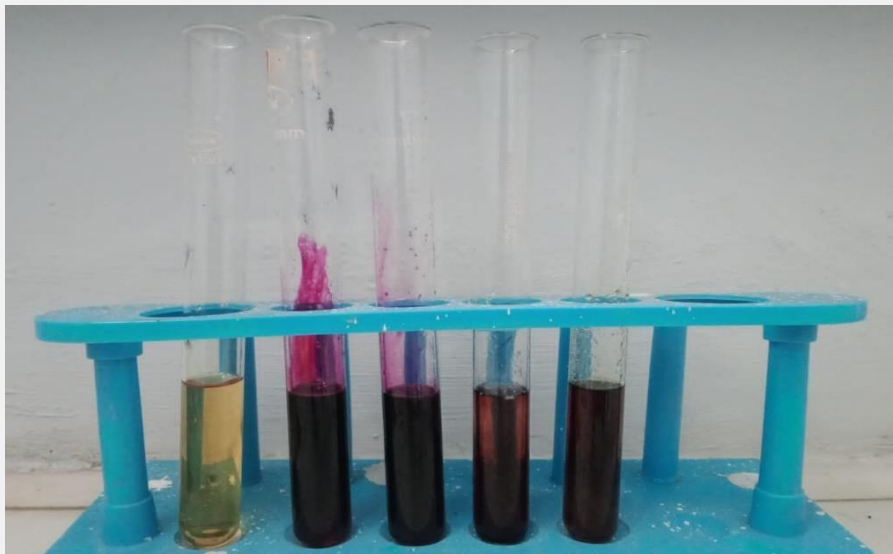


Figure 5.5 Estimation of lipids (before heat shock)

Table5.1 Estimation of lipids (before heat shock)

Blank	Absorbance of olive oil (OD)	Concentration Of olive oil (mg)	Absorbance of lipids (OD)	Concentration of lipids (mg)
0	0.10	100	0.20	200
0	0.15	150	0.30	300

Table5.1 for concentration of lipid and absorbance of lipids and absorbance of olive oil and concentration of olive oil.

5.1.5.1 CALCULATION

$$\frac{\text{Absorbance of unknown}}{\text{Concentration of unknown}} = \frac{\text{Absorbance of standard}}{\text{Concentration of standard}}$$

$$\begin{aligned}\text{Conc. Unknown} &= \frac{\text{A unknown} \times \text{conc. Standard}}{\text{A standard}} \\ &= \frac{0.20 \times 200\text{mg/dl}}{0.30} \\ &= 133\text{mg/dl}\end{aligned}$$

5.1.5.2 Graph

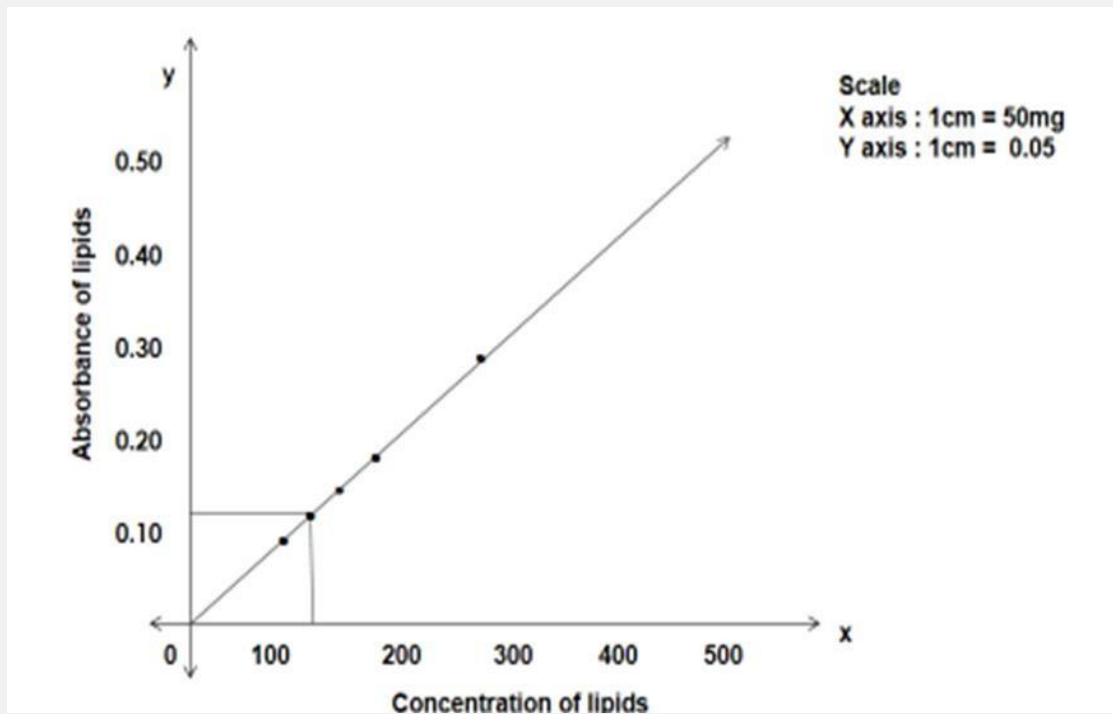


Figure 5.6 X axis is concentration of lipids(mg) and y axis is absorbance of lipids(OD).The standard curve obtained by plotting lipid content(mg)of olive oil as a function of optical density (OD).The optical density was measured using colorimeter.

