"DEVELOPMENT OF BIOFUEL FROM ALGAE"

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BACHELOR OF TECHNOLOGY

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APPLIED PETROLEUM ENGINEERING

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CERTIFICATE

This is to certify that the work contained in this thesis titled "**Development of biofuel from algae**" has been carried out by **Mr. Agam Gupta (R040207004)** and **Mr. Ravi Bhandari (R040207044)** under my/our supervision and has not been submitted elsewhere for a degree. They have successfully completed the project for the fourth year at **University of Petroleum & Energy Studies, Dehradun.**

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ABSTRACT

In order to decrease greenhouse gas emissions from industrial combustions and transports, biodiesel demand is constantly increasing. Biodiesel has received considerable attention in recent years as it is biodegradable, renewable and non-toxic fuel. It emits less gaseous pollutants than conventional diesel fuel, and can work directly in diesel engines with no required modifications.

The most common way to produce biodiesel is by transesterification of the oils with an alcohol in the presence of a catalyst to yield fatty acid methyl esters and glycerin. A microalgae (chlorella vulgaris) sample is used to produce biodiesel from algal oil and was experimented in the laboratory and results are discussed. Microalgae are photosynthetic renewable resources. They have fast growth rates, high lipid content and are capable to grow in saline waters which are unsuitable for agriculture. This project provides an overview in the production of biodiesel from microalgae including different systems of cultivation such as open ponds and closed Photobioreactors, the methods of harvesting biomass and extracting the oil content. It was found that, whereas there are outstanding issues related to photosynthetic efficiencies and biomass output, microalgae-derived biofuels could progressively substitute a significant proportion of the fossil fuels required to meet the growing energy demand.

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During the period that we carried out this project, we received valuable support and able guidance from numerous sources that helped to shape it and provide continuous feedback and kind support.

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CHAPTER 1: INTRODUCTION

1.1. Introduction

Conventional energy generation has brought on the risk of climate change, inevitable fuel shortages, and compromised national security. Along with these impending issues, energy demand is continually increasing. To protect the environment and maintain a sufficient energy for the supply, a concerted effort is being made to advance renewable energy alternatives. This project focused on the replacement of petroleum oil with renewable biodiesel. Therefore, finding a replacement would have the biggest impact on reducing fossil fuel demand. Waste grease, animal fats, and vegetable oils can be used for the production of biofuel. Most plants contain oil as a portion of their biomass, some more than others. An attractive source for biofuel would contain high amounts of oil, have the least impact on the environment, and minimize process operating costs. Additionally, the object for any renewable energy is to harness the energy of the sun at the most efficient rate. Vegetation that is already being grown in agriculture is an obvious target. Weed-like vegetation, such as Jatropha, is another attractive source for biodiesel because of its fast growth and low nutrient requirement to flourish. However, when looking to the optimal conversion of the sun's energy, microalgae achieve a photosynthetic efficiency of 11.6% whereas most plants harvest the sun's energy at a rate of 1-2%.Algae appear to have the most potential as a biodiesel source. However, technical challenges exist for the cultivation of high oil content algae. The most economical culturing set-up would be an open system, but these systems have not been feasible for culturing high oil content algae due to invasive species and temperature fluctuations. Though a closed system is more costly, controlled condition could optimize oil yields. One way of optimizing these oil yields is to utilize algae's natural process for storing energy in the form of lipids. When algae are put into a stressful environment, such as nutrient starvation, carbon uptake is used for storing energy rather than reproduction, thus producing more lipids. Yet, this may be a limiting factor in the optimization of overall oil yield due to the lack of cell reproduction. Overall biomass yield frequently decreases under environmentally stressed conditions.

1.2. Significance Of Research

This research utilizes both natural processes of algae for rapid growth and to develop a biofuel from algae.The research aims the development of a process for the use of micro-algae as a source of biofuel. The first stage would be culture of selected strain of algae in a continuous closed reactor for the exponential growth by providing light conditions nutrients and air agitation while a second stage would be filtration and drying of algal biomass and extraction of oil from algal biomass.

1.3. Objectives

The specific objectives of this project are to:

- Demonstrate that the microalgae chlorella vulgaris can be used to develop biofuel.
- Optimize culture conditions to maximize algal oil content.
- Examine the method of biofuel production via extraction from the microalgae.

Chapter 2: LITERATURE REVIEW

2.1 Algal Basics

Algae is almost everywhere around us. We see it flourish in both fresh and saline water, in cold mountain streams and hot inland swamps and ponds. Algae grow in almost any aquatic environment and use light and carbon dioxide (CO2) to create biomass. Algae range in size from a few micrometers to over 30 m in length.

2.1.1 Microalgae vs. macro algae

Generally, algae are classified into types: macroalgae and microalgae. Macroalgae are multicellular plants that are larger in size, which can well grow over 30 m in length. They produce small amounts of lipids that can be converted into fuel. Macroalgae can be often seen growing in ponds. The largest known multi-cellular algae are called seaweed. Microalgae, on the other hand, are microscopic (in the size of micrometers) unicellular algae that normally grow in water body. They can be grown in wastewater treatment ponds and saline aquifers. They are capable of converting CO_2 into energy. Microalgae cells grow at an exponential rate and can double every few hours during their exponential growth period. Microalgae grow so quickly and are known to contain large amounts of lipids which make them a promising crop for human use and so they are increasingly becoming an interest as a biofuel feedstock. This portion of the report discuss only about microalgae.

2.1.2 Biology of microalgae

Algae are known as one of the oldest life-forms on earth. They are primitive plants (thallophytes), i.e. they have no roots stems or leaves and have no covering of cells around the reproductive cells and have chlorophyll and can manufacture their own food through the process of photosynthesis. There are several main groups of microalgae, which differ primarily in pigment composition, biochemical constituents and life cycle. The most important algae in term of abundance are classified in four groups, explained below and shown in table 1.

Table 1: Four most important microalgae groups in terms of abundance

Microalgae	Known species	Storage material	Habitat
Diatoms	100 000	Carbohydrates and TAGs	Oceans, fresh water and brackish water
Green Algae	8 000	Starch and TAGs	Freshwater
Green algae	2 000	Starch and TAGs	Different habitats
Golden Algae	1 000	Carbohydrates and TAGs	Freshwater

Algae can either be autotrophic or heterotrophic; the former require only inorganic compounds such as CO_2, salts and a light energy source for growth; while the latter are non-photosynthetic therefore require an external source of organic compounds as well as nutrients as an energy source. Some algae are mixotrophic, i.e. they are capable of existing as either an autotroph or heterotroph. For autotrophic algae, photosynthesis is a key component of their survival, whereby they convert solar radiation and CO_2 absorbed by chloroplasts into adenosine triphosphate (ATP) and O_2 the usable energy currency at cellular level, which is then used in respiration to produce energy to support growth.

2.1.3 Major composition of microalgae biomass

The biomass of microalgae contains a number of complexes such as proteins and lipids, which make up the organelles. The composition of the biomass is useful for characterizing how the microalgae species is best useful. For example, with the knowledge that biodiesel is made from oils, a microalgae with a very high protein content and low lipid content would not be useful as a biofuel feedstock.

Algal biomass contains three main components: carbohydrates, protein and lipids/natural oil. Because the bulk of the natural oil made by microalgae is in the form of triacylglycerides (TAGs), which is the right kind of oil for producing biodiesel, microalgae is the exclusive focus in the algae-to-biofuel arena. Microalgae grow very quickly compared to terrestrial crops. They commonly double every 24 hrs. During the peak growth phase, some microalgae can double every 3.5 hrs. Oil contents of microalgae are usually between 20-50% (dry weight), while some strains can reach as high as 80%.

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$$CHO\text{-}OCH_2\ CH_2.....CH_2CH_3$$
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$$CH_2O\text{-}OCH_2\ CH_2.....CH_2CH_3$$

Molecular structure of triacylglycerol (TAG)

The fatty acids attached to the TAG within the algal cells can be both short and long chain hydrocarbons. The shorter chain length acids are ideal for the creation of biodiesel, and some of the longer ones can have other beneficial uses.

Table2: Oil content of some microalgae

Microalgae	Oil content (% dry weight)
Chlorella protothecoides	23-30
Botryococcusbraunii	25-80
Chlorella vulgaris	14-40
Crypthecodiniumcohnii	20
Cylindrotheca sp.	16-37
Dunaliella salina	14-20
Neochlorisoleoabundans	35-65
Nitzschiasp	45-47
Phaeodactylumtricornutum	20-30
Schizochytrium sp.	50-77
Spirulina maxima	4-9
Tetraselmissuecia	15-23

2.2. Biofuel

Oil prices have always been a concern. Recent events, along with increased awareness of the environment, have shown us the need for the creation of alternative means of energy. Many different options have been proposed. Nuclear power is possible but comes with obvious safety concerns. Solar and wind look like viable options, but don't seem to be getting large amounts of support. Another option is biofuel, which involves using the energy of organic materials to replace the function of fossil fuels. Biofuel fuel that is derived from biomass that is, plant material or animal waste. Since such feedstock material

can be replenished readily, biofuel is considered to be a source of renewable, unlike fossils fuels such as petroleum, coal, and natural gas. Biofuel is getting attention as a cost-effective and environmentally friendly nature. It serves as an alternative to petroleum and other fossil fuels, particularly within the context of rising petroleum prices and increased concern over the contributions made by fossil fuels to global warming. Another kind of biofuel is biodiesel, which is made from either vegetable oils or animal oils. As with ethanol, it can be used purely on its own but is commonly just a supplement to be added with other fuel. It's currently the most common biofuel in Europe. The process of turning animal and vegetable oils into usable fuel is known as transesterification. 1.8% of the world's transport fuel was biofuel in 2008. This figure seems small, but investment in these technologies is continually increasing, and will inevitably create new technological breakthroughs and a rise in popularity. Biofuels come in many different forms, and are commonly categorized into first, second and third generation.

