A DISSERTATION ON

COLLECTION, ISOLATION AND PURIFICATION OF CYANOBACTERIA AND THEIR BIOCHEMICAL CHARACTERIZATION

SUBMITTED TO THE

DEPARTMENT OF BIOSCIENCE

INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILLMENT

FOR THE

DEGREE OF MASTER SCIENCE IN MICROBIOLOGY

BY

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Enrollment No. 2000100619

M. Sc. Microbiology (IV Semester)

Department of Biosciences

Integral University, Lucknow

UNDER THE SUPERVISION OF

Mr. Manish Kr. Pandey

Biotech Park, Lucknow

Biotech Park in Biotechnology City, Lucknow

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CERTIFICATE

This certificate is issued to **Ms. Hoori Tahzeeb, D/o Mr. Mohd. Murshil Siddqui** on successful completion of an advanced Training Course/Project Work at Biotech Park, Lucknow.

Registration No./ Receipt No	:	**Offline/9550**
Sponsoring Institution	:	**Integral University**
Course & Department	:	**M.Sc.**
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Category of Training	:	**Project Work**
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Project Title	:	**"Collection, isolation and purification of cyanobacteria and their biochemical characterization."**

The candidate has fulfilled prescribed requirements of the laboratory & project work, faculty consultation and has completed the assigned project.

June 24, 2022

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TO WHOM IT MAY CONCERN

This is certified that **Ms. Hoori Tahzeeb,** a student of M. Sc. Microbiology (IV Semester), Integral University has completed her four months dissertation work entitled **"Collection, Isolation, and Purification of Cyanobacteria and Their Biochemical Characterization"**. Successfully she has been completed this work from **2 march to 24 June 2022 at the Biotech Park, Lucknow** under the guidance of Mr. Manish Panday. The dissertation was a compulsory part of M.Sc. degree. I wish her good luck and a bright future.

Dr. Snober S. Mir

Head Department of Bioscience

Integral University, Lucknow

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DECLARATION

I hereby declare that the present work on **"Collection, Isolation and Purification of Cyanobacteria and their Chemical Characterization."** Is record of original work done by me under the guidance of **Mr. Manish k Pandey (Technical Manager). Biotech Park, Lucknow. During 2- march 2022 to 24 June 2022**. All the data which were provided in this were through my original work.

I also declare that not any part of this thesis has previously been submitted to my university.

HOORI TAHZEEB
M.Sc. (Microbiology)
Integral University

AKNOWLEGMENT

I would like to extend my heartiest gratitude towards my university Integral University, Lucknow as well as my teachers who have been a constant source of inspiration, support and guidance. I was very thankful and express my sincere and deepest gratitude towards **DR. A.K. Singh, CEO Biotech Park, Lucknow,** for giving me the opportunity of completing my project work.

I also express my sincere thanks to my project supervisor **Dr. Shashank Mishra Scientist C** and **Mr. Manish K. Pandey, (Technical Manager), biotech park, Lucknow** for his resolute guidance abiding interest constructive criticism tremendous enthusiasm and meticulous supervision throughout the period of my project.

Finally, I take this opportunity to extent my deep appreciation to my family and friends, for all that they meant to me during the crucial completion of this training and project.

Hoori Tahzeeb

M. Sc (Microbiology)

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OVERVIEW OF BIOTECH PARK, LUCKNOW

The biotechnology park, Lucknow located in city of nawabs. It decorates Lucknow with a futuristic state of art facility for biotechnology led enterprises. It is the only functional Biotechnology Park in the north India serving the state of Uttar Pradesh to bring biotechnology as the way of cultivating crops, living a healthy life enriching the skills and boosting biotech industry. The park was set up jointly by the Department of biotechnology. Government of India and Department of Science and Technology, Government of Uttar Pradesh in the year 2002 and become fully functional in the year 2007.

Biotech Park Lucknow is registered under the Indian Act 21 (1868). The Park is run under the guidance of the society. Governing Board, Advisory-cum-managing committee and other duly constituted committees.