5.1.6 Estimation of lipids (after heat shock)

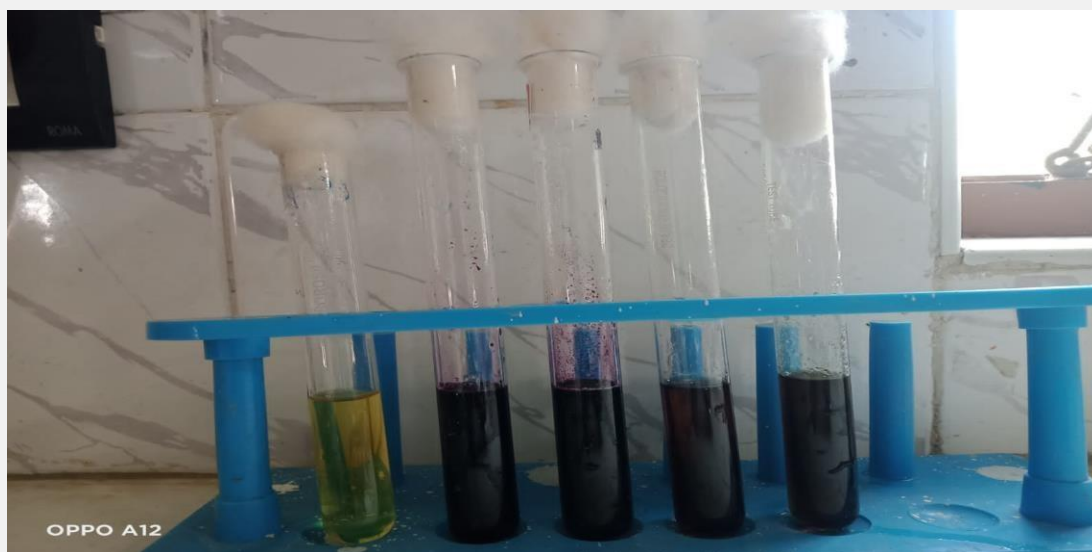


Figure 5.7 Estimation of lipids (after heat shock)

Table 5.2 Estimation of lipids (after heat shock)

Blank	Absorbance of olive oil (OD)	Concentration of olive oil (mg)	Absorbance of lipids (OD)	Concentration of lipids (mg)
0	0.25	250	0.10	100
0	0.50	500	0.20	200

Table 5.2 for concentration of lipid and absorbance of lipids and absorbance of olive oil and concentration of olive oil.

5.1.6.1 CALCULATION

$$\frac{\text{Absorbance of unknown}}{\text{Concentration of unknown}} = \frac{\text{Absorbance of standard}}{\text{Concentration of standard}}$$

$$\text{Conc. Unknown} = \frac{A_{\text{unknown}}}{A_{\text{standard}}} \times \text{conc. Standard}$$