2.2.1. First generation fuels

First generation fuels are made from food crops such as sugar, starch and animal or oil fats. Grains can be made into bioethanol, and sunflower seeds into vegetable oil and then biodiesel. These are the most common first generation biofuels: Biodiesel, bioalcohols, vegetable oil, bioethers, solid biofuels, Syngas and biogas.

2.2.2. Second generation fuels

From non-food crops like waste, stalks of wheat and corn we get the second generation of biofuels. Since first generation biofuels are made from edible sources, the hunt is on to create more second-generation technology that can avoid a food shortage that may occur. They include biohydrogen, biomethanol, mixed alcohols and wood diesel.

2.2.3. Third generation fuels

Algae fuel, also called oilgae or third generation biofuel, is a biofuel from algae. Algae are low-input, high-yield feedstock to produce biofuels. Based on laboratory experiments, it is claimed that algae can produce up to 30 times more energy per acre than land crops such as soybean but these yields have yet to be produced commercially. With the higher prices of fossil fuels (petroleum), there is much interest in algaculture (farming algae). One advantage of many biofuels over most other fuel types is that they are biodegradable, and so relatively harmless to the environment if spilled. Algae fuel still has its difficulties

though, for instance to produce algae fuels it must be mixed uniformly, which, if done by agitation, could affect biomass growth. The United States Department of Energy estimates that if algae fuel replaced all the petroleum fuel in the United States, it would require only 15,000 square miles (38,849 square kilometers), which is roughly the size of Maryland or less than one seventh the amount of land devoted to corn in 2000. Algae, such as Botryococcus braunii and Chlorella vulgaris are relatively easy to grow, but the algal oils hard to extract. There are several approaches, some of which work better than others. Macroalgae (seaweed) also have a great potential for bioethanol and biogas production.

- **Ethanol from living algae**

Most biofuel production comes from harvesting organic matter and then converting it to fuel but an alternative approach relies on the fact that some algae naturally produce ethanol and this can be collected without killing the algae. The ethanol evaporates and then can be condensed and collected. The company Algenol is trying to commercialize this process.

- **Distillates**

However, if biocatalytic cracking and traditional fractional distillation are used to process properly prepared algal biomass, i.e. biocrude, then distillates can be produced, such as jet fuel, gasoline, diesel and others.

2.2.4. Fourth generation biofuels

A number of companies are pursuing advanced "bio-chemical" and "thermo-chemical" processes that produce "drop in" fuels like "green gasoline," "green diesel," and "green aviation fuel." While there is no one established definition of "fourth-generation biofuels," some have referred to it as the biofuels created from processes other than first generation ethanol and biodiesel, second generation cellulosic ethanol, and third generation algae biofuel. Some fourth generation technology pathways include: pyrolysis, gasification, upgrading, solar-to-fuel, and genetic manipulation of organisms to secrete hydrocarbons.

2.3 Types of biofuel

Some long-exploited biofuels, such as wood, can be used directly as a raw material that is burned to produce heat. The heat, in turn, can be used to run generators in a power plant to produce electricity. Liquid biofuels are of particular interest because of the vast infrastructure already in place to use them, especially for transportation. The liquid biofuel

in greatest production is ethanol (ethyle alcohol), which is made by fermenting starch or sugar. Brazil and the United States are among the leading producers of ethanol. In the United States, ethanol biofuel is made primarily from corn (maize) grain, and it is typically blended with gasoline to produce "gasohol," a fuel that is 10 percent ethanol.

In Brazil, ethanol biofuel is made primarily from sugarcane, and it is commonly used as a 100-percent-ethanol fuel or in gasoline blends containing 85 percent ethanol. The second most common liquid biofuel is biodiesel, which is made primarily from oily plants (such as the soybean or oil palm) and to a lesser extent from other oily sources (such as waste cooking fat from restaurant deep-frying). Biodiesel, which has found greatest acceptance in Europe, is used in diesel engines and usually blended with petroleum diesel fuel in various percentages.

Other biofuels include methane gases which can be derived from the decomposition of biomass in the absence of oxygen and methanol butanol, and dimethyl ether—which are in development. At present, much focus is on the development of methods to produce ethanol from biomass that possesses high cellulose content. This cellulosic ethanol could be produced from abundant low-value material, including wood chips, grasses, crop residues, and municipal waste.

2.4 Potential role of biofuels from microalgae

Microalgae can provide several different types of renewable biofuels. They are composed mainly of carbohydrates, proteins and lipids. Algae having the ability to synthesize TAGs are considered as a second generation feedstock for biofuels production. The lipid content of algal oil can be processed into biodiesel. Also, by anaerobic digestion of the algal biomass they can provide biogas and fertilizers. An important algal characteristic for biodiesel production is the suitability of lipids in terms of type, chain length, degree of saturation and proportion of total lipid made up by triglycerides.)

Table 3. Oil yields based on crop type (adapted from Chisti, 2007).

Crop	Crop Oil yield (gallons/acre)
Corn	18
Soybeans	48
Canola	127
Jatropha	202
Coconut	287
Oil Palm	636
Microalgae [a]	6283-14641

a. Oil content ranging from 30% to 70% (w/w) of dry biomass

Microalgae have also a high technical potential to decrease greenhouse gases, given their ability to use carbon dioxide in their photosynthetic efficiency, and the possibility of achieving faster growth as compared to any energy crop. They reproduce quickly and can be harvested day after day. However, the lipid content in microalgae required to be high, otherwise the economic performance would be hard to achieve. Algae can grow in salt water, freshwater and even contaminated water. They can grow at the sea, lakes, ponds, and on lands not suitable for food.

The advantages of using microalgae-derived biofuels are:

- Microalgae are capable of all year round production, therefore, oil productivity of microalgae cultures exceeds the yield of the best oilseed crops, e.g. biodiesel yield of 12,000 l ha^{-1} for microalgae (open pond production) compared with 1190 l ha^{-1} for rapeseed.
- They grow in aqueous media, but need less water than terrestrial crops. therefore reducing the load on freshwater sources.
- Microalgae can be cultivated in saline water on non-arable land, and therefore may not incur land-use change, minimizing associated environmental impacts while not compromising the production of food, fodder and other products derived from crops.
- Microalgae have a rapid growth potential and many species have oil content in the range of 20–50% dry weight of biomass, the exponential growth rates can double their biomass in periods as short as 3.5 hrs.
- With respect to air quality maintenance and improvement, microalgae biomass production can effect biofixation of waste CO2 (1 kg of dry algal biomass utilize about 1.83 kg of CO2).
- Nutrients for microalgae cultivation (especially nitrogen and phosphorus) can be obtained from wastewater; therefore, apart from providing growth medium, there is dual potential for treatment of organic effluent from the agro-food industry.
- Algae cultivation does not require herbicides or pesticides application.
- They can also produce valuable co-products such as proteins and residual biomass after oil extraction, which may be used as feed or fertilizer or fermented to produce ethanol or methane.
- The biochemical composition of the algal biomass can be modulated by varying growth conditions; therefore, the oil yield may be significantly enhanced.
- Microalgae are capable of photo biological production of 'biohydrogen'.

The outlined combination of potential biofuel production, CO2 fixation, biohydrogen production, and bio-treatment of wastewater underscore the potential applications of microalgae.

Despite its inherent potential as a biofuel resource, many challenges have impeded the development of algal biofuel technology to commercial viability that could allow for sustainable production and utilization. They include:

- Species selection must balance requirements for biofuel production and extraction of valuable co-products.
- Attaining higher photosynthetic efficiencies through the continued development of production systems.
- Development of techniques for single species cultivation, evaporation reduction, and CO2 diffusion losses.
- Potential for negative energy balance after accounting for requirements in water pumping, CO2 transfer, harvesting and extraction.
- Few commercial plants in operation; therefore, there is a lack of data for large scale plants.
- Incorporating flue gases which are unsuitable in high concentration owing to the presence of poisonous compounds such as NOx and SOx.

2.5 Growing, Harvesting and Processing algae

2.5.1 Monoculture

Most growers prefer monoculture production and go to considerable lengths to maintain the purity of their cultures. With mixed cultures, one species comes to dominate over time and if a non-dominant species is believed to have particular value, it is necessary to obtain pure cultures in order to cultivate this species. Individual species cultures are also needed for research purposes.

A common method of obtaining pure cultures is serial dilution. Cultivators dilute a wild sample or a lab sample containing the desired algae with filtered water and introduce small aliquots into a large number of small growing containers. Dilution follows a microscopic examination of the source culture that predicts that a few of the growing containers contain a single cell of the desired species. Following a suitable period on a light table, cultivators again use the microscope to identify containers to start larger cultures.