Molecular Biology & amp; Analytical Facility:

The sophisticated analytical testing facility is located in an area 2280 sq. ft. The analytical Quality. Assurance and Control Laboratory is NABL accredited and meet ISO 9000 requirements. The facility is equipped with High Pressure Liquid Chromatography (HPLC), Nanodrop-spectrophotometer, Polarimeter, High Pressure Thin Layer Chromatography (HPTLC), and other support equipment. HPLC has a versatile functioning since it is equipped with UV/VIS, Florescence PDA and Ion Chromatography detectors. A wide range of metabolites and solvent recovery unit along with metabolites or molecules can be quantitatively analysed using HPLC and HPTLC. It also houses common facility for storage.

Extraction unit:

The solvent extraction unit comprises of solid-liquid solvent extraction and solvent recovery system for extraction of photochemical / lead molecules from high value medicinal plants. Multipurpose reaction cum hydrolysis chromatography.

Distillation unit:

- Feature
- Improved design
- All contact parts of stainless steel
- Oil yield is more than convention
- Low oil loss
- Utilizes spent marc/agro waste as fuel
- Environmentally friendly

Capacity: 1000 kg fresh herbs/batch

Distillation time: One batch can be run in 3-10 hrs. depending on the plant material.

Bioinformatics centre:

The bioinformatic centre, Biotech City is a sun DIC centre of DBT under BTIS programme, was originally setup in November 2001, at Indian Institute of Toxicology Research (IITR), Lucknow, as a networking site, the centre was moved to Biotech Park in 2007. Bioinformatics Centre has been setup to establish a close network with various institution and provide information to industries regarding technology, facilities and expertise available with them in the area of biotechnology city. Biotechnology City Map, Biology Institutions in the City, Advanced Research Facilities, Resources Persons Biotechnology Products and Processes available and being developed, Patenting of Biotechnology Product/Processes, Biotechnology Tutorial.

Tissue culture:

Tissue culture facility at Biotech Park, Lucknow is spread over 4071 sq. ft. area having the capacity to raise and multiply Banana, Potato, Jatropha seedlings. The facility has a modern Polycarbonate house for macro-propagation.

Advantages of Propagation by Tissue Culture

- The elimination of diseases and the production of disease-free plantlets
- The rapid production of large no. of genetically identical plantlets
- Introduction of new varieties and/or genotypes
- Preservation of germplasm
- Production of haploid plants which can be used for plant breeding
- Production of plantlets from species in which plant development from seed is difficult

Capacity

One 2 mill10,000 to 100,000 plants per batch and can production plants / annum.

Hardening facility

Hardening facility consist of Poly houses, Net house and Glass houses. Glass house is a facility to protect and maintain the plants of temperate climate. These plants can also be multiplied in large scale during off season. The value crops like Geranium, Patchouli, Pyrethrum could be saved during rainy season when the climate is hot and humid.

Advantages

- It is maintained under the guidance of trained technical staff
- Compartmentalized Glass house for different condition
- Provision for addition of specialized facilities the automatic temperature/ humidity/ mist
- control

Capacity

For primary and secondary hardening of up to 2000000 plants, 20000 mother plants per Glass house

KNOWLEDGE NETWORK AND COLLABRATION

ABSTRACT:

Microalgae are industrially valuable microorganism, especially in the production of biodiesel feedstocks. However, there are several biological and culture system and limitations that result in low productivity of microalgae cultures. hence, there is an urgent need to enhance the yield and productivity of microalgae mass cultures. The growth of microalgae on the surface of materials, together with other microorganism allows for the formation of microalgae biofilms. Microalgae have ability to convert atmospheric CO_2 to carbohydrates, lipids, and other valuable bioproducts by using rich source for biofuels and bioactive compounds. Microalgae are convincing materials to promote bio-based sustainable economy. Microalgae have the ability to perform ecological and environmental services. The report provides a brief and simple explanation of the methodology for sampling, purification, isolation and growth of algae. The algae are being collected from various locations and habitats and viewed under microscope and then the desirable algae could be isolated. Procedures described includes: Performed plating procedures without contaminating media. Performed soft agar overlays when working with algae. Used pour-plate and spread- plate methods to spread the collected crude sample from the field. Isolated single colonies by the streak-plate method. All of the cultivations were performed under controlled condition.

