

IMPROVING GROWTH AND LIPID PRODUCTION OF MICROALGAE FOR BIODIESEL PRODUCTION VIA VARIOUS LIGHT CONDITION

Submitted in partial fulfilment of the requirements for the award of Bachelor of
Technology degree in Biotechnology

by

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INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited with Grade “A” by NAAC
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of V. Ashwin Sundar (37230001) who carried out the project entitled "Improving growth and lipid production of microalgae for biodiesel production via various light condition" under our supervision from September 2020 to March 2021.

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DECLARATION

I, V. Ashwin Sundar (37230001), hereby declare at the Project Report entitled **“IMPROVING GROWTH AND LIPID PRODUCTION OF MICROALGAE FOR BIODIESEL PRODUCTION VIA VARIOUS LIGHT CONDITION”** done by me under the guidance of **Dr. V. Ramesh Kumar** is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

the assorted attributes in regards to biodiversity and flexibility of microalgae alongside their higher development rate contrasted and earthly plants, the capacity to develop on non-gainful land and utilize low quality water, the capacity to eliminate toxins from wastewater and to sequester CO₂ from pipe gases, and so forth, microalgae have been considered as a promising future biofuel feedstock. There are a few pathways for handling microalgae into biofuel: biodiesel creation through transesterification of lipids, bioethanol creation through aging of the algal biomass, biogas creation through anaerobic assimilation, and bio-unrefined creation through thermochemical transformation are among the options measures. Synchronous creation of biodiesel and biogas from microalgae has gotten interest as it empowers the usage of lipid-separated green growth for additional handling and biogas creation with the goal that it could assist with empowering a most extreme use of the green growth biomass.

Anaerobic processing has likewise become an uncommon concentration in the usage of microalgae for biofuel creation especially from the bio-treatment facility perspective. For a feasible creation of biofuel from microalgae, a few difficulties, for example, dealing with a high-energy and capital-escalated collecting/dewatering measure, adapting to the high measure of build-ups left after lipid extraction on account of lipid-based biofuel creation (microalgae biomass contains 30–40% lipid, and up to 70% of the remaining biomass is left after the extraction cycle), and the requirement for manures, should be survived. Anaerobic processing can give a pathway to stay away from a portion of these issues by recuperating supplements from the separated leftover biomass and delivering power from the methane biogas.

The creation of biofuel from microalgae anyway has not yet been acknowledged in enormous scope creation. Significant examination holes, for example, diminishing energy input, expanding yield, and those identified with a productive material and energy utilization, are holding back to be tended to. In microalgae development, the supplement supply fundamentally affects cost, maintainability, and creation sitings, while the significant supplements.

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LIST OF ABBREVIATION

FTIR	-	Fourier Transform Infrared Spectroscopy
OD	-	optical density
EPA	-	Eicosapentaenoic corrosive and
DPA	-	docosapentaenoic corrosive
DHA	-	docosahexaenoic corrosive
UV-vis	-	Ultraviolet visible

CHAPTER – 1

INTRODUCTION

1.1. Introduction

Microalgae are the algae that are not visible for naked eyes. These algae can only see by eyes using microscopes. Algae is a group eukaryotic organism which does photosynthesis by themselves. These algae can be in the form unicellular and multicellular. Algae can be in the size of 50mts. Algae can grow in freshwater and in sea waters. Algae can produce their oxygen by photosynthesis. These algae are mostly aquatic. There are some algae which is complex and large like seaweeds. Algae have chlorophyl which is responsible for doing photosynthesis. Algae reproductive cell is fertile which is surrounded by infertile cells. The reproductive cell is gamete cell which as a half chromosome which helps to form whole organism using it. Some belongs to isogamy and other belongs to oogamy. Algae are helps human in production of crude oil and beauty products and more. Phycology is study of algae and phycologist is the person who study phycology. Algae are in vary in size structure and in colour. Algae does not contain structure like root, stem, leaf. Algae colours varied because its chloroplast pigments. In 1830's this is the type of classification used to classify the algae.

Microalgae is mostly used in many countries because of its uses. It used in various industries like biopharma, biodiesels, nutraceutical because of its potential. For production biofuels it plays a vital role in substitute of fossil fuel. These algae have capacity to convert carbon-di-oxide into useful form that helps to produce these fuels, and this helps in pollution and it cost less, and it is renewable. Algae can grow in any type of habitats in aquatic like freshwater, pond water, river water, sea water. It also grows in land. Irrespective of climate condition its capable to grow in large amount. It as capacity to withstand the land temperature and light intensity.

Algae are differentiated by their colours the red algae are Rhodophyta, brown algae are Phaeophyta, and green algae is Chlorophyta. The algae can be classified as micro algae and macro algae. The algae colours are varied due to the chloroplast present in the algae. Algae can be used for wastewater treatment. This alga can produce various vitamins, proteins. Algae produces substance for textile industry and nutrition that helps humans in plenty of ways. Various industries get benefited by culturing the algae.

1.2 Classification of Microalgae

In early years they have classified using the colour they seen, and they named after that the red algae are Rhodophyta, brown algae and green algae. The size of the algae is also used to differentiate the algae as macro algae and micro algae. A by and large concurred arrangement of green growth perceives nine wide scientific classifications, called Divisions. These are Chlorophycophyta, Xanthophycophyta, Bacillariophycophyta, Phaeophycophyta, Rhodophycophyta, Chrysophycophyta, Euglenophycophyta, Cryptophycophyta and Pyrrophyphyta. Among these, the individuals from the initial five Divisions are unmistakably plant-like life forms, yet the others, especially the beats, have some creature like characters. For instance, Euglena is remembered both for green growth and in the protozoa under the class Phytomastigophora. the main six sorts of Classification of Algae. A portion of the kinds are: 1. Atomic Organization 2. Nature of Cell Wall Components 3. Pigmentation and Photosynthetic Apparatus 4. Nature of Reserve Food.

1.2.1 Atomic Organization

Based on atomic association green growth can be prokaryotic or eukaryotic. Cyanophyceae or blue green growth are prokaryotic in nature though any remaining classes of green growth are eukaryotic. In prokaryotic e.g., Cyanophyceae—core isn't coordinated as atomic film is missing. DNA fibrils are free in nucleoplasm and are not related with histones. Cell division by mitosis and meiosis isn't found. Film bound cell organelle like chloroplast, mitochondria and ER are missing. Eukaryotic green growth has very much separated core, mitochondria, chloroplast and endoplasmic reticulum in their cell structure.

1.2.2 Nature of Cell Wall Components

The cell divider in green growth is for the most part comprised of polysaccharides. Sometimes lipids and proteins are additionally present in them. The inward layer of cell divider in green growth is by and large comprised of cellulose, which is insoluble polysaccharide, and the external layer is made of pectic substances. The cell divider in Chlorophyceae is comprised of cellulose. In Xanthophyceae pectic substance is more normal. In Chrysophyceae cell divider is non-cellulosic which is silicified or calcified. In Phaeophyceae cell divider contains alginic corrosive and fucinic corrosive. In Rhodophyceae the cell divider is made of non-cellulosic polysaccharides like xylans and galactans. In Cyanophyceae or blue green growth the cell divider is more like microorganisms and is comprised of mucopeptides.