2.5.2 Microalgae Production systems

Producing biomass from microalgae is generally more expensive than growing crops. Photosynthetic growth requires light, carbon dioxide, water and inorganic salts. Algal biomass contains generally three components: carbohydrates, proteins and lipids. Growth medium must provide the inorganic elements that constitute algal cells. These essential elements are nitrogen (N), phosphorous (P), iron, and in some cases Silica. Minimal nutritional requirements can be estimated using the formula $CO_{0.48}\ H_{1.83}\ N_{0.11}\ P_{0.01}$. This formula is based on the data presented by Grobbelaar in 2004. Microalgae biomass contains approximately 50% carbon by dry weight. All of this carbon is typically derived from carbon dioxide. 100 ton of algal biomass produced can fixed roughly 183 ton of carbon dioxide. Carbon dioxide must be fed continuously during light day hours. Microalgae cultivation can be done in open production systems such as lakes or ponds and in controlled closed systems called Photobioreactor (PBRs). The most important methods include batch, continuous and semi-continuous modes.

a) Batch mode:

This is a closed, volume limited system in which there is no inputs or output of materials. The algal population cell density increases constantly, whereas nutrient components of culture medium decrease over time. Other products produced by the cells during growth also increase in culture medium. When resources are used by the cells the cultures die unless supplied with new medium. This is done by transferring a small volume of existing culture to a large volume of fresh culture at regular intervals. With this method algae are allowed to grow and reproduce in closed containers. In batch mode microalgae shows a typical growth dynamic pattern according to a sigmoid curve (figure 18), consisting of a succession of six phases of growth:

- **Adaption:** in this phase the culture has to be adapted to the environment. It takes some time before algae can start growing.
- **Acceleration** and **Exponential growth:** It starts the exponential logarithmic growth. During this phase an increase of algal biomass per time is proportional to the biomass in the culture at any given time. A steady-state is reached and the cells are divided at constant rate. The cell density increases as a function of time according to the exponential function:

  $N2=N1.e^{\mu}$, where N2 and N1 are the number of cells at successive times and μ is the growth rate.

- **Decreasing growth:** The logarithmic point declines when nutrients are depleted and when de culture density reaches its critical point. The increase in algal biomass becomes linear.
- **Stationary:**

The light supply per algal cell becomes limited. The cell population continues to increase but the growth rate decreases until it reaches zero, where the culture enters the stationary phase. The cell concentration remains constant at its maximum value. The growth curve approaches a limiting value.

- **Decline and death:**

This phase is caused by unfavourable conditions, like limited supply of light and nutrients or an infection by other microorganisms. This phase is characterized by negative growth rate and it becomes an exponential death of the population.

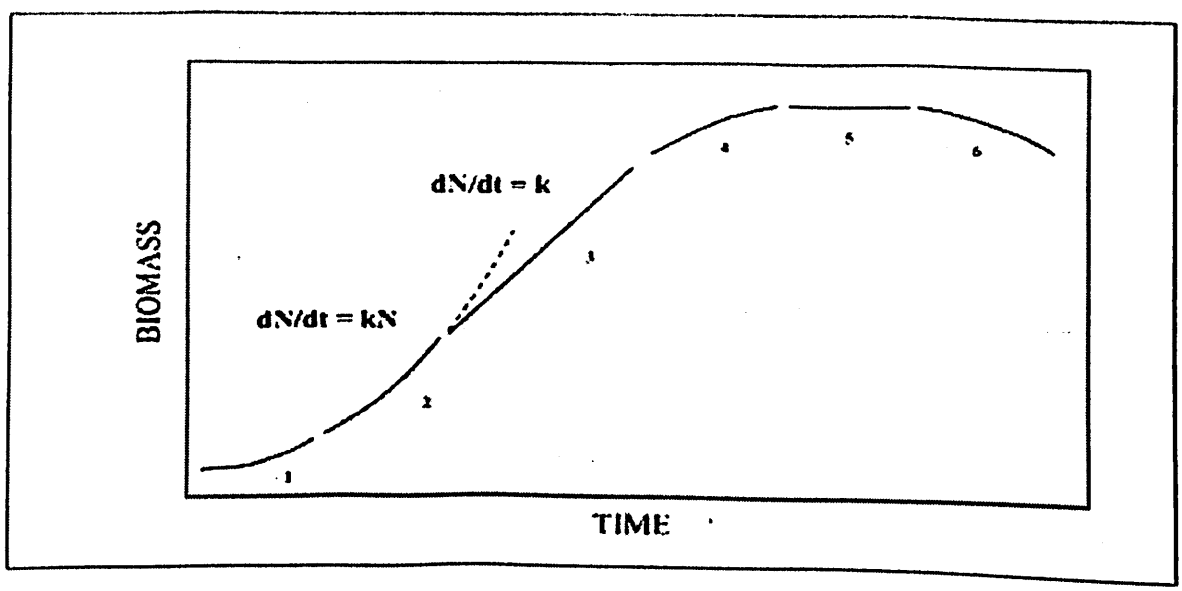


Figure 1: Schematic representation of algae growth rate in batch culture

b) Continuous system:

In continuous culture systems, resources are potentially infinite: cultures are maintained at a chosen point on the growth curve by the regulated addition of fresh culture medium. A volume of fresh culture medium is added at a rate proportional to the growth rate of algae, while an equal volume of culture is removed. This method maintains the cultures very close to the maximum growth rate, because the culture never runs out of nutrients. The

early development of a continuous culture system was developed back to the 1950s with the first chemostat, called "bactogen".

c) Semi-continuous systems:

In these systems the fresh medium is delivered to the culture all at once, by simply opening a valve in the medium delivery line. Fresh medium flows into the culture vessel, and spent culture flows out into a collecting vessel. Once the required medium has entered the culture, the valve is closed, and the culture is allowed to grow for 24h. Then, it becomes a partial periodic harvesting followed by topping up the original volume and supplementing with nutrients to achieve the original level of enrichment. The culture is grown up again and partially harvested. As the culture is not harvested completely, the semi-continuous method yields more algae than batch methods for a given tank size.

2.6 Algae Mass-Cultivation Systems

Most microalgae are strictly photosynthetic, i.e., they need light and carbon dioxide as energy and carbon sources. This culture mode is usually called photoautotrophic. Some algae species, however, are capable of growing in darkness and of using organic carbons (such as glucose or acetate) as energy and carbon sources. This culture mode is termed heterotrophic. Due to high capital and operational costs, heterotrophic-algal culture is hard to justify for biodiesel production. In order to minimize costs, algal-biofuel production usually must rely on photoautotrophic-algal growth using sunlight as a free source of light—even though it lowers productivity due to daily and seasonal variations in the amount of light available.

Photoautotrophic microalgae require several things to grow. Because they are photosynthetic, they need a light source, carbon dioxide, water, and inorganic salts. The water temperature should be between 15°C and 30°C (approximately 60°F to 80°F) for optimal growth. The growth medium must contribute the inorganic elements that help make up the algal cell, such as nitrogen, phosphorus, iron, and sometimes silicon. For large-scale production of microalgae, algal cells are continuously mixed to prevent the algal biomass from settling, and nutrients are provided during daylight hours when the algae are reproducing. However, up to one-quarter of algal biomass produced during the day can be lost through respiration during the night.

There are a variety of photoautotrophic-based, microalgal culture systems. For example, the algae can be grown in suspension or attached on solid surface. Each system has its own advantages and disadvantages. Currently, suspend-based open ponds and enclosed photobioreactors are commonly used for algal-biofuel production. In general, an open pond is simply a series of outdoor "raceways," while a Photobioreactor is a sophisticated reactor design that can be placed indoors (greenhouse) or outdoors. The details of the two systems are described below.

2.6.1 Open Pond:

Open ponds are the oldest and simplest systems for mass cultivation of microalgae. In this system, the shallow pond is usually about one-foot deep, and algae are cultured under conditions identical to their natural environment. The pond is designed in a raceway configuration, in which a paddlewheel circulates and mixes the algal cells and nutrients (figure 2). The raceways are typically made from poured concrete, or they are simply dug into the earth and lined with a plastic liner to prevent the ground from soaking up the liquid. Baffles in the channel guide the flow around the bends in order to minimize space.

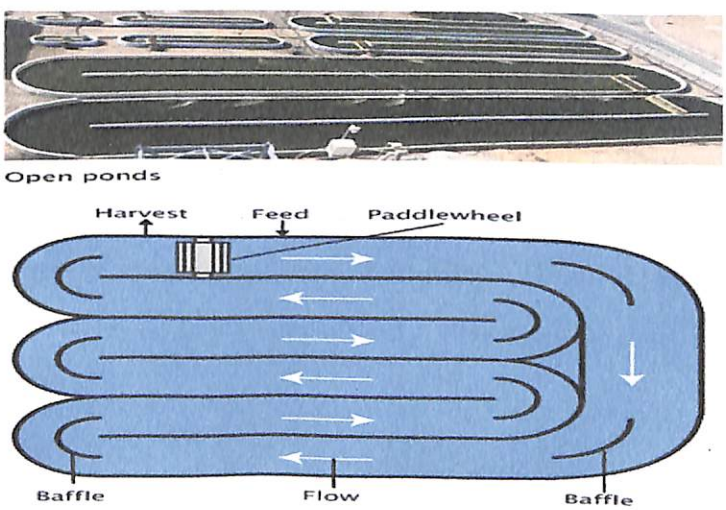


Figure 2: Open pond system

The system is often operated in a continuous mode, i.e., the fresh feed (containing nutrients including nitrogen phosphorus and inorganic salts) is added in front of the paddlewheel, and algal broth is harvested behind the paddlewheel after it has circulated through the loop. Depending on the nutrients required by algal species, several sources of wastewater—such as dairy/swine lagoon effluent and municipal wastewater—can be used for algal culture. For some marine-type microalgae, seawater or water with high salinity can be used.