INTRODUCTION

Algae are defined as a group of predominantly, aquatic, photosynthetic and nucleus-bearing organisms. Algae are lower plants that means lack or absence of roots, leaves and stems. There is so many types of algae and they range in size from microscopic micro monas species to giant kelps that reach 60 meters (200 feet) in then length. Algae can be found in both fresh and marine water. In the beginning in 1830s, algae were classified into major groups based on color e.g., red, brown, and green. There are few characteristics of algae mention below.

- They are eukaryotic motile and non-motile.
- Algae are unicellular, filamentous and multicellular.
- Found in wide range of shape and size.
- Reproduction in algae can be occur in both forms sexual and asexual.

Algal pigments are- chlorophylls, carotenoids, phycobilin. Algae can be cultivated to produce a variety of products for large to small markets. Like plastics, chemical feedstocks, lubricants, fertilizers, pharmaceuticals, and algal fuels even in cosmetic.

MICROALGAE:

Microalgae are microscopic algae invisible to the naked eyes. Microalgae are unicellular photosynthetic microorganism, living in saline or fresh water environments, that convert sunlight, water and carbon dioxide to algal biomass. They can be classified as eukaryotic microorganism or prokaryotic cyanobacteria (blue-green algae), with more than 25,000 species already isolated and identified.

Microalgae were used as food in ancient civilizations such as Aztecs in Mexico. They used culture of Arthospira (Spirulina) maxima. A, cyanophycean, to prepare a type of cake.

The first scientific studies on microalgae started at the end of the 19^{th} century, when the microbiologist beijerinck (1890) succeeded in growing pure culture of *chlorella vulgaris.*

Microalgae is a capable of performing photosynthesis, are important for life on earth, they produce approximately half of the atmospheric oxygen and use simultaneously the greenhouse gas carbon dioxide to grow photo autotrophically.

Microalgae have to date found a number of industrial applications like include formulation of food feed, cosmetics, health products and fertilizer and a tool for wastewater treatment. Microalgae have been recognized as a promising platform for biofuels production and bio

refiners. Microalgae have very high growth rates when compared with that of terrestrial plants, thereby demonstrating high CO_2 fixation efficiency and high biomass productivity.

CYANOBACTERIA: cyanobacteria are also known as cyanophytes, are the phylum of gram-negative bacteria. That obtain energy via photosynthesis. The name cyanobacteria refer to their color (from ancient Greek 'kuanos' blue). Giving them their other name blue-green algae. Cyanobacteria occurs in various environments including (fresh and marine water, oceans, hot springs), terrestrial environment (soil, deserts, and glacier), and symbiosis (with plants, lichens and primitive animals). They appear to have originated in freshwater or a terrestrial environment. Cyanobacteria are morphologically diverse, including unicellular and filamentous forms (branched and unbranched). some filamentous species produce specialized cells including heterocyst's, trichomes, hormogonia, and akinetes. As prokaryotes, cyanobacteria lack a nucleus and membrane-bound organelles. Cyanobacteria use photosynthesis pigment, such as **carotenoids**, **phycobilin's** and various form of **chlorophyll,** which absorb energy from light. A distinguishing feature of cyanobacteria is their photosynthetic pigment content. In addition to chlorophyll cyanobacterial thylakoids include phycobilin protein complexes, which give cyanobacteria their characteristic blue-green coloration.

Some cyanobacteria have been consumed by humans for centuries while other are known for their toxicity. The initial metabolic products of photosynthesis are sugar phosphate. Excess photosynthates in cyanobacteria are stored as polysaccharides (primarily glycogen) and may constitute up to 60% of the biomass. Cyanobacteria produce a limited number of sugar compounds.