1.2.3 Pigmentation and Photosynthetic Apparatus

The shade is quite possibly the main standards utilized in separation of classes in green growth, as green growth was at first and essentially isolated based on shading e.g., green growth, red green growth, earthy coloured green growth or blue green growth. The colours in green growth can be chlorophylls, carotenoids and biloproteins. These colours are available in sac like constructions called thylakoids. The thylakoids are masterminded in stacks in granum of the chloroplasts. Various gatherings of green growth have various sorts of colours and association of thylakoids in chloroplast.

1.2.4 Nature of Reserve Food

The essential result of photosynthesis i.e., starch is same on the whole gatherings of green growth however because of aggregation of food over extensive stretch the idea of insoluble save food might be extraordinary. The idea of save food can be a measure for qualification of various gatherings of green growth. In Chlorophyceae the save food is starch. In Xanthophyte oil and leucosine are hold food materials. In Rhodophyceae Rhodophyceae or floridean starch and in Cyanophycean myxophycean starches are the hold food sources. In Phaeophyceae the save food material is laminarin or mannitol.

1.3 PRODUCTION METHOD

A scope of microalgae animal groups are created in incubation facilities and are utilized in an assortment of ways for business purposes. Studies have assessed principal factors in the accomplishment of a microalgae incubation facility framework as the components of the holder/bioreactor where microalgae is refined, openness to light/illumination and centralization of cells inside the reactor.

Open lake framework

This strategy has been utilized since the 1950s. There are two fundamental benefits of refined microalgae utilizing the open lake system. Firstly, an open lake framework is simpler to construct and operate. Secondly, open lakes are less expensive than shut bioreactors in light of the fact that shut bioreactors require a cooling system. However, a disadvantage to utilizing open lake frameworks is diminished profitability of certain monetarily significant strains, for example, *Arthrospira* sp., where ideal development is restricted by temperature. That said, it is feasible to utilize squander warmth and CO₂ from modern sources to remunerate this.

Carrier technique

This technique is utilized in outside development and creation of microalgae; where air is moved inside a framework to circle water where microalgae is growing. The way of life is filled in straightforward cylinders that lie on a level plane on the ground and are associated by an organization of pipes. Air is gone through the cylinder with the

end goal that air escapes from the end that rests inside the reactor that contains the way of life and makes an impact like stirring.

Shut reactors

The greatest benefit of refined microalgae inside a shut framework gives power over the physical, compound and natural climate of the culture. This implies factors that are hard to control in open lake frameworks like vanishing, temperature slopes and security from surrounding defilement make shut reactors preferred over open systems. Photobioreactors are the essential illustration of a shut framework where abiotic variables can be controlled for. A few shut frameworks have been tried to date for the reasons for refined microalgae, scarcely any significant ones are referenced underneath:

Flat photobioreactors

This framework incorporates tubes laid on the ground to shape an organization of circles. Blending of microalgal suspended culture happens through a siphon that raises the way of life vertically at coordinated stretches into a photobioreactor. Studies have discovered beat blending at spans creates preferable outcomes over the utilization of nonstop blending. Photobioreactors have likewise been related with preferable creation over open lake frameworks as they can keep up better temperature gradients. A model noted in higher creation of *Arthrospira* sp. utilized as a dietary enhancement was ascribed to higher profitability in view of a more qualified temperature range and an all-inclusive development period over summer months.

Vertical frameworks

These reactors utilize vertical polyethylene sleeves swung from an iron edge. Glass cylinders can likewise be utilized then again. Microalgae are likewise refined in vertical alveolar boards (VAP) that are a kind of photobioreactor. This photobioreactor is portrayed by low efficiency. Notwithstanding, this issue can be overwhelmed by adjusting the surface zone to volume proportion; where a higher proportion can increment productivity. Mixing and deoxygenation are downsides of this framework and can be tended to by percolating air consistently at a mean stream rate. The two fundamental kinds of vertical photobioreactors are the Flow-through VAP and the Bubble Column VAP.

Level plate reactors

Level plate reactors (FPR) are assembled utilizing tight boards and are set evenly to amplify daylight contribution to the system. The idea driving FPR is to build the surface zone to volume proportion to such an extent that daylight is proficiently used. This arrangement of microalgae culture was initially thought to be costly and unequipped for coursing the culture. Therefore, FPRs were viewed as impractical generally for the business creation of microalgae. Notwithstanding, an exploratory FPR framework during the 1980s utilized course inside the way of life from a gas trade unit across flat panels. This defeats issues of flow and gives a benefit of an open gas move unit that diminishes oxygen fabricate up. Examples of effective utilization of FPRs can be found in the creation of *Nannochloropsis* sp. utilized for its significant degrees of astaxanthin.

Fermenter-type reactors

fermentor-type reactors (FTR) are bioreactors where maturation is done. FTRs have not grown gigantically in the development of microalgae and represent a weakness in the surface territory to volume proportion and a diminished effectiveness in using sunlight. FTR have been created utilizing a blend of sun and counterfeit light have prompted bringing down creation costs.[19] However, data accessible for huge scope partners to the research centre scale frameworks being created is very limited.[19] The principle advantage is that outward factors for example light can be controlled for and profitability can be upgraded with the goal that FTR can turn into an option for items for the drug industry.

1.4 Application of Microalgae

1.4.1 BIOFUEL CREATION

To fulfil the needs of petroleum products, elective methods for energizes are being researched. Biodiesel and bioethanol are inexhaustible biofuels with much potential that are significant in flow research. Be that as it may, agribusiness based inexhaustible energizes may not be totally economical and along these lines will most likely be unable to supplant petroleum products. Microalgae can be astoundingly wealthy in oils (up to 80% dry load of biomass) reasonable for change to fuel. Besides, microalgae are more profitable than land based agrarian yields and could in this way be more reasonable over the long haul. Microalgae for biofuel creation is fundamentally delivered utilizing rounded photobioreactors.

1.4.2 DRUGS AND BEAUTIFIERS

Novel bioactive substance mixtures can be detached from microalgae like sulphated polysaccharides. These mixtures incorporate fucoidans, carrageenans and ulvans that are utilized for their helpful properties. These properties are anticoagulants, cell reinforcements, anticancer specialists that are being tried clinical research. Red microalgae are described by shades called phycobiliproteins that contain normal colorants utilized in drugs and additionally cosmetics.

1.4.3 BIOFERTILIZER

Blue green alga was first utilized as a method for fixing nitrogen by permitting cyanobacteria to increase in the dirt, going about as a biofertilizer. Nitrogen obsession is significant as a method for permitting inorganic mixtures, for example, nitrogen to be changed over to natural structures which would then be able to be utilized by plants. The utilization of cyanobacteria is a monetarily stable and harmless to the ecosystem technique for expanding profitability. This strategy has been using for rice creation in India and Iran, utilizing the nitrogen fixing properties of free-living cyanobacteria to enhance nitrogen content in soils.