Although open ponds cost less to build and operate than enclosed photobioreactors, this culture system has its intrinsic disadvantages. Because they are open-air systems, they often experience a lot of water loss due to evaporation. Thus, open ponds do not allow microalgae to use carbon dioxide as efficiently, and biomass production is limited. Biomass productivity is also limited by contamination with unwanted algal species as well as organisms that feed on algae. In addition, optimal culture conditions are difficult to maintain in open ponds, and recovering the biomass from such a dilute culture is expensive. The necessary for a large-scale cultivation area has been pointed out as a limitation in using open ponds to grow microalgae for mitigating the CO2 released from power plants. It has been estimated that a raceway pond requires 1500 m2 to fix the CO2 emitted from a 150 MW thermal power plant.

2.6.2 Enclosed Photobioreactors

Algae can be grown in closed systems called Photobioreactors (PBRs). These devices are bioreactors which incorporates some type of light source. PBRs are flexible systems that can be optimized according to the biological features of the algal species that are cultivated. PBRs provide a protected environment with safety from contamination by other microorganisms and culture parameters can be better controlled. They allow more species to be grown than open systems, and permit especially single-species culture of microalgae. They also prevent evaporation and reduce water use, lower CO2 losses due to outgassing and permit higher cell concentration and consequently higher productivity. As algae are grown, excess culture is overflowed and harvested. If sufficient care is not taken, continuous bioreactors often collapse very quickly. PBRs can be divided into four groups:

- Vertical tubular
- Horizontal tubular
- Plate
- Plastic bag systems

Horizontal tubular photobioreactor is explained here. A horizontal tubular PBR consists of an array of straight transparent tubes made of plastic or glass. This tubular array is the solar collector, where the sunlight is captured. The ground of the solar collector is often painted white to increase reflectance to the tubes. Tube diameter is limited at 0.1m or less because light does not penetrate too deeply in the culture that is necessary to ensure high biomass productivity in the PBR. Microalgae cultures circulate from a vessel to the solar collector and go back to the vessel. In the solar collector is where photosynthesis occurs, is where

algae absorb solar radiation or artificial light through the transparent plastic tubes. This operation is in continuous mode. The water is transported by pumps from the photosynthesis part of the PBR to the feeding vessel. The algae are not illuminated in the vessel, so that a natural light-dark system between the photosynthesis part and the vessel can occur. Biomass sedimentation in tubes is prevented by maintaining highly turbulent flow. Flow is produced using a mechanical pump or an airlift pump. Artificial illumination of tubular PBRs is technically feasible but expensive compared with natural illumination.

As the culture goes along the PBR tube, pH increases because of consumption of carbon dioxide. Additional carbon dioxide injection is necessary to prevent carbon limitation and an excessive rise in pH. PBR requires cooling during daylight hours and control of the temperature during the night. In outdoor PBR is effectively and inexpensive use heat exchanges for cooling the cultures. A heat exchanger may be located in the degassing column. To select the suitable biomass production method for making biodiesel it is necessary to compare between open ponds and closed PBRs.

Figure 3: Photobioreactors

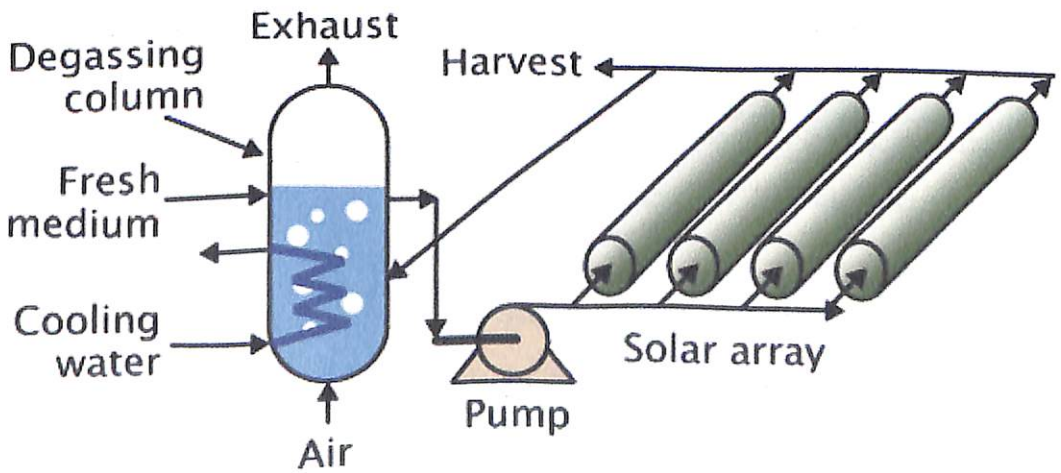


Figure 4: Schematic tubular photobioreactor

Table 4: Comparison of closed and opened production system

Parameter	Open ponds and raceways	Photobioreactors
Required space	High	Low
Water loss	Very high, may also cause salt precipitation	Low
CO2 loss	High(depends on pond depth)	Low
Oxygen concentration	Usually low enough because of continuous spontaneous outgas.	Closed systems requires gas exchange devices
Temperature	Highly variable	Temperature can be controlled by cooling
Shear	Low (gentle mixing)	High (fast and turbulent flows required for good mixing, pumping through gas exchange devices)
Cleaning	No issue	Required (wall-growth and dirt reduce light intensity)
Contamination Risk	High (limiting the number of species that can be grown)	low
Biomass quality	Variable	Reproducible
Biomass concentration	Low, between 0.1 and 0.5 g/l	High, between 2 and 8 g/l
Production flexibility	Only few species possible, difficult to switch	High, switching possible
Process control and reproducibility	Limited (flow speed, mixing, temperature only by pond depth)	Possible within certain tolerances
Weather dependence	High (light intensity, temperature, rainfall)	Medium (light intensity, cooling required)
Startup	6 – 8 weeks	2 – 4 weeks
Capital costs	High	Very high
Operating costs	Low	Very high

Harvesting cost	High, species dependent	Lower due to high biomass concentration and better control over species and conditions
Current commercial applications	5000 t of algal biomass per year	Limited to processes for high added value compounds or algae used in food and cosmetics

2.7 Microalgae production and biofuels productivity factors

Microalgae like other plant-based biofuel resources provide the mechanism for collection, conversion and storage of solar energy into chemical form. For biofuel production the major factors which are economically viable for production include: productivity (such as strain selection, photosynthetic efficiency, and productivity of lipids), production and harvesting costs. Photosynthetic efficiency is only relevant for autotrophic algae; for heterotrophically cultivated algae, the utilization of sugars is more relevant.

2.7.1 Photosynthesis

Photosynthesis is defined as the synthesis of organic molecules which are using the energy of light. In this process many inorganic compounds and light energy are converted to organic matter by photoautotroph. Light (natural or artificial) provides the energy to:

- Transfer of electrons from water to $NADP^+$ forming NADPH (nicotinamide adenine dinucleotide phosphate)
- Generate ATP (adenine tri-phosphate).

ATP and NADPH provide the energy and electrons to reduce carbon dioxide into organic molecules. Microalgae are normally grown using light energy to fix carbon dioxide into hydrocarbons with oxygen discharged as a waste product according to the formula:

$$CO_2 + H_20 \rightarrow C_n (H_2O)_n + O_2$$

Normally, light energy is harvested by chlorophyll molecules. This reaction can be expressed as an oxidation-reduction reaction, where carbon dioxide and water are converted to carbohydrates and oxygen. The conversion is traditionally divided in two stages, light reactions and dark reactions. The main scheme of these stages it is shown in Figure6.

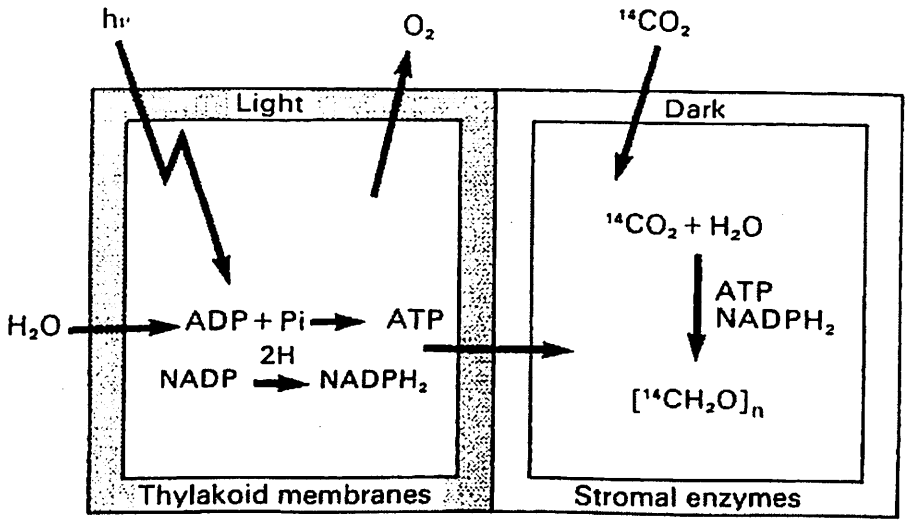


Figure 5: Light and dark reactions of oxygenic photosynthesis

In light reactions, which are bound on photosynthetic membranes of the chloroplasts, the light energy is converted into chemical energy providing $NADPH_2$ and a high energetic compound ATP. Dark reactions or enzymatic reactions, take place in the stroma of the chloroplast. $NADPH_2$ and ATP are utilized in the sequential biochemical reduction of carbon dioxide to carbohydrates, more commonly called the Calvin cycle.