Review of Literature

The objective of this report is to present a discussion of the literature review performed on methods of harvesting microalgae. There is no single best method of harvesting microalgae. The choice of preferable harvesting technology depends on algae species, growth medium, algae production, end product, and production cost benefit. Algae size is an important factor since low-cost filtration procedures are presently applicable only for harvesting fairly large microalgae. Small microalgae should be flocculated into larger bodies that can be harvested by one of the methods mentioned above. However, the cells' mobility affects the flocculation process, and addition of nonresidue oxidants to stop the mobility should be considered to aid flocculation. The decision between sedimentation or flotation methods depends on the density difference between the algae cell and the growth medium. For oil-laden algae with low cell density, flotation technologies should be considered. Moreover, oxygen release from algae cells and oxygen supersaturation conditions in growth medium support the use of flotation methods. If high-quality algae are to be produced for human consumption, continuous harvesting by solid ejecting or nozzle-type disc centrifuges is recommended. These centrifuges can easily be cleaned and sterilized. They are suitable for all types of microalgae, but their high operating costs should be compared with the benefits from their use. Another basic criterion for selecting the suitable harvesting procedure is the final algae paste concentration required for the next process. Solids requirements up to 30% can be attained by established dewatering processes. For more concentrated solids, drying methods are required. The various systems for algae drying differ both in the extent of capital investment and the energy requirements. Selection of the drying method depends on the scale of operation and the use for which the dried product is intended.

INTRUMENTATION

ANALYTICAL BALANCE

Make & Model: - Sari torus

An analytical balance is a weighing device used to measure and weigh even minute quantities with precision (up to 5 decimal). It can weigh in gram as well as milligrams. The device has slits on either side of the weighing platform to prevent entry of dust or unwanted particles in the weighing area.

Micropipette:

Micropipette are utilized in the laboratory to transfer small quantities of liquid, usually down to 0.1µL they are most commonly used in chemistry, biology, forensic, pharmaceuticals, and drug discovery labs, among others. Common pipette sizes used in labs includes Not only do micropipette differ in size and volume dispensed but depending on those particular aspects they also require specific pipett4e tips. Micropipettes use a disposable pipette tip to aspirate liquid, note that the tip is the only part of pipett4e that makes contact with the solution. A new tip is utilized for every sample in order to prevent cross contamination. The most essential aspects of pipette tips are its quality, if you are looking for a filter, low retention, gel loading tip, make sure that the pipette tip will perform accordingly and as precise as your micropipette. Make sure to research the purity of your pipette tip.

pH Meter:

The instrument gives an accurate determination of pH in any solution. The instrument consists of two electrodes, one glass electrode and other calomel electrode. Glass electrode is not absolute in its measurements and should be repeatedly standardized with solution of known pH.

Millipore Water Purification System:

It is an ultra-purification water system which provided the purest form of water for laboratory use. The device deploys a series of filtration & de-ionization processes to remove impurities and unwanted ions from water. It uses minute filters to remove impurities and ion exchange resins to exchange unwanted ions with H + & OH - ion. accuracies within parts per million (ppm).

Hot air oven

Make & Model: - JSGW

Hot air oven is drying equipment used to sterilized as well as dry various laboratory apparatuses. It works on the principle of dry heat sterilization where the articles to be sterilized or dried is subjected to temperature ranging from 50-300 o C. Since it can be operated at such high temperature, it is primarily used for glass and metal articles. A thermostat keep temperature within set limits while a fan fitted inside the oven ensures uniform distribution of heat.

Spectrophotometer:

Make and model: Labtronics (LT-2800)

Ultraviolet-visible spectrophotometry refers to the absorption spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near infrared (NIR) ranges. The absorption in the visible range directly affects the perceived colour of the chemical involved. In the region of the electromagnetic spectrum, molecules undergo electronic transition. This technique is complementary to fluorescence spectroscopy, in that florescence deal with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state. The Beer-Lambert Law state that the absorbance of a solution is directly proportional to the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/Vi's spectroscopy can be used to determine the concentration of the absorber in the solution. It is necessary to know how to

quickly the absorbance change with concentration. This can be taken from reference, or more accurately, determine from a calibration curve.

Autoclave:

Autoclave widely used in microbiology, medicine, podiatry, tattooing, body piercing, veterinary science, mycology, dentistry and prosthetics fabrication. They vary in size and function depending on the media to be sterilized. Typical load includes laboratory glassware, and other equipment and waste, surgical instruments and medical waste. A notable growing application of autoclave is the pre-disposable treatment and sterilization of waste materials such as pathogenic hospital waste. Autoclave is based on the same principle as conventional autoclave is that they are able to neutralize potentially infectious agents by utilizing pressurized steam and superheated water. A new generation of waste converter is capable of achieving the same effect without a pressure vessel to sterilize culture media, rubber material, gowns, dressing, gloves, etc. It is particularly useful for materials which cannot withstand the higher temperature of a hot air oven. Autoclaves are widely used to cure composite and in the vulcanization of rubber. The high heat and pressure that autoclaves allow help to ensure that the best possible physical properties are repeatedly attain.