1.4.4 COSMETICS

microalgae are oxygenic photosynthetic creatures that are basically found in sea-going conditions and wetlands. There is an expanding pattern in the usage of photosynthetic microorganisms including large scale and miniature green growth in the field of Cosmeceuticals by joining the mass items separated from its biomass into corrective formulations. There is numerous logical confirmation that demonstrates the skill of green growth obviously, it relies upon explicit sort of concentrate, how it is prepared and its application. Because of the developing financial part of the restorative business, the requirement for innocuous and proficient characteristic crude fixings has become a most extreme necessity. According to certain exploration reports, algal items utilized in cosmeceuticals have been known to be appropriate choices with valuable impact even after delayed utilization. Different algal species are presently being utilized broadly for the treatment of different skin related issues by going about as a cream or surface upgrading, sunscreens, against wrinkling, and so on This survey essentially centers around the business estimation of different algal items extricated from both full scale and microalgal species for its utilization as a substitute in the biotechnology and corrective industry.

1.4.5 BIO-DIESEL

Biodiesel is a diesel fuel got from creature or plant lipids (oils and fats). Studies have shown that a few types of green growth can deliver 60% or a greater amount of their dry load as oil. Since the cells fill in fluid suspension, where they have more productive admittance to water, CO₂ and broken up supplements, microalgae are equipped for delivering a lot of biomass and usable oil in either high-rate algal lakes or photobioreactors. This oil would then be able to be transformed into biodiesel which could be sold for use in autos. Provincial creation of microalgae and preparing into biofuels will give financial advantages to country networks.

As they don't need to deliver primary mixtures like cellulose for leaves, stems, or roots, and in light of the fact that they can be developed gliding in a rich healthful medium, microalgae can have quicker development rates than earthly yields. Likewise, they can change over a lot higher part of their biomass to oil than regular yields, for example 60% versus 2-3% for soybeans. The per unit territory yield of oil from green growth is assessed to be from 58,700 to 136,900 L/ha/year, contingent upon lipid content, which is 10 to multiple times as high as the following most elevated yielding harvest, oil palm, at 5 950 L/ha/year.

The U.S. Branch of Energy's Aquatic Species Program, 1978–1996, zeroed in on biodiesel from microalgae. The last report proposed that biodiesel could be the lone suitable strategy by which to deliver sufficient fuel to supplant current world diesel use. On the off chance that green growth inferred biodiesel were to supplant the yearly worldwide creation of 1.1bn huge loads of customary diesel then a land mass of 57.3 million hectares would be required, which would be profoundly great contrasted with other biofuels.

CHAPTER 2

LITERATURE SURVEY

[1]. Enhancing growth and lipid production of marine microalgae for biodiesel production via the use of different LED wavelengths.

Microalgae equipped for amassing high lipid content were separated photoautotrophic, heterotrophic and mixotrophic societies. The biomass and lipid creation of all tried strains in mixotrophic culture were eminently improved in examination with photoautotrophic and heterotrophic societies. Among the tried strains, marine *Chlorella* sp. what's more, *Nannochloropsis* sp. were ideal possibility for biodiesel creation due to their high lipid creation. The impact of light force and starting glucose fixation on development and lipid substance of the two strains were researched. Albeit expanding light power and starting glucose fixation improved the development of the two strains, it diminished their lipid content. To create a high measure of biomass with high lipid content, a took care of bunch development with stepwise expanding light power was performed. The lipid creation by this procedure was around double that of customary cluster development. The fundamental unsaturated fat arrangements of the two microalgae were C16–C18 (>80%) which are proper for biodiesel creation.

[2]. Enhanced growth and lipid production of microalgae under mixotrophic culture condition: Effect of light intensity, glucose concentration and fed-batch cultivation.

The emergency of energy delivering particles (fills) is relied upon to expansion in future, which is at present created from unrefined mineral oil. Biodiesel is generally dependable, non-poisonous, biocompatible fluid fuel that can supplant the current impractical wellsprings of energy. Among all the known sources, microalgae show high potential for the creation of biodiesel attributable to their various advantages like higher biomass productivities than plants, no agrarian land prerequisite, development in squander water and gathering of 20-half triacylglycerols. Microalgae biomass and lipid content assumes a significant part in business creation of biodiesel. The present work was done to build up an axenic culture of a potential microalga *Chlorella* sp. for high biomass and improved lipid collection. The significant development boundaries like pH, light tone, light power and photoperiod were read for better creation of *Chlorella* biomass. The impact of saltiness on cell development was likewise examined and contrasted and ordinary Fogg's medium developed cells. The principle biomolecules like starch, protein, lipid and chlorophyll content were additionally assessed with the assistance of standard biochemical techniques in salt enhanced and without salt Fogg's medium. The cell lipid content was expanded by developing the cells under various salt focuses. The miniature algal strain showed most elevated development of 0.822 g L⁻¹ and 1.021g L⁻¹ in Fogg's medium and under 0.2 M NaCl enhanced medium separately. In any case, the greatest lipid creation of 0.1842 g L⁻¹ was assessed by developing the cells in Fogg's medium incorporating 0.5 M NaCl with slight trade off in cell development (0.858 g L⁻¹). The lipid substance of *Chlorella* sp. was discovered to be 26.84% as contrasted with 14% acquired under typical culture

condition. Hence, developing *Chlorella* sp. under salt enhanced medium and advancing light necessity will create high biomass and oil for biodiesel creation [2].

[3]. Effect of Salinity, pH, Light Intensity on Growth and Lipid Production of Microalgae for Bioenergy Application.

Explicitly development state-based plans for development streamlining of microalgae *Chlorella* sp. were explored for the lipid improvement in biodiesel creation, contrasted and normal advancement tests. The enhanced states of beginning cell thickness, light power and air circulation stream in like manner improvement were about 0.45 cm^{-1} (in OD686, for example 0.15 g/L), 6000 lux and $0.2\text{ vvm (mL mL}^{-1}\text{ min}^{-1})$ separately, considering the lipid yield and energy utilization. The most extreme dry weight and lipid content were 1.2 g/L and 33.6% separately. At that point three explicit plans for development conditions change were directed to examine the impacts of changing conditions alongside development state for the expected upgrade of biomass and lipid creation. The outcomes showed that incredible improvement of both biomass dry load of 1.9 g/L and lipid yield of 0.6 g/L was accomplished. The new and explicit development strategy essentially improved the biomass and lipid creation by 52.7% and 64.7% separately, contrasted and the previously mentioned three basic enhancement tests. The GC–MS investigation showed that C16–C18 segments represented over 90% of unsaturated fat, and the unsaturated fat shared preferable properties over that practically speaking development.

[4]. Optimization and lipid production enhancement of microalgae culture by efficiently changing the conditions along with the growth-state.

In response to the energy crisis, global warming, and climate changes, microalgae have received a great deal of attention as a biofuel feedstock. Due to a high lipid content in microalgal cells, microalgae present as a promising alternative source for the production of biodiesel. Environmental and culturing condition variations can alter lipid production as well as chemical compositions of microalgae. Therefore, application of the strategies to activate lipid accumulation opens the door for lipid overproduction in microalgae. Until now, many original studies regarding the approaches for enhanced microalgal lipid production have been reported in an effort to push forward the production of microalgal biodiesel. However, the current literature demonstrates fragmented information available regarding the strategies for lipid production improvement. From the systematic point of view, the review highlights the main approaches for microalgal lipid accumulation induction to expedite the application of microalgal biodiesel as an alternative to fossil diesel for sustainable environment. Of the several strategies discussed, the one that is most commonly applied is the design of nutrient (e.g., nitrogen, phosphorus, and sulfur) starvation or limitation. Other viable approaches such as light intensity, temperature, carbon dioxide, salinity stress, and metal influence can also achieve enhanced microalgal lipid production.