2.7.2 Impact of strain selection

The selection of appropriate algae strains is an important factor in the overall success of biofuel production from microalgae. The ideal algal strain for biofuel production should:

- Have high lipid productivity.
- Be robust and able to survive the shear stresses common in photobioreactors.
- Be able to dominate wild strains in open pond production systems.
- Have high CO2 sinking capacity.
- Have limited nutrient requirements.
- Be tolerant to wide range of temperatures resulting from the diurnal cycle and seasonal variations.
- Provide valuable co-products.
- Have a fast productivity cycle.
- Have a high PE.
- Display self-flocculation characteristics.

2.7.3 Lipid productivity

While many microalgae strains naturally have high lipid content (20–50% dry weight), it is possible to increase the concentration by optimizing the growth determining factors such as the control of nitrogen level, light intensity, temperature, salinity, CO2 concentration and harvesting procedure. The Lipid accumulation refers to increased concentration of lipids within the microalgae cells without consideration of the overall biomass production. Lipid productivity takes into account both the lipid concentration within cells and the biomass produced by these cells and is therefore a more useful indicator of the potential costs of liquid biofuel production.

The most effective method of improving microalgae lipid and oil content accumulation is nitrogen limitation, which not only results in the accumulation of lipids, but also results in a gradual change of lipid composition from free fatty acids to triacylglycerol (TAG). TAGs are more useful for conversion to biodiesel. Lipid accumulation in microalgae occurs when a nutrient (typically nitrogen, but can be silicate for diatoms) is exhausted from the medium or becomes the growth limiting factor. Cell proliferation is prevented but carbon is still assimilated by the cell and converted to TAG lipids that are stored within existing cells thereby increasing the concentration. The effects of salinity, nitrogen concentration and light intensity on lipid productivity can result to 76% increase in production of lipids for specific growth conditions when compared to more typical growth processes.

2.8 Culture parameters

The most important parameters which are regulating algal growth are nutrient quantity and quality, light, pH, salinity, temperature and mixing intensity.

- **Temperature:**

The culture should be in a temperature as close as possible to the temperature at which the microorganisms are collected. Polar organisms has temperature (<10°C), so temperature should be between (10 – 25°C) and tropical should be (>20°C). Most commonly used species tolerates temperatures between 16 and 27°C. Temperatures lower than 16°C will slow down growth, whereas those higher than 35°C are harmful for a number of species.

- **Light:**

Light intensity play an important role, most often important light intensities range between 100 and 200 $\mu E\ sec^{-1}\ m^{-2}$, which corresponds to about 5-10% of full daylight (2000 $\mu E\ sec^{-}$

^{1} m^{-2}). Light may be natural or supplied by fluorescent tubes emitting blue or red light spectrum (most active portions of light for photosynthesis). Light intensity and quality can be manipulated by filters.

- **pH:**

The pH range for most growing algal species is between 7 and 9, with the optimum range being 8.2-8.7, because the pH of natural seawater is around 8. The control the pH in culture media is important because certain algae grow only within pH ranges in order to prevent the formation of precipitates. The problem of CO2 depletion in dense cultures could cause a significant decline in pH.

- **Salinity:**

Marine algae are extremely tolerant to changes in salinity. Every algae has different optimum salinity range that can increase during hot weather conditions due to high evaporation. This Salinity changes normally affect microalgae in three ways: osmosis stress, ion stress and changes of the cellular ion ratios due to the selective ion permeability of the membrane. The easiest way for salinity control is by adding freshwater or salt as required. Salinities of 20-40 $g \cdot L^{-1}$ have been found to be optimal.

- **Mixing:**

It is necessary to prevent sedimentation of the algae and ensure that all cells of the population are equally exposed to the light and nutrients. It is important to avoid thermal stratification and to improve gas exchange between the culture medium and the air. However, high speed and turbulence can damage microalgae due to shear stress. If an alga does not come to the surface its growth can be effected and it could be the major reason for its death.

Table 5: A generalized set of conditions for culturing micro-algae

Parameters	**Range**	**Optima**
Temperature (°C)	16-27	18-24
Salinity ($g.l^{-1}$)	12-40	20-24
Light intensity (lux)	1,000-10,000	2,500-5,000
Photoperiod (light: dark, hours)		16:8(minimum) 24:0 (maximum)
pH	7-9	8.2-8.7

2.9 Nutrients for algal growth

- **Carbon:**

Hydrocarbons are composed 90% of carbon and 10% of hydrogen. The source of hydrogen is water and the source for the enormous quantity of carbon needed is fulfilled from atmospheric CO2, present at the very low concentration of 0.03%.

- **Nitrogen:**

The other important nutrient is nitrogen, which comprises of 10-13% of the organic dry weight of the biomass of growing cultures. It can be utilized by algae in organic (urea) or inorganic forms (nitrate, nitric, ammonia).The nitrogenous fertilizer name ammonium carbonate is a major by-product of the conversion of biomass into oil. It is very important to remove nitrogen from the product to get high quality oil. The nitrogen removed can be reintroduced into the growth ponds. Nitrite is toxic when used in larger amounts and thus not very convenient to use. The assimilation of organic nitrogen is highly related to the pH, since ammonia may decrease the pH to a low 3. Nitrogen deficiency results in a growth limitation.

- **Phosphorous:**

Phosphorous is another significant nutrient for algal growth. A limitation in phosphorous will also cause a decreased growth rate. The phosphorous forms PO_4^{3-} , $H_2PO_4^-$ and $H_2PO_4^{2-}$ are the most important inorganic sources for algae. However, algae can obtain phosphorous from organic compounds, which have to be hydrolyzed with the phosphatases. The ideal phosphorous concentration in the growth medium varies among the different species. The average concentration is in range of 20 to 50µg/l.

- **Silica:**

Silica is also necessary for the growth of diatoms which is a part of the cell wall. The assimilation takes places in the later phase of cell growth, when the cell wall is built. Sodium metasilicate is used as a silica source for diatoms. Silica deprivation increases lipid content in diatoms algal cells.

- **Vitamins**

Some algae require additional vitamins for optimal growth. The most important vitamins are B12, thiamine and biotin etc. Concentrations may range from 1/10 to 1/100 µg/l.

2.10 Harvesting

Algal harvesting is done to recover biomass from the culture medium that may contribute to 20-30% of the total biomass production cost. Therefore, it is important to select algae with properties that simplify harvesting, like algae with large cell size or high specific gravity etc. This is considered as an expensive part of industrial production of biomass. Microalgae have a very nice green-looking suspension. However, in fact from industrial point of view is very thin. The optimal material for industrial conversion is containing at least 300-400 g dry weight/ L. The most common known harvesting processes are flocculation, filtration, centrifugation and flotation.

- **Flocculation:**

The effluent algal suspension needs to be concentrated. It is proceed with flocculation and flotation combination. Flocculation is used to aggregate the microalgae cells to increase the effective particle sizes. Flocculation step is one of the important and effective steps which are used by many companies However, the cell wall is a big barrier to facilitate the extraction and the thickness of the cell wall is affected by the conditions of the cells at the time of harvesting. Alum and ferric chloride are chemical flocculants which are used to harvest algae. This method is often too expensive in large dimensions. However, interrupting the carbon dioxide supply to an algae system can cause microalgae to flocculate on its own, which is called "auto-flocculation".

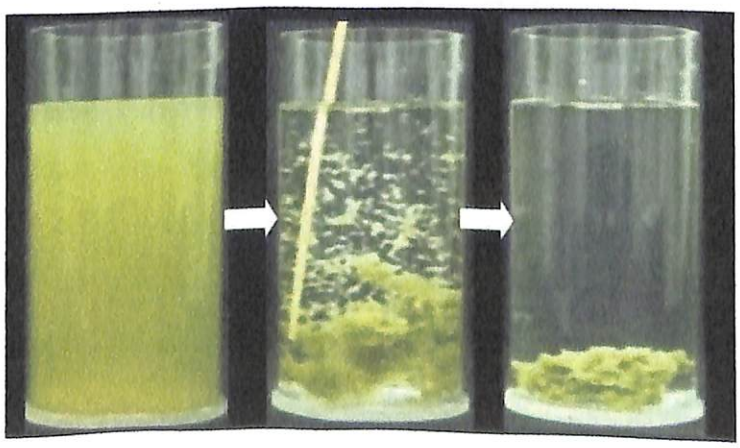


Figure 6: Microalgae flocculation process

- **Filtration:**

This method is carried out commonly on membranes of modified cellulose with the aid of a suction pump. The greatest advantage of this method is that it is able to collect microalgae cells with very slow density. However, concentration by filtration is limited to small volumes with eventual clogging in the filters by the package cells when vacuum is applied. Filtration is best suited for large microalgae, but cannot recover organisms with smaller dimensions such Scenedesmus, Dunaliella or Chlorella.