heat to the surrounding. Insulation from outside create an isolated condition inside the cabinet, which allow the microbes grow effectively. Similarly, other parameters like humidity and airflow are also maintained through different mechanism that create an environment similar to the natural environment of the organisms. Similarly, they are provided with adjustments for maintaining the concentration of CO_2 to balance the pH and the humidity required for the growth of the organisms. Variation of the incubator like a shaking incubator also available, which allows

BOD Incubator:

Make and model: YSL440

Serial no.: - 08A0268

Incubator is an insulated and enclosed device that provide an optimal condition of temperature, humidity, and the other environmental condition requirement for growth of organisms. An incubator is a piece of vital laboratory equipment necessary for the cultivation of microorganisms under artificial conditions. An incubator can be used for the cultivation of both unicellular and multicellular organisms. An incubator is based on the principle that microorganisms require a particular set of parameters for their growth and development. In an incubator the thermostat maintains a constant temperature that can be read from the outside via the thermometer. The temperature is maintained by utilizing the heating and no-heating cycles. During the heating cycle, the thermostat heats the incubator, and during no-heating cycle, the heating is stopped, and the incubator cooled by radiating heat to the surrounding. Insulation from outside create an isolated condition inside the cabinet, which allow the microbes grow effectively. Similarly, other parameters like humidity and airflow are also maintained through different mechanism that create an environment similar to the natural environment of the organisms. Similarly, they are provided with adjustments for maintaining the concentration of CO 2 to balance the pH and the humidity required for the growth of the organisms.

Centrifuge:

A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a potentially strong face perpendicular to the axis of spin (outward). The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substances and particles to move outward in the radial direction. At the same time, objects that are less dense are displaced and move to the centre. In a laboratory centrifuge that uses sample tubes, the radial acceleration causes denser particles to settle to the bottom of the tube, while low density substances rise to the top.

Types;

- **Industrial scale centrifuge** - used in manufacturing and waste processing to sediment suspended solids, or to separate immiscible liquids.
- **High speed centrifuge** - provide very high accelerations to separate fine particles down to the nano-scale, and molecules of different masses.
- **Large centrifuge** - used to stimulate high gravity or acceleration environments.
- **Medium sized centrifuge** - used in washing machine. **Gas centrifuge -** used for isotope separation, such as to enrich nuclear fuel for fissile isotopes.

Hot Plate with Magnetic Stirrer:

Make & Model: Remi 5MLH PLUS

A hot plate with magnetic stirrer is a laboratory device that can heat a liquid at constant temperature as well as stir by causing a bar magnet to rotate within the liquid in presence of the rotating magnetic field. It is preferred over mechanical stirrers since it is not prone to wear & tear and doesn't interfere with the flow of liquid.

Vortex mixer:

Make and Model: - CM 101

It is a relatively simpler device which is used to decrease the time required to dissolve a substance in a liquid by creating a small vortex within the container of the liquid. The device consists of a rubber end which moves in a circular motion at very high speeds. When the container of a liquid comes in contact with it, the motion is transferred to it.

LAMINAR AIR FLOW (LAF):

A laminar air flow cabinet or tissue culture hood is a carefully enclosed bench designed to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive materials. Air is drawn through a HEPA filter and blown in a very smooth, laminar flow towards the user. Due to the direction of air flow, the sample is protected from the user but is not protected from the sample. The cabinet is usually made of stainless steel with no gaps or joints where spores might collect. Such hoods exist in both horizontal and vertical configurations, and there are many different types of cabinets with a variety of airflows patterns and acceptable uses. Laminar flow cabinet may have a UV-C germicidal lamp to sterilize the interior and content before usage to prevent contamination of the experiment. Germicidal lamps are usually kept on for 15 minutes to sterilize the interior before the cabinet is used. The light must be switched off when the cabinet being used, to limit exposure to the skin and eyes as stray UV light emissions can cause cancer and cataract.

Materials and Method:

SAMPLE COLLECTIONS:

Sample 1: ALGAL SAMPLE FROM SOIL AND WATER.