[5]. Strategies for Lipid Production Improvement in Microalgae as a Biodiesel

This examination researched nitrogen and phosphorus digestion and lipid creation of microalgae in modern wastewater. Two local strains of freshwater microalgae were assessed their biomass development and lipid creation in altered BBM medium. *Chlamydomonas* sp. TAI-2 would be wise to biomass development and higher lipid creation than *Desmodesmus* sp. TAI-1. The ideal development and lipid collection of *Chlamydomonas* sp. TAI-2 were tried under various nitrogen sources, nitrogen and CO₂ focuses and light period in altered BBM medium. The ideal CO₂ air circulation was 5% for *Chlamydomonas* sp. TAI-2 to accomplish maximal lipid aggregation under consistent light. Utilizing mechanical wastewater as the medium, *Chlamydomonas* sp. TAI-2 could eliminate 100% NH₄⁺-N (38.4 mg/L) and NO₃⁻-N (3.1 mg/L) and 33% PO₄³⁻-P (44.7 mg/L) and amass the lipid up to 18.4%. More than 90% of complete unsaturated fats were 14:0, 16:0, 16:1, 18:1, and 18:3 unsaturated fats, which could be used for biodiesel creation [5].

CHAPTER-3

AIM AND OBJECTIVE

3.1 AIM

The extraction of bio-diesel from the microalgae is the main aim of this study. The Microalgae are grown, extracted and processed for the production of bio-diesel.

3.2. OBJECTIVE

- The microalgae are collected from pond with pond water.
- To growth the microalgae under three various light condition.
- To evaluate the growth of microalgae.
- Lipid extraction from the grown microalgae.
- Bio-diesel production from the extracted lipids.

CHAPTER-4

MATERIALS AND METHOD

4.1. COLLECTION OF MICROALGAE

The microalgae are collected from pond water of munjarpattu village from Vellore district in Tamil Nadu during the month of January 2021. The collected samples are taken in can along with the water of pond. The algae are freshly taken in the can and they were cultivated and harvested. These algae are cultivated in the presences of three different light condition.

4.2. CULTIVATION OF MICROALGAE

The microalgae are grown inside the can with collected pond water. During the cultivation period the algae are in the presences of three light condition for 16 hours for 14 days in rotation and they are given with aeration for the breath of algae. The three conditions are sunlight, dark region, artificial light. The small quantity from collected algae are filtered using the filter paper then they are identified under the compound microscope then they are identified as microspora, nannochloropsis, oscillaoria this identification is based on the shape and with their unique feature.

4.2.1 IDENTIFIED MICROALGAE

4.2.1.1 MICROSPORA

The microsporidia utilize an exceptional organelle called the polar cylinder in have cell intrusion. This mechanical assembly is appended to the foremost finish of the spore and fills in as an extension through which the sporuloplasm is infused into the host cell cytoplasm. Like connection of the Apicomplexa, lectin–carb associations seem, by all accounts, to be significant for beginning connection of the spore to the host epithelium and for flagging release of the polar cylinder.

Intrusion for microsporidia starts with spore release, which in itself requires three cycles to happen: actuation following change in osmotic pressing factor inside the spore, realignment of the polar cylinder, and infusion of the sporuloplasm into the host cell. Actuation of the spore requires an assortment of conditions, explicit to every species. These conditions differ from an acidic pH to a basic pH; a few animal groups require an emotional change between the two. When actuated, paying little heed to mode, the spore encounters a change in osmotic pressing factor. This pressing factor change triggers spore release, during which the polar fiber is expanded. This prompt puncturing of the host cell film, and section of the sporuloplasm into the host cell at that point follows. Numerous speculations exist on how this complex pressing factor change happens inside the small spore. Changes in the proton angle, diminished trehalose levels, and the dislodging of calcium from the polarplast film have all been examined.

The polar cylinder gives safe section to the sporuloplasm as it enters the host cell by connecting the distance between have cytoplasm and parasite. Customarily, the polar

cylinder is portrayed as penetrating the cell. Indeed, the specific system of this cooperation isn't all around portrayed. A few agents have hypothesized that have cell actin intervenes this trade. The polar cylinder may likewise empower ingested spores to escape from macrophage phagosomes, permitting the living being to get away from one cell and taint a neighboring cell, subsequently spreading the contamination. Reliable with this hypothesis, specialists have shown the capacity of these spores to develop inside a phagosome.

4.2.1.2 NANNOCHLOROPSIS

Types of *Nannochloropsis* have gotten mainstream for investigations of lipid creation for biofuels and additionally the creation of long-chain polyunsaturated unsaturated fats, particularly, and endeavors are in progress to popularize this alga. *Nannochloropsis* species are unicellular, planktonic, with either 2–4 µm distance across subspherical or 3–4 × 1.5 µm tube shaped cells. They contain a yellow-green chloroplast with the fundamental shades being chlorophyll and the xanthophylls, violaxanthin and vaucheriaxanthin. Sexual generation has never been noticed and the class seems to come up short on the qualities for meiosis. *Nannochloropsis* happens in both freshwater and marine conditions. As of now 5 species are perceived (*Nannochloropsis australis*, *Nannochloropsis granulata*, *Nannochloropsis limnetica*, *Nannochloropsis oceanica*, *Nannochloropsis oculata*) as the species *Nannochloropsis salina* and *Nannochloropsis gaditana* as of late have been moved to the sort *Microchloropsis*.

Nannochloropsis is a variety of green growth containing six known species. The variety in the current ordered arrangement was first named by Hibberd (1981). The species have for the most part been known from the marine climate yet in addition happen in new and salty water. The entirety of the species is little, nonmotile circles which don't communicate any unmistakable morphological highlights that can be recognized by one or the other light or electron microscopy. The characterisation is for the most part done by *rbcl* quality and 18S rRNA succession examination.

The green growth of the family *Nannochloropsis* vary from other related microalgae in that they have chlorophyll and totally need chlorophyll b and chlorophyll c. Furthermore, they can develop a high convergence of a scope of shades, for example, astaxanthin, zeaxanthin and canthaxanthin. They have a distance across of around 2 to 3 micrometers and an exceptionally basic ultrastructure with diminished primary components contrasted with adjoining taxa.

Nannochloropsis is viewed as a promising alga for mechanical applications due to its capacity to gather undeniable degrees of polyunsaturated greasy acids.[7][8] Moreover, it shows promising highlights that can permit hereditary control focused on the hereditary improvement of the current oleaginous strains. Different types of *Nannochloropsis* for sure are transfectable and there has been proof that a few strains can perform homologous recombination. Right now, it is mostly utilized as an energy-rich food hotspot for fish hatchlings and rotifers. By and by, it has raised developing revenue additionally for the examination of biofuel creation from photosynthetic living beings. (see *Nannochloropsis* and biofuels). *Nannochloropsis* is really being used as food added substance for human nourishment and it is likewise served at Restaurant

"A Poniente" of El Puerto de Santa María near the common habitat where *Nannochloropsis gaditana* was first confined and still grows. A recent report proposes it very well may be utilized for an exceptionally performant, reasonable sans fish feed for cultivated fish.