$$= \frac{0.10}{0.20} \times 200\text{mg/dl}$$

$$= 100\text{mg/dl}$$

GRAPH

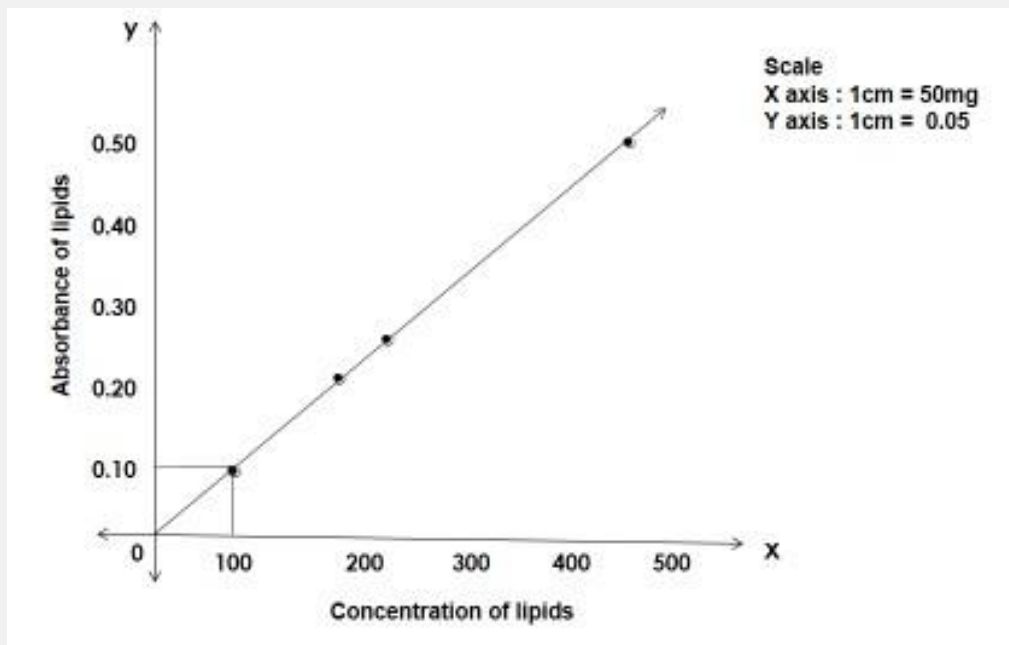


Figure 5.8 X axis is concentration of lipids(mg) and y axis is absorbance of lipids(OD).The standard curve obtained by plotting lipid content(mg)of olive oil as a function of optical density (OD).The optical density was measured using colorimeter.

PRODUCTION OF BIODIESEL



Figure5.9 Production of biodiesel from microalgae (before heat shock)



Figure5.10 Production of biodiesel from microalgae (after heat shock)

Result

Microalgae can be used as a feedstock for the production of biodiesel, which can then be used as a heating fuel. The heating value of biodiesel made from microalgae dependson several factors, including the lipid content of the microalgae, the method used for converting the lipids into biodiesel.

Results of microalgae biodiesel research include:

Before Heating:

The amount of biodiesel was 133mg/dl.

After Heating:

The amount of biodiesel was 100mg/dl.

5.2 DISCUSSION

Biodiesel is a renewable, clean-burning alternative to petroleum based diesel fuel. One potential source of biodiesel is microalgae, which are small aquatic organisms that are capable of photosynthesis and can be grown in a variety of environments. The production of biodiesel from microalgae involves several steps. First, the microalgae must be cultivated in a controlled environment, such as a photo bioreactor or an open pond. During cultivation, the microalgae are typically exposed to light, nutrients and carbon dioxide, which stimulate their growth and lipid production. Once the microalgae have reached maturity and accumulated sufficient lipids, they are harvested and processed to extract the oil.

The oil is then converted into biodiesel through a chemical process called transesterification, which involves reacting the oil with an alcohol, such as methanol or ethanol, in the presence of a catalyst. There are several advantages to producing biodiesel from microalgae. First, microalgae can be grown on non-arable land and can use wastewater or other sources of nutrients, reducing competition for resources with food crops.

However, there are also several challenges associated with producing biodiesel from microalgae. One of the biggest challenges is the high cost of production, which is due in part to the energy-intensive process of cultivating and harvesting microalgae. Additionally, scaling up microalgae production to a commercial level is a complex and expensive process that requires significant investment in infrastructure and technology.

Another challenge is the variability in the lipid content of different strains of microalgae, which can make it difficult to achieve consistent yields of biodiesel. Additionally, the high water content of microalgae biomass can make it difficult to extract the oil and can result in high processing costs.

CHAPTER-6

SUMMARY AND CONCLUSION

6.1 SUMMARY

Algae are an economical choice for biodiesel production, because of its availability and low cost. Our results prove that biodiesel can be produced from macroalgae. In this way algae can be used as renewable energy. Many researchers reported that microalgae might better for higher biodiesel production. But research has not done yet in this regard. So our results newly highlighted by producing biodiesel from macroalgae though it contains lower lipid content. Further research should be done having macroalgae and microalgae to compare the ratio of biodiesel production, chemical analysis and statistical significance. With the increase of the price of crude oil in the late 00s, blending biodiesel with petro diesel appears a sustainable solution to reduce the dependency on oil producing countries.

6.2 CONCLUSION

For now, the 1st generation of biofuels currently used could have economic, environmental and social negative consequences. To overcome these problems, producing biodiesel from microalgae lipids seems to be a sustainable solution as microalgae could be used to reduce the CO₂ emissions from coal power plants or wastewater pollution. Researchers are working to engineer super lipids producing microalgae strain in order to increase the yield of biodiesel. Producing biodiesel by Tran's esterification of lipids remains relatively costly compared to 1st generation biodiesel. Moreover, the polyunsaturated content of these www.intechopen.com 260 Advances in Chemical Engineering biodiesels could be profitable for cold properties, but seems to cause problems with the cetane number and the oxidative stability to meet the American standard (ASTM). Thus, more efforts must be made to reduce the process costs and to increase the biodiesel quality.

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