Microalgal samples were collected with the help of forceps, collecting spoon into the bottles. All the samples were collected from different locations and habitats some of them from biotech park, and some of them from other locations. Oscillatoria are found abundance in this sample, some diatoms are also found.

Sample 2: (from tank-1), **SAMPLE 3:** (Tank-2), **SAMPLE 2:1** (taking from lower surface of culture). **Sample 4:** (bottle sample).

Sample 5: (Wall sample). **Sample 6:** (Home Drainage), **Sample 7:** (Road side sample). **Sample8** (Animal Drinking water).

Location A

Location B

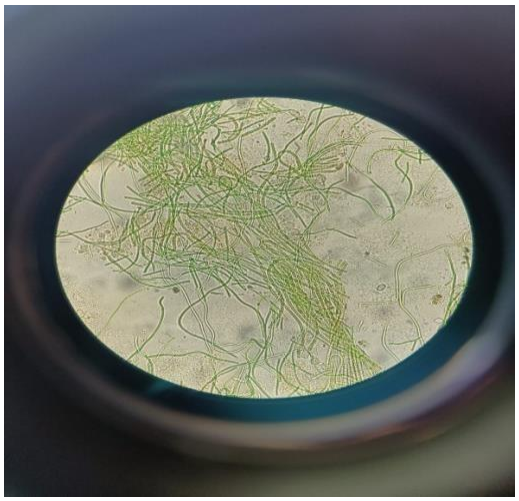
Location C

Location D

Location E

Location F

Microscopy of Collected algal samples: After collecting the sample micron microscopy were done form each sample. Sample 1, abundance of *Oscillatoria* and diatoms are also found. Sample 2, tank-1 found *phormedium* sp. and diatoms are formed. Sample 3, Tank-2, found diatoms and *Oscillatoria*. Sample 4, bottle sample, found chlorella. Sample 5, Wall sample, found Oscillatoria and abundance of *Nostoc* some other filamentous algae. Sample 6, Animal drinking, diatoms. Sample 7, Road side, *Oscillatoria*, abundance of *phormedium* sp. and *nostoc* formed, a smaller number of diatoms. Sample 8, home drainage, *Oscillatoria* and *phormedium* are found.



After the microscopy all the collected sample were poured into the test tube or flask in which BG11 media were also added, then these test tubes were kept into the B.O.D for incubation and algal growth from 10 to 15 days. When the appropriate growth of algae was found then

procedure for poring and plating was started. Making plates for spreading the sample first autoclave the petri dish and spreader for pouring and spreading. All these processes were done in the LAF (Laminar Air Flow). Laminar air flow chamber is utilized for creating aseptic environment in the laboratory conditions and have variety of uses.

MEDIA PREPRATION:

BG11 Medium supports the growth of photoautotrophic growth of blue green algae. They require light as a source of energy. And it was also universal medium for the cultivation and maintenance of cyanobacteria. For the composition of BG11 Medium add each chemical components in series so, that they appear here and ensure each is visually dissolved. Firstly, weigh each sample after that these sample were added to the volumetric flask and add distilled water in the flask and maintain the proper level. Shake it well. After that transfer the prepared media into the conical flask. And maintain the pH between (6.8 to 7.1).

Autoclave are also known as steam sterilizer, and are typically used for Laboratory or industrial applications. An autoclave is a machine that uses steam under pressure to kill harmful bacteria, viruses, fungi and spores on items that are placed inside a pressure vessel.

BG-11 MEDIA COMPOSITION:

COMPOUNDS	**Quantity**
$NaNO_3$	1.500 gm
K_2HPO_4	0.040 gm
$MgSO_4.7H_2O$	0.075 gm
$CaCl_2.2H_2O$	0.036 gm
Citric Acid	0.006 gm
Ferric Ammonium Citrate	0.006 gm
EDTA (Disodium Salt)	0.001 gm
$NaCO_3$	0.020 gm
Trace Metal Mix AS	1.000 mL
Agar	10.000gm
Distilled Water	1000 mL

Culturing Condition: To check the pH of the nutrient broth medium conical flask was taken in which 500 ml BG- 11 media was added the pH obtain was 6.8.