4.2.1.3 OSCILLAORIA

Oscillatoria is a class of filamentous cyanobacteria. It has in excess of 100 species. It is named for the swaying in its development. Fibers in the provinces can slide to and for against one another. Consequently, the entire mass is reoriented to its light source. It is regular in wet spots wealthy in rot natural mailer. It is regularly found in water tanks waters like streams, side of the road trench, channels and sewers. It is primarily blue-green or earthy colored green. It shapes meager blue green adhesive covering on the outside of streaming water. It's one specie is found in natural aquifers. A few animal groups like *O. formosa* and *O. princeps* are harmonious. They structure relationship with the nitrogen fixing microorganisms. Some regular species are *O. fomsa*, *O. prolificn* and *O. formosa*.

All cells have very much evolved cell divider. The cell divider comprises of an internal dainty cell layer a medium pectic layer and external adhesive. All around characterized core is missing. Mitochondria, endoplasmic reticulum and Golgi bodies are additionally missing. The proptoplasm comprises of fringe and focal cytoplasm. Shades are found in the fringe, so it is known as chromoplasm. A few little granules are likewise present. Pseudo-vacuole is also found at times. Glycogen like substance is the save food material. Barely any oil drops may likewise be found put away as save food.

Its body is made from single column of cells. These cell structure trichomes. Its trichomes are unbranched fibers. They are covered by slim adhesive sheath. All cells of a trichome are comparable fit as a fiddle aside from apical cells. The apical cells are raised at the tip. Any remaining cells bend more extensive and tube shaped. In certain species, the apical cells may end in subacute point. At times, it might have cap or calyptra at the tip. A few animal varieties have limited trichome. They have cylidrical cells with their length equivalent or more prominent than the breath

4.3 GROWTH ANALYSIS (OD METHOD)

The growth of microalgae is observed once in every two days for 14 days to ensure the growth of the microalgae. The sample are collected from the can are observed under the UV-vis spectrophotometer and the growth is identified. The growth under three lights is noted and the values are plotted and graphed. These are used to compare the growth of microalgae in different light conditions.

4.3.1. ULTRAVIOLET-VISIBLE (UV-VIS) SPECTROPHOTOMETER

Ultraviolet-visible (UV-Vis) spectrophotometry is a procedure used to gauge light absorbance across the bright and obvious scopes of the electromagnetic range. At the point when occurrence light strikes matter it can either be assimilated, reflected, or sent. The absorbance of radiation in the UV-Vis range causes nuclear excitation, which alludes to the progress of particles from a low-energy ground state to an energized state. An UV-Vis spectrophotometer estimates the force of light sent

through an example contrasted with a reference estimation of the occurrence light source. The prestigious DS-11 arrangement of UV-Vis spectrophotometers from DiNovo utilizes a beat Xenon streak light to grill microvolume tests with light across a frequency scope of 190 – 840nm. The communicated light is procured by a CCD optical identifier with a frequency precision of inside 0.5nm. According to Beer-Lambert Law, the measure of light consumed is straightforwardly relative to the convergence of the example and the distance the light goes through the example; the pathlength.

4.4 LIPID EXTRACTION

4.4.1 LIPIDS

Lipids are particles that contain hydrocarbons and make up the structure squares of the design and capacity of living cells. Instances of lipids incorporate fats, oils, waxes, certain nutrients (like A, D, E and K), chemicals and the majority of the cell film that isn't comprised of protein. Lipids are not dissolvable in water as they are non-polar, however are subsequently solvent in non-polar solvents like chloroform. Lipids are chiefly made out of hydrocarbons in their most diminished structure, making them a phenomenal type of energy stockpiling, as when processed the hydrocarbons oxidize to deliver a lot of energy. The sort of lipid found in fat cells for this object is a fatty oil, an ester made from glycerol and three unsaturated fats.

Lipids created by microalgae can be partitioned into two fundamental gatherings: polar lipids, as glycerophospholipids, which have a significant job in cell structure; and non-polar lipids, as triacylglycerols, primarily answerable for energy stockpiling. Primary lipids (polar lipids) generally have long chains of unsaturated fats which could be changed to get polyunsaturated fats (PUFAs), this sort of unsaturated fat incorporates Eicosapentaenoic corrosive (EPA), docosapentaenoic corrosive (DPA) and docosahexaenoic corrosive (DHA).

4.4.2 EXTRACTION OF LIPIDS (SOXHLET EXTRACTOR)

In this technique the sample is dried, ground into little particles and put in a permeable cellulose thimble. The thimble is put in an extraction chamber, which is suspended over a carafe containing the dissolvable and under a condenser. The carafe is warmed and the dissolvable vanishes and climbs into the condenser where it is changed over into a fluid that streams into the extraction chamber containing the example. The extraction chamber is planned with the goal that when the dissolvable encompassing the example surpasses a specific level it floods and streams down into the bubbling carafe. Toward the finish of the extraction cycle, which keeps going a couple of hours, the carafe containing the dissolvable and lipid is eliminated. In some gadget a pipe permits to recuperate the dissolvable toward the finish of the extraction in the wake of shutting a stopcock between the channel and the extraction chamber. The dissolvable in the jar is then vanished and the mass of the leftover lipid is estimated. The level of lipid in the underlying example would then be able to be determined.

40g of dry harvested microalgae are placed in the thimble inside the filter paper. Then hexane is used as the solvent for the lipid extraction. This process is done overnight for 16 hr. cold water are used for cooling the extractor. The hexane gets heated and

floods to the thimble containing the solid sample. Then the condenser ensure that all the hexane vapour are drips into the chamber. Finally, the lipids are drips into the distillation flask.

4.5 BIO-DIESEL PRODUCTION

4.5.1 BIO-DIESEL

Biodiesel is a type of diesel fuel got from plants or creatures and comprising of long-chain unsaturated fat esters. It is regularly made by synthetically responding lipids like creature fat (tallow), soybean oil, or some other vegetable oil with a liquor, delivering a methyl, ethyl or propyl ester. Dissimilar to the vegetable and waste oils used to fuel changed over diesel motors, biodiesel is a drop-in biofuel, which means it is viable with existing diesel motors and appropriation foundation. Biodiesel can be utilized alone or mixed with Petro diesel in any proportions. Biodiesel mixes can likewise be utilized as warming oil.

Biodiesel can be utilized in unadulterated structure (B100) or might be mixed with oil diesel at any fixation in most infusion siphon diesel motors. New limit high-pressure (29,000 psi) regular rail motors have exacting production line cut-off points of B5 or B20, contingent upon producer. Biodiesel has diverse dissolvable properties from Petro diesel, and will corrupt characteristic elastic gaskets and hoses in vehicles (for the most part vehicles produced before 1992), albeit these will in general destroy normally and, in all probability, will have effectively been supplanted with FKM, which is nonreactive to biodiesel. Biodiesel has been known to separate stores of build-up in the fuel lines where Petro diesel has been used. As an outcome, fuel channels may get obstructed with particulates if a fast change to unadulterated biodiesel is made. Consequently, it is prescribed to change the fuel channels on motors and radiators soon after first changing to a biodiesel mix.