- **Centrifugation:**

Centrifugation is a method of separating algae by using a centrifuge force by which algae settles down to the bottom of the tank from where the algae can be removed. This method may prove useful on a commercial and industrial scale, but is expensive for personal use. A centrifuge is a device that puts an object in rotation around a fixed axis, applying a force perpendicular to the axis. The centrifuge works using the sedimentation principle. This method is reasonably efficient, but sensitive algal cells may be damaged by granulation against the rotor wall which is a drawback involved in this type of process.

- **Flotation:**

Usually flotation is used in combination with flocculation for algae harvesting in waste water. It is a simple method in which air is supplied from the bottom of the tank by which algae can be made to float on the surface medium and then removed. It is used alum to flocculate algae/air mixture, with fine bubbles supplied by an air compressor.

2.11 Algae oil extraction

After harvesting another step which is used is algae oil extraction. Under optimal conditions of growth, algae synthesize fatty acids for esterification into glycerol-based membrane lipids, which constitute about 5-20% of their dry cell weight. Fatty acids can be classified in medium chain (C10-C14), long chain (C16-C18) and very long chain (>C20) species and fatty acids derivatives. However, under unfavourable environmental conditions, many algae alter their lipid biosynthetic pathways to the formation and accumulation of neutral lipids (20-50% DCW), mainly in the form of triglycerides (TAGs). For biodiesel production, these neutral lipids have to be extracted from microalgae biomass. Extraction algal oil is one of the most costly processes which can determine the sustainability of microalgae-based biodiesel. It is common to apply dehydration of algal biomass to increase its shelf-life and for the final product (figure 8). Several methods have

been employed to dry microalgae, where the most common include spray-drying, drum-drying, freeze-drying and sun-drying etc.

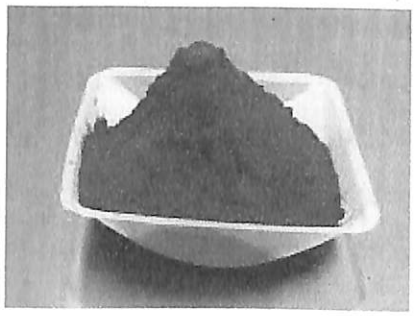


Figure 7: Algae biomass dehydrated

After drying it follows the cell disruption of microalgae. Several methods can be used depending on the microalgae wall and the type of product to be obtained. For biofuel and from biofuel to biodiesel production, lipids and fatty acids have to be extracted from the microalgae biomass. Algal oil can be extracted using chemical methods or mechanical methods:

2.11.1 Mechanical methods

These methods are classified in mechanical expeller press and ultrasonic assisted extraction.

- **Expeller press:**

Algae are dried to retain its oil content and it can be pressed out with an oil press. Commercial manufactures use a combination of mechanical press and chemical solvents in extracting oil.

- **Ultrasonic extraction:**

This method is a brand of Sonochemistry. Ultrasonic waves are used to create bubbles in a solvent material, when these bubbles collapse near the cell walls, it creates shock waves and liquid jets that cause those cells walls to break and release their contents into the solvent. This method can be done with dry or wet microalgae, with wet is necessary to extract part of the water from the mash before extraction oils with a solvent.

2.11.2 Chemical methods

Neutral lipids or storage lipids are extracted with non-polar solvents such as diethyl ether or chloroform but membranes associated lipids are more polar and require polar solvents such as ethanol or methanol to disrupt hydrogen bondings or electrostatic forces. The chemical extraction solvents are Hexane, benzene and ether. Hexane is the most popular and inexpensive but is a good solvent only for lipids of low polarity. Benzene is no more used since it is now considered as a potent carcinogenic substance. This it may be replaced by toluene. By working with chemicals care must be taken to avoid exposure to vapours and contact with the skin.

- **Solvent method:**

Solvent can be used together with a mechanical extraction method, first pressing the oil. After the oil has been extracted using an expeller, the remaining product can be mixed with hexane to extract all the oil content. Then, Oil and hexane are separated by distillation. Different solvents can be also used such as ethanol (96%) and hexane-ethanol (96%) mixture. With these solvents it is possible to obtain up to 98% quantitative extraction of purified fatty acids.

- **Soxhlet extraction**:

Oils from algae are extracted through repeated washing, with an organic solvent such as hexane or petroleum ether, under reflux in special glassware or Soxhlet extractor. A Soxhlet extractor is a laboratory apparatus which was designed for the extraction of lipid from a solid material. It is only required where the desired compound has a limited solubility in a solvent and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. Normally a solid material containing some of the desired compound is placed inside a thimble which is made from thick filter paper which is placed in the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent namely hexane. The Soxhlet is then equipped with a condenser and is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent some of the desired compound will then dissolve in the warm solvent.

When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

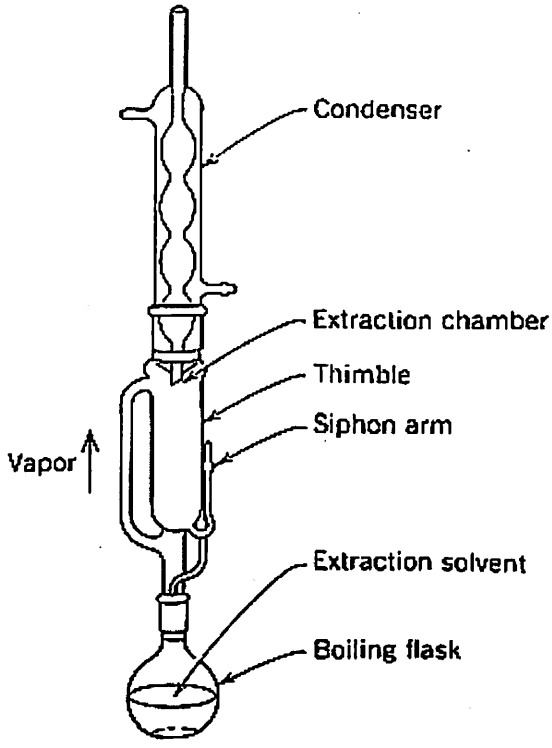


Figure 8: Soxhlet extractor

After extraction the solvent is removed, typically by means of rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

- **Supercritical fluid extraction:**

In supercritical fluid/CO2 extraction, CO2 is liquefied under pressure and heated to the point that it has the properties of both liquid and gas. This liquefied fluid then acts as a solvent for extracting the oil. CO2 is the most used supercritical solvent because the compounds can be obtained without contamination by toxic organic solvents and without thermal degradation. Microalgae contain significant quantities of protein, carbohydrates, lipids and other nutrients. The residual biomass can be used as animal and fisheries feed, and another anaerobic digestion can be used as fertilizers and composts.

Table 6: Chemical composition of Algae expressed on a dry matter basis (%)

Strain	Protein	Carbohydrates	Lipids	Nucleic acid
Scenedesmus obliquus	50-60	10-17	12-14	3-6
Scenedesmus dimorphus	8-18	21-52	16-40	-
Chlorella vulgaris	51-58	12-17	14-22	4-5
Chlorella pyrenoidosa	57	26	2	-
Spirulina maxima	60-71	13-16	6-7	3-4.5
Scenedesmus quadricauda	47	-	1.9	-
Spirogyra sp.	6-20	33-64	11-21	-

2.12 Yield parameters

2.12.1 Specific growth rate

Biomass productivity is the mass of oil produced per unit volume of microalgae broth per day, and it depends on the algal growth rate and the oil content of biomass. It can be calculated dividing the difference between the dry weights at the start and at the end of the experiment by its duration (days). The specific growth rate can be calculated by the equation:

$$\mu = 1/t * \ln (X_f/X_o)$$

Where,

X_f is the microalgae biomass concentration at the end of culturing process (g/L).

X_o is the microalgae biomass concentration at the beginning of the process.

T is the time (in hours) of culturing process.

μ the specific growth rate ($hours^{-1}$)

2.12.2 Lipid productivity

Lipid productivity can be calculated as the product of biomass productivity (g/L/day) and lipid content, to give an indicator of oil produced on a basis of volume and time. The lipid productivity can be calculated also by the equation:

$$V = Cl/t$$

Where,

Cl is the concentration of lipids at the end of process and

t the time running the process (mg/L/day).

Table 7: Biomass productivity, lipid content and lipid productivity of 3 microalgae cultivated in 250-ml flasks

Microalgae strains	Habitat	Biomass productivity (g/L/day)	Lipid content (%Biomass)	Lipid Productivity (mg/L/day)
Chlorella vulgaris F&M-M49	freshwater	0.20	18.4	36.9
Chlorella sp. DM	freshwater	0.23	18.7	42.1
Scenedesmus. Sp	freshwater	0.26	21.1	53.9

2.13 Reasons why are algae so exciting from a renewable energy standpoint?

- The yields of oil and fuels from algae are much higher (10-25 times) than competing energy crops.
- Algae can grow in diverse regions, thus ensuring that there is no competition with food crops.
- Algae are excellent bioremediation agents - they have the potential to absorb massive amounts of CO_2 and can play an important role in sewage and industrial wastewater treatment.
- Algae are the only feedstock that has the potential to completely replace world's demand of transportation fuels.
- Algae are already being used in a wide variety of industries and applications, and many newer applications are being discovered. Such a wide range of end-uses enable companies to produce both fuels and non-fuel products from the same algae feedstock.
- Algae biodiesel has virtually no sulfur content.
- Biodiesel has superior lubricating properties, reducing fuel system wear, and increases the life of fuel injection equipment.