4.5.2 TRANSESTERIFICATION

Methanol was blended in with 0.5g of sodium hydroxide (NaOH) And mixed for 20 min at 400rpm at 65degree Celsius. The proportion of methanol to oil in the combination was kept 6:1 proportion. The combination of methanol and NaOH Is at that point poured into the tapered cup containing the algae oil in order to start the transesterification process. The funnel shaped cup was steered constantly for 4h at 400 rpm and permitted to agree to 16h so as acquire 2 separate layers the supernatant layer(biodiesel) and dregs layer (methanol) the biodiesel was isolated from the residue layer by jar separator.

4.5.3 BIOREFINERY PRODUCTES

In the structure of a biorefinery approach, which will make scale-up of microalgae-based frameworks financially plausible. To this end, usage of wastewater and effluents to deliver important mixtures, including proteins, shades, nutrients, and various bioactive substances addresses an arising and profoundly encouraging field of microalgal innovation. Since antiquated occasions, individuals have been utilizing microalgae as a food and feed.

while right now microalgae have been considered as a novel wellspring of protein. This kind of protein is regularly called single-cell protein or microbial protein. In the event that effluents are utilized as development media for microalgal creation, a few concerns emerge as far as security and cleanliness of the delivered protein source.

Microalgal biomass additionally comprises an important wellspring of shades, principally chlorophylls and carotenoids, which have been corresponded with various medical advantages, including anticipation of malignancy and cardiovascular illnesses, cancer prevention agent, calming, and antidiabetic movement, just as a few applications as regular colorants, makeup, food, and feed supplements.

farming addresses another space of use for microalgal biomass, because of the similitudes and surprisingly improved attributes contrasted with traditional manures. A few microalgal animal types, incorporating *Chlorella* and *Arthrospira* with high supplement evacuation limit, can return significant supplements and micronutrients, just as soil natural carbon, considerably improving soil quality and yield development and adapting to full scale and micronutrient lacks. In conclusion, high debris substance of microalgae can be a favourable trademark, such if there should arise an occurrence of biomass of *Scenedesmus* and *Desmodesmus* sp. filled in emanating from an adjustment tidal pond, which debris focus near 20% and low centralization of HMs can bring about an excellent digestate utilized as biofertilizer.

4.6 FTIR ANALYSIS

Fourier Transform Infrared Spectroscopy, otherwise called FTIR Analysis or FTIR Spectroscopy, is a scientific strategy used to distinguish natural, polymeric, and, sometimes, inorganic materials. The FTIR investigation strategy utilizes infrared light to examine test tests and notice synthetic properties.

The FTIR instrument sends infrared radiation of around 10,000 to 100 cm^{-1} through an example, with some radiation consumed and some went through. The consumed radiation is changed over into rotational or potentially vibrational energy by the example atoms. The subsequent sign at the identifier presents as a range, ordinarily from 4000 cm^{-1} to 400 cm^{-1} , addressing an atomic unique finger impression of the example. Every atom or synthetic design will create a novel ghostly unique mark, making FTIR investigation an incredible device for substance ID.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 COLLECTION MICROALGAE SAMPLE

The microalgae are collected from pond water of munjarpattu from Vellore district in Tamil nadu. The microalgae are collected by cans with pond water for the cultivation of microalgae. The microalgae are cultivated in three different light condition. after the cultivation of microalgae, they are sent to Vellore institution technology for the identification of microalgae. They are identified as microspora, nannochloropsis, Oscillatoria on the basis of shape.



Fig 5.1 Microspora

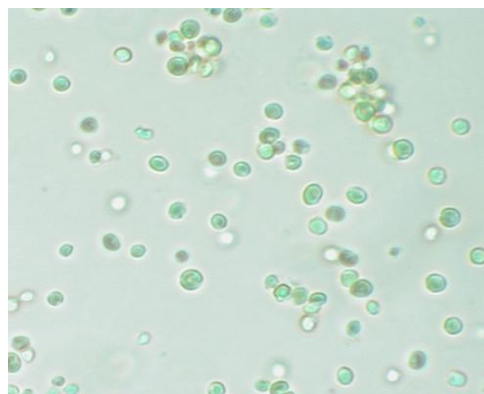


Fig 5.1.1 Nannochloropsis



Fig 5.1.2 Oscillatoria



Fig 5.1.3 sample with pond water

5.2 CULTIVATION OF MICROALGAE

Microalgae are cultivated in three different light condition with the constant supply of air into it. due to aeration the microalgae get agitated continuously for 24x7. nitrogen and phosphorus limitations. Algal productivity is often correlated to levels of nitrogen (N) and phosphorus (P) (See N:P ratio, above), but other nutrients are required including carbon, silica, and other micronutrients. Biomass is usually measured by the amount of chlorophyll a in the water column (measurement of gross level of algae) and/or as mass per area for attached species. Chlorophyll a is a photosynthetic pigment that serves as a measurable parameter for all algae production. Quantitative biomass estimates can be made noting that on average 1.5% of algal organic matter is chlorophyll a.

The nutrients for growth of microalgae is provided via pond water. The pond water contain all the nutrients for the growth of microalgae (These animals lives in pond keep the submerged biological system adjusted and give a lot of supplements like nitrates, phosphates, and silicates to the green growth).



Fig 5.2 microalgae placed in dark region Fig 5.2.1 microalgae placed in sunlight

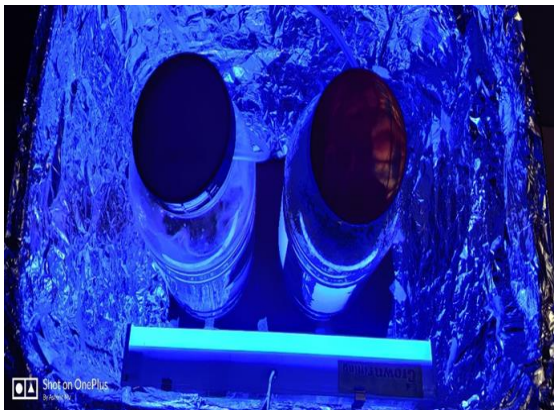


Fig 5.2.2 microalgae placed in artificial lamp

5.3 growth analysis (OD method)

The marine microalgae developments were estimated utilizing the optical thickness (OD) strategy. Tests were required at regular intervals for 14 days where OD readings were taken utilizing an UV–vis spectrophotometer (Shimadzu UVmini-1240) at 540 nm. The blank are set using water.

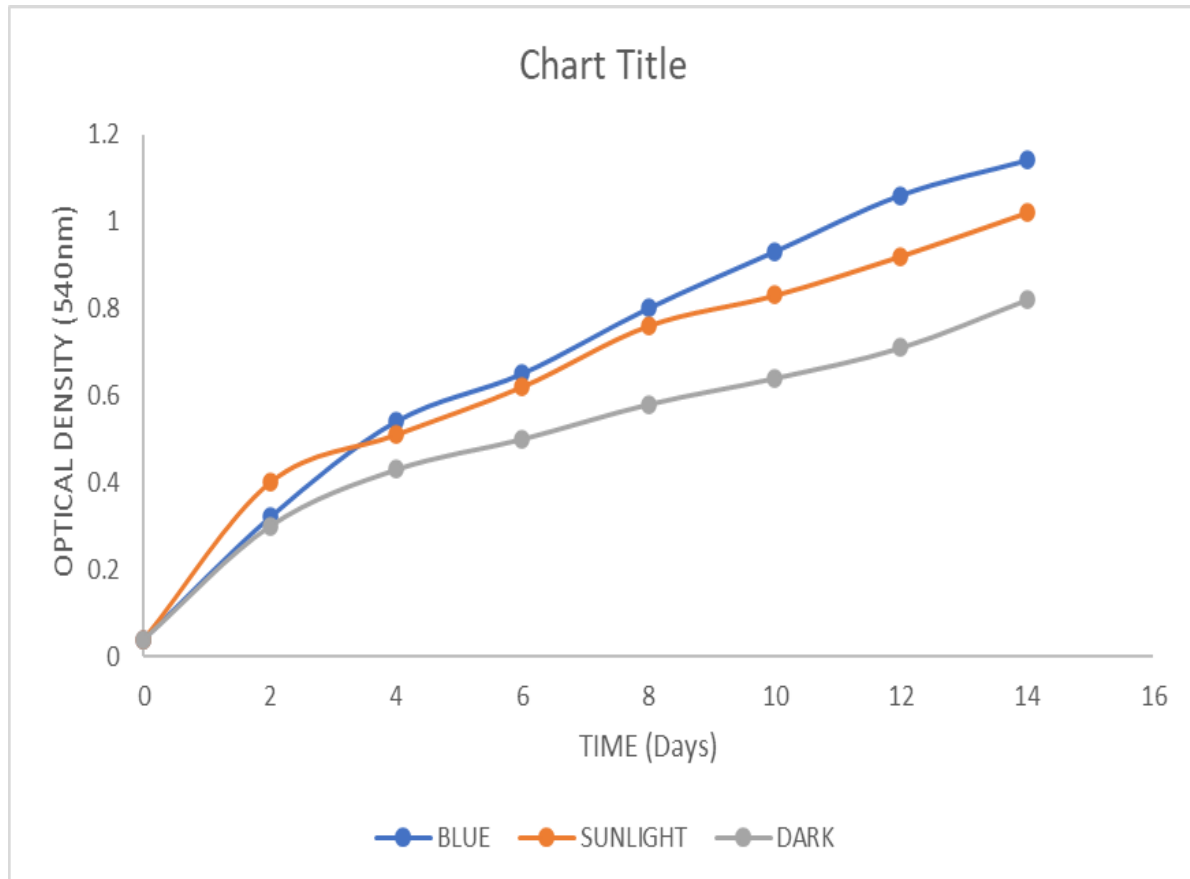


Fig 5.3 uv-vis spectrophotometer

Table 5.3 optical density vs time of various light condiction

Time(days)	Blue light	sunlight	Dark region
0	0.039	0.039	0.039
2	0.34	0.43	0.28
4	0.53	0.48	0.39
6	0.64	0.60	0.43
8	0.79	0.70	0.54
10	0.85	0.79	0.61
12	0.93	0.84	0.68
14	1.17	0.95	0.78

Fig 5.3.1 growth curve of microalgae using od method



shows microalgae development bend under various light condition during the 14 days development. For the most part, the optical thickness of bunch societies expanded from day 1 to 14. The way of life was tested at 48 h span with beginning optical thickness perusing around 0.045. From the general pattern of development rates, microalgae becomes better under blue light contrasted with other light condition. Higher development rate noticed for microalgae Fig. (a) obviously shows that the blue enlightenment condition brings about higher biomass beginning from the eighth day when contrasted with the other light conditions. After day 14 the development will in general log jam and stayed fixed with time because of supplement exhaustion and squanders begin to amass in the way of life medium.

5.4 HARVESTING OF MICROALGAE

The microalgae are harvested using cotton cloth. Then the microalgae are dried under sunlight for 12 hrs. After harvesting the microalgae are taken for lipid extraction. The lipid extraction are done for the highest microalgae produced from the highest growth method (artificial lamp produce high quantity of microalgae).



Fig 5.4 harvested microalgae using cotton cloth

5.5 LIPID EXTRACTION (USING SOXHLET EXTRACTOR)

In this technique the sample is dried, ground into little particles and put in a permeable cellulose thimble. The thimble is put in an extraction chamber, which is suspended over a carafe containing the dissolvable and under a condenser. The carafe is warmed and the dissolvable vanishes and climbs into the condenser where it is changed over into a fluid that streams into the extraction chamber containing the example. The extraction chamber is planned with the goal that when the dissolvable encompassing the example surpasses a specific level it floods and streams down into the bubbling carafe. Toward the finish of the extraction cycle, which keeps going a couple of hours, the carafe containing the dissolvable and lipid is eliminated. In some gadget a pipe permits to recuperate the dissolvable toward the finish of the extraction in the wake of shutting a stopcock between the channel and the extraction chamber. The dissolvable in the jar is then vanished and the mass of the leftover lipid is estimated. The level of lipid in the underlying example would then be able to be determined.

40g of dry harvested microalgae are placed in the thimble inside the filter paper. Then hexane is used as the solvent for the lipid extraction. This process is done overnight for 16 hr. cold water are used for cooling the extractor. The hexane gets heated and floods to the thimble containing the solid sample. Then the condenser ensure that all the hexane vapour are drips into the chamber. Finally, the lipids are drips into the distillation flask.



Fig 5.5 sample loaded in Soxhlet extractor



Fig 5.5.1 lipids extracted

40g of dry microalgae are taken for the extraction.

The microalgae for the extraction are taken from the artificial light which shows higher growth rate. Initially, the oil is extracted from the algal biomass (mixed algae culture) with a nonpolar solvent hexane by Soxhlet extraction method. The yield of oil extracted is 10% (4ml of algal oil are produced).

5.6 BIO-DIESEL PRODUCTION

TRANSESTERIFICATION PROCESS

Methanol was blended in with 0.5g of sodium hydroxide (NaOH) and mixed for 20 min at 400rpm at 65degree Celsius. The proportion of methanol to oil in the combination was kept 6:1 proportion. The combination of methanol and NaOH is at that point poured into the tapered cup containing the algae oil in order to start the transesterification process. The funnel shaped cup was stirred constantly for 4h at 400 rpm and permitted to agree to 16h so as to acquire 2 separate layers the supernatant layer (biodiesel) and dregs layer (methanol) the biodiesel was isolated from the residue layer by jar separator.