- Algae biodiesel has more aggressive solvent properties than petro diesel and will dissolve left over varnish residue. Fuel filters should be changed shortly after introducing biodiesel into systems formerly running on petrodiesel to avoid clogging.
- Biodiesel has about 5-8 percent less energy density than petrodiesel, but with its higher combustion efficiency and better lubricity to partially compensate, its overall fuel efficiency decrease is only about 2 percent.
- The cloud point, or temperature at which pure (B100) biodiesel starts to gel, is about 32 ^{0}F. A blend of B20 (20% biodiesel, 80% petrodiesel) generally does not gel in cold weather. Various additives will lower the gel point of B100.
- Biodiesel's flash point (lowest temperature at which it can vaporize to form an ignitable mixture in air) is 266^{0}F, significantly higher than petro diesel's 147^{0}F, or gasoline's 52^{0}F.
- Biodiesel reduces particulate matter by about 47 percent as compared to petroleum diesel. Biodiesel has less dangerous particulate matter because it reduces the solid carbon fraction on the particulate matter while increasing the amount of oxygen.
- Higher yield and hence hopefully lower cost.
- The most significant benefit is however in the yield of algal oil, and hence biodiesel. According to some estimates, the yield (per Acre say) of oil from algae is over 200 times the yield from the best-performing plant/vegetable oils. While soybean typically produces less than 50 gallon of oil per acre and rapeseed generates less than 130 gallon per acre, algae can yield up to 10,000 gallons per acre.
- Algae can grow practically in every place where there is enough sunshine.
- The biodiesel production from algae also has the beneficial by-product of reducing carbon and NOx Emissions from power plants, if the algae are grown using exhausts from the power plants.

2.14 Algae for carbon dioxide mitigation

Another importance of algae is that it is used for reducing the CO2 concentration in the atmosphere is known as algae-based carbon capture technology. This technology offers a safe and sustainable solution to the problems associated with global warming. Microalgae have the ability to fix CO2 while capturing solar energy with efficiency of 10-15 times greater than that of terrestrial plants, and produced biomass for biofuels production. The algae production can be fed with the exhaust gases from coal, power plants, cement plants,

steel plants, and other polluting sources, to increase the algal productivity and clean up the air. Flue gases from power plant are responsible for more than 7% of the total world CO2 emissions.

2.15 Current limitation for Algae biofuel production

The major limitation for algal biofuel and finally to biodiesel is the production cost and energy input required while producing and harvesting the microalgal biomass. Different researchers claim different theories on this topic. When considering net energy inputs and life cycle fossil fuel inputs, one researcher concluded that biofuels from microalgae were not even capable of out-producing terrestrial plants. Other studies concluded that the high moisture content of microalgae means drying it before using it for energy production would be too great an energy usage. While the academic world is still debating the cost factor, some companies such as Aurora Biofuels and Solazyme are turning algae into biofuel; at a profit. Cost has been one of the largest inhibitors to algal biodiesel research. The people now working on these and several similar commercial ventures are clearly eager to make growing algae a going business in this country. Yet it's not hard to find experts who view such prospects as dim indeed.

2.16 Is Jatropha curcas biofuel losing out to algae biofuel?

A recent post on Chemically Green **"The truth about Jatropha curcas"** lists the prospects and cons for growing Jatropha Curcas. India, which has researched extensively in growing Jatropha, has been investing heavily in Jatropha Curcas for biofuel production for their own automobile transportation. Early reports on Jatropha Curcas stated this bush could be grown on marginal land and little irrigation and this has not proven to be factual. Now, India is changing directions for biofuel production and is taking a look at perhaps the real answer for producing biofuels and biodiesel: Growing algae to produce biodiesel. A real biofuel revelation coming from India: Algae biodiesel will out-produce Jatropha Curcas for biodiesel production. Also, when you compare the advantages of growing biodiesel vs. Jatropha, algae has many more positive traits than Jatropha including production yields by 10:1. No intense crop management, no soil preparation, grow in any environment and less labour intense handling of Jatropha for processing of biodiesel.

New Delhi, in the endeavour to reduce dependence on fossil fuels and cut carbon emissions to achieve a clean environment, humble algae appears to be taking a lead over the more-talked-about biodiesel source Jatropha. Experts say that "algae farming in less

than 1 per cent of India's total land can make the country self-sufficient in liquid fuel. Algae yield from one acre of wasteland can be 10 times more than Jatropha and by a conservative estimate over 10,000 litres of oil can be produced from one acre of waste/degraded land".

CHAPTER 3: EXPERIMENTAL METHODS

This section describes the experimental aspects of "Biofuel Production from Algae" on a small scale in biodiesel laboratory.

3.1 Strain used for culture

- Chlorella vulgaris

3.2 Reagents

There have been some raw materials used:

- Urea
- Glucose
- Potassium nitrate
- Magnesium sulphate
- Potassium dihydrogen phosphate

3.3 Material used

The instruments which have been used are:

- Algae culture vessel
- Thermometers
- Graduated cylinders
- Watch glass
- Graduated flasks
- Filter paper
- funnel
- Volumetric flasks
- Florescent light
- Aerator
- Stoppers
- Electronic balance
- Soxhlet extractor

3.4 Experimental procedure

Step1: Algae subculture

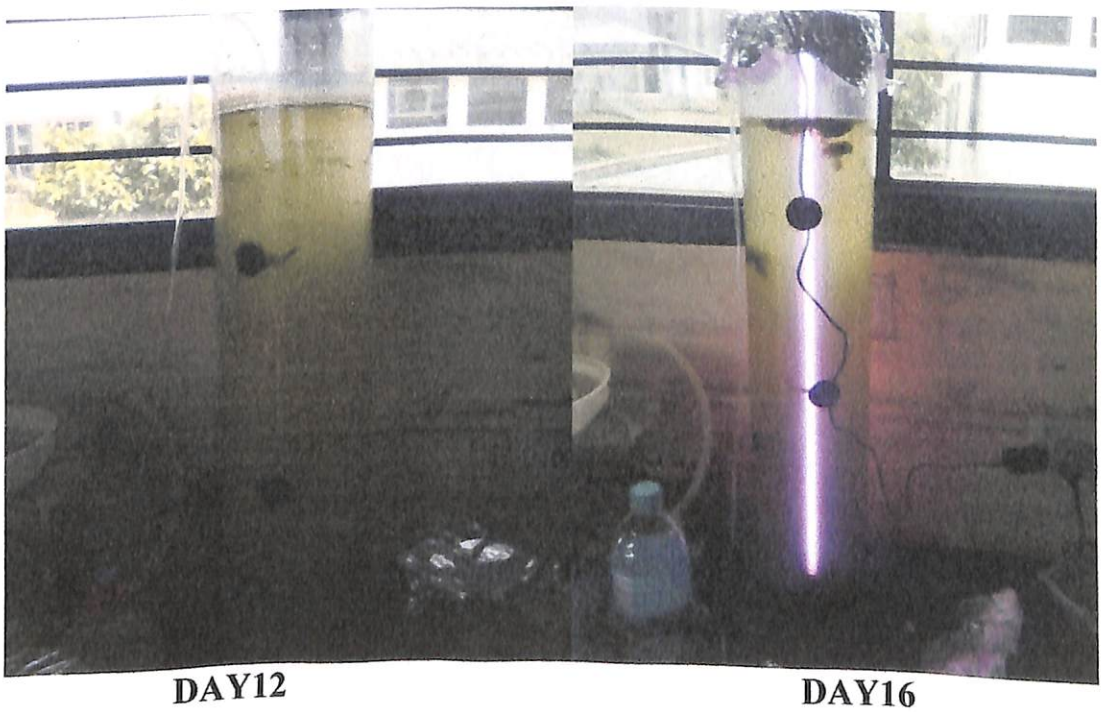
A microalgae sample (chlorella vulgaris) was used in this work. The alga used was donated by **Prof. G.R.S.Bisht** from "**Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences & Research**". Algae cells were maintained in a solution containing fresh water and 1gram solution of urea. The main culture was conducted in 15litre algae culture vessel which contains 10litre of fresh water and maintained at 20°C.The medium was not sterilized prior to cultivation. The aeration rate was maintained constant at 6000 L/hr and speed of agitation were maintained at 100rpm, and is provided by air pump through 7 mm air line and the CO_2 content of the aeration gas was fixed at approximately 3 %, throughout the culture experiment. The water tanks were placed on shelves with fluorescent lamps. Light was provided for 16 hours in a day, enabling the irradiation intensity to be altered. The initial pH of the culture broth was adjusted to around 8.0 in the main culture.The illumination was provided by 25 W 220v Cool White Plus fluorescent lights at 110-120 μmol s^{-1} m^{-2} measured with an LI-250A light meter(it simplifies operation by automatically selecting the range with the best accuracy for a given sensor input signal).