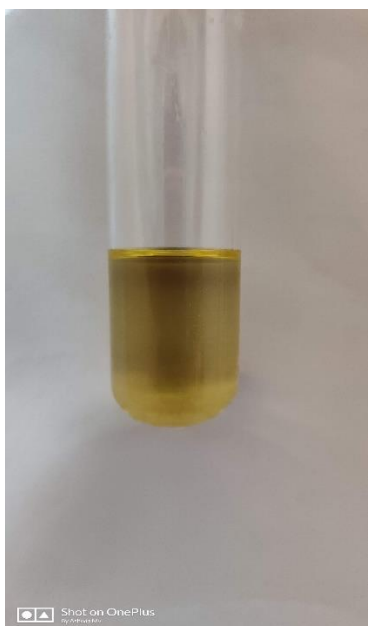


Fig 5.6 bio-diesel

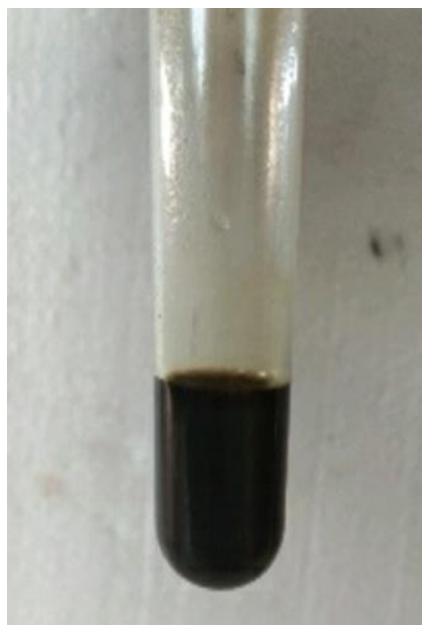


Fig 5.6.1 sedimented biomass

5.7 FTIR ANALYSIS

Fourier Transform Infrared Spectroscopy, otherwise called FTIR Analysis or FTIR Spectroscopy, is a scientific strategy used to distinguish natural, polymeric, and, sometimes, inorganic materials. The FTIR investigation strategy utilizes infrared light to examine test tests and notice synthetic properties.

The FTIR instrument sends infrared radiation of around 10,000 to 100 cm^{-1} through an example, with some radiation consumed and some went through. The consumed radiation is changed over into rotational or potentially vibrational energy by the example atoms. The subsequent sign at the identifier presents as a range, ordinarily from 4000 cm^{-1} to 400 cm^{-1} , addressing an atomic unique finger impression of the example. Every atom or synthetic design will create a novel ghostly unique mark, making FTIR investigation an incredible device for substance ID.

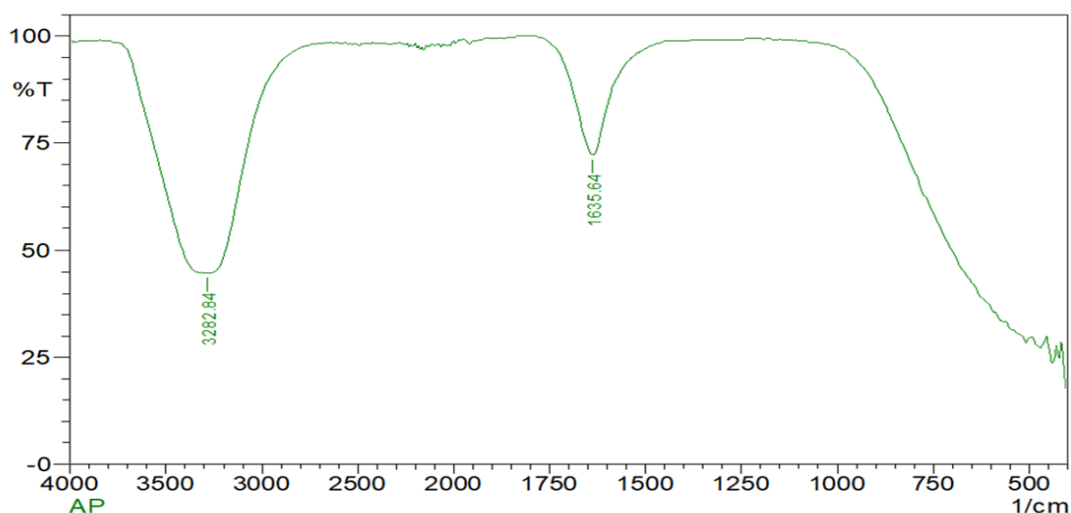


Fig 5.7 FTIR analysis

The FTIR analysis of the product sample gives the frequency value and the corresponding peaks at 3282.84, 1635.64 indicates the presence of alkenes.

DISCUSSION

Biodiesel is at present created from oil combined by regular fuel crops that reap the sun's energy and store it as substance energy. This presents a course for inexhaustible and carbon-impartial fuel creation.

Benefits of microalgal frameworks: Microalgal frameworks: have a higher photon change productivity (as confirmed by expanded biomass yields per hectare), can be reaped bunch astute essentially lasting through the year, giving a dependable and nonstop inventory of oil, can use salt and waste water streams, consequently decreasing freshwater use, can couple CO₂-unbiased fuel creation with CO₂ sequestration, 5) produce non-poisonous and profoundly biodegradable biofuels.

Benefits of biodiesel from green growth oil :Green growth oil/green growth biofuel: gives fast development rates, develops essentially anyplace, a high for each section of land yield (7-31 times more noteworthy than the following best yield—palm oil), green growth biofuel is non-harmful, green growth biofuel is profoundly biodegradable, undeniable degrees of polyunsaturated in green growth biodiesel is reasonable for chilly climate environments, can diminish fossil fuel by-products dependent on where it's grown, green growth biomass can be created at high volumes and this biomass can yield a higher level of oil than different sources, 8) green growth oil has restricted market rivalry, green growth can be developed ashore, new water, or seawater, and advancements of green growth creation permit it to turn out to be more beneficial while burning-through assets that would somehow be viewed as waste, have a higher photon change proficiency, can be gathered clump savvy virtually throughout the entire year, giving a solid and nonstop stock of oil, and can use salt and waste water streams, consequently enormously decreasing freshwater use. Green growth has the capability of filling in places from the farmlands and woods, along these lines limiting the harms to the biological system and evolved way of life supply. Green growth can likewise be filled in sewage and close to control plant smokestacks, where they can process the poisons and convey the oil.

SUMMARY AND CONCLUSION

The microalgae are taken from the pond along with the water of pond these are taken to the laboratory and identified under the compound microscope the identified microalgae are Microspora, Nannochloropsis, Oscillatoria then they are cultivated under the three conditions of light like dark, sunlight, artificial light. The growth of algae is compared in the presence of the condition and the growth of microalgae are higher in the presence of the artificial light and less in the dark light. Aeration is given to the growth of the algae. This is process involved in the cultivation of the microalgae. the growth is analysed using the growth analyser and the graph are plotted and the growth of the algae is monitored and noted. The harvest of microalgae is done to extract the lipid from the microalgae using the Soxhlet extractor then the lipid is Methanol was blended in with 0.5g of sodium hydroxide (NaOH) And mixed for 20 min at 400rpm at 65degree Celsius. The proportion of methanol to oil in the combination was kept 6:1 proportion. The combination of methanol and NaOH Is at that point poured into the tapered cup containing the algae oil in order to start the transesterification process is produced this diesel which is environment friendly and they are pollution free comparatively from the diesel extracted from mining. The growth of microalgae is higher in the artificial LED light comparing to the other light. The Soxhlet is used to extract the lipid which is more essential and quite good amount of lipid is extracted. The lipid is mixed with Methanol and blended in with 0.5g of sodium hydroxide (NaOH) to produce the bio diesel. This diesel is environment friendly compare to other extracted diesel. the artificial light source show higher growth rate when compare to other two light condition.

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