The following nutrients were provided directly to the culture medium for algal growth KNO_3 (42.5 gm), $MGSO_4$ (4.30gm), KH_2PO_4 (0.65gm) per 100 ml. and exposed to fluorescent light that provides light for photosynthesis. The culture temperature was maintained at 20°C which can be measured by the thermometer.

Apparatus was usually kept sealed from the top to prevent the culture from contamination and was opened only at the time when nutrients were added. Light intensity is a very important factor for the cultivation process of algae. Cell growth occurred normally within a CO_2 concentration range of 3 to 10 % However, limited cell growth occurred at very low CO_2 concentrations (0.03% CO_2).

Figure 9: Culturing of algae in biodiesel lab

DAY 1 **DAY6**



DAY12 **DAY16**

DAY 24

DAY 30

Step2: Algae harvesting

The algae was harvested in the biodiesel lab is by the method of filtration. It is done by pouring a measuring amount of solution in the funnel in which a filter paper of less than 4 micron (μ) is placed through which the liquid solution is passed out and collected in a beaker under the funnel and the algae is collected on the filter paper from where it is removed by scratching. It is than placed in the oven at very low temperature for drying the algae. From there it is taken to the Soxhlet extractor for extracting oil.

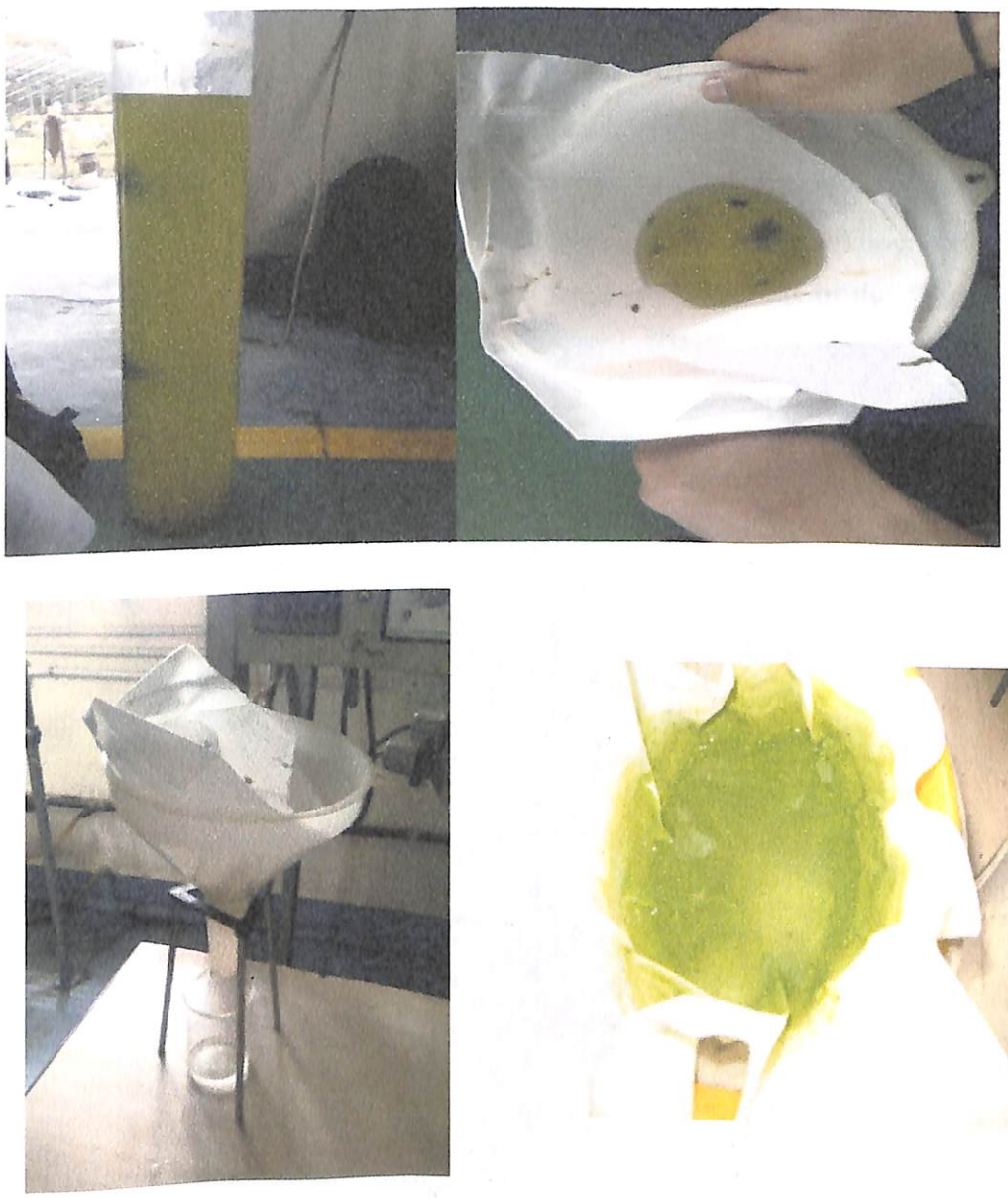


Figure10: Filtering method

Step3: Algae oil extraction

After filtration and drying of algae it is sent to the Soxhlet extractor for extracting oil from it. Dry algae are grinded by grinding machine and are sent into Soxhlet apparatus. Normally a solid material containing some of the desired compound is placed inside a thimble which is made from thick filter paper which is placed in the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent namely hexane. The Soxhlet is then equipped with a condenser and is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side. During each cycle, a portion of the non-volatile compound dissolves in the solvent and this cycle repeats many times. After extraction the solvent is removed, typically by means of rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

Figure11: Soxhlet extractor

3.5 Result and discussion

Algal biomass was successfully produced in a tubular bioreactor providing sufficient nutrients, light and CO_2. The result was found that the maximum yield of dry algae was 50gm and lipid content of the biomass was determined to be 23% of dry weight on weight/volume basis

The oil produced was found to be 11.5ml.

But when we culture algae in nutrient and light deficient condition, the growth of algae was very poor. After some time it become whitish colour from green colour, which was the indication of death of algae. Thus, it was observed from the experiment that optimum light, nutrients and carbon dioxide are essential for proper growth and high lipid content of algae.

3.6 Method of data analysis

Once biofuel is obtained, a series of tests will be carried out to know some physical properties such as density, refraction index, viscosity and yield.

- **Density**

To determine the density of the biofuel is necessary to know its mass and volume. Before deposited the cleaned biofuel in the flask, the reaction product is put down in a graduated cylinder where the volume is measured. The mass is calculated weighting the flask empty and full with biodiesel. The difference will be the mass of the fuel. With these two values the density is obtained, which is mass divided by volume.

- **Refraction index**

Refraction index is the ratio of the speed of light in air or in a vacuum to the speed of light in another medium; it is a measure of how much the speed of light is reduced inside a medium. It is symbolized by 'n' and it is a dimensionless value:

$$N=c/v$$

Where:

c= speed of light in vacuum

v= speed of light in a medium whose index is calculated

The refractometer is the device which allows us to measure the refractive index.

- **Dynamic viscosity**

Viscosity is a measure of the internal friction or resistance of an oil to flow.

Viscosity coefficients can be defined by:

- Dynamic viscosity, also called absolute viscosity, the more usual one;
- Kinematic viscosity is the dynamic viscosity divided by the density.

Dynamic viscosity is calculated with the following formula:

$$\eta = k * t * (\rho \text{ ball} - \rho \text{ medium})$$

Where:

η = dynamic viscosity of the medium which has been studied

k = geometric constant

t = fall time of the object

ρ ball = density of the object

ρ medium = density of the medium

The viscometer is used to calculate this physical property. The equipment has a hollow tube with an object within it. This tube is filled with the medium that is being studied. The density of the medium, in this case biofuel, is known. The density of the object, ρball, is calculated from its mass and volume (obtained by volume of water displaced). To know the constant value of k it is necessary to use the viscometer in a medium with density and viscosity known.

- **Kinematic viscosity**

Kinematic viscosity represents the properties of the fluid throwing away the forces that generate its motion. It is obtained through the ratio of absolute viscosity and density of the product:

$$V = \eta / \rho$$

Where:

v = kinematic viscosity of the medium

η = dynamic viscosity of the medium

ρ = density of the medium

CHAPTER 4: CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

4.1. Conclusion

The work presented in this project leads to a deeper understanding of the use of algal biomass as a biodiesel feedstock, in areas related to the entire process from growth to reaction methodology. The work conducted in Chapter 3 provides a "proof-of-concept" that the algae Chlorella vulgaris is capable of developing the biofuel. The results shows that a 30 day repetitive growth and harvest schedule allow for optimal fatty acid production.

Algae are an economical choice for biofuel and further to biodiesel production because of its availability and low cost. Our results prove that biofuel can be produced from microalgae. 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 that biofuel can be produced from microalgae though it contains lower lipid content and further research should be done having Macroalgae and microalgae to compare the ratio of biofuel production, chemical analysis and statistical significance.

The large uptake of nutrients by biomass indicates that the biomass debris left after oil extraction will be an effective and easily transportable crop fertilizer.

This technology could benefit not only the India and other industrialized countries but also other developing countries that lack sanitation and energy infrastructure. In both settings it would give people the power to grow a clean burning renewable fuel while simultaneously cleaning agriculture wastewater. These results can have significant impacts on the way wastewater is treated in the future and the sources of our renewable energy

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