Culturing algal samples:

1. Firstly, 500ml of culture medium i.e., BG-11 was prepared in a 1000ml conical flask then 3 test tube were taken of 55 ml capacity in which 5 ml media was poured in each test tube and then inoculated with 1 ml of collected samples.

2. After inoculation was completed, flask was kept in BOD incubator for 12 days in order to get algal growth.

3. Specific photoperiods was maintained during the 12 days for proper growth of algae.

POURING AND SPREADING

Pour plate prepared by mixing the inoculum with the cooled but still molten medium before pouring the latter into the petri dish. Pour plate is a technique that allows quantifying the colony-forming units of bacteria in a sample.

Spread plate a technique used to count or isolate bacterial colonies on the surface of the agar.

Firstly, autoclave the BG11 media and let them little bit cool down. All these processes were done under LAF (Laminar Air Flow). Before starting the procedure to avoid the contamination in plates and media clean the Laminar Air Flow with ethanol wipe all over the surface. And wear mask and gloves for proper work. When plates are fix and solidified then by the help of pipette 1,2 drop of sample were poured into the plates. And, sample is spread on the solidified agar on a plate by the help of spreader. Spread at least 3 to 5 mints when all the sample was mix or absorb into the agar media then kept all these plates into the incubator for proper growth for 6 to 8 days. After the proper growth of algal sample again done microscopy, and makes 2 or 3 clean slides for observing or isolate the proper algae.

Plating

Spreading

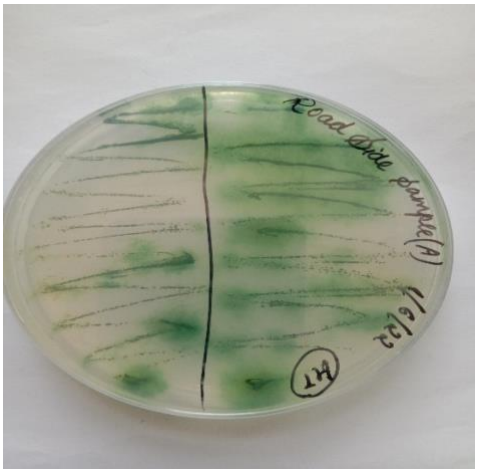
STREAKING:

Streaking plate technique is the process used to isolate a pure stain from single species of microorganisms. In this process microbiological culture can be grown on a new plate so, that the organism can be isolated, studied, or tested. For streaking poured the media into the plates, wait 15 to 20 mints for solidified. After that, sample was taken from the spreading plates take

single colony of algae and streak by the help of inoculating loop before streaking loop is first sterilized by passing it through the flame. The inoculation loop is then dragged across the of the agar back and forth in a zig zag motion until approximately 30% of the plate has been covered. All these processes were done under the Laminar Air Flow. After that all the streaking plates was kept into the incubator for algal growth. For 10 days. Then for observing specific algae make a clean slide form the streak plates and observe under the microscope. After observing in the microscope some of the algae are found like *Nostoc, Chlorella, Diatoms, Oscillatoria, Phormedium.* After inoculation of single algae form streaking plates poured algae in the media for proper biomass production.

Importance of Streaking:

Streak plate technique is used to grow algae on a growth media surface so that individual algae colonies are isolated and sampled. Isolated colonies indicate a clone of cells, being derived from a single precursor cell. When the selected culture media is inoculated using a single isolated colony, the resulting culture grows from that selected single clone, which involves the dilution of algae by systematically streaking them over the surface of the agar in a petri dish to obtain isolated colonies which will subsequently grow into mass of cells, or isolated colonies. If the agar surface grows microorganisms which are all the genetically same, the culture is then considered as a pure culture.



Isolation Of Algal Sample:

After 5-6 days the growth of algal sample was observed. All the growing algal sample were transferred into the test tubes for obtained a pure sample. isolated algae were *Nostoc, Chlorella, Scenedesmus, and Phormedium.*

Cultivation: For cultivation of the algae, suitable conditions are required. These conditions are given below-

- **Nutrient Medium:** For algal culture, nutrient medium is required. Nutrient medium or culture media is an artificial or synthetic medium in which algae can be grown. For media, we have used BG11, which is a universal medium for the cultivation and maintenance of green algae and blue green algae (cyanobacteria).

- **pH range**: A pH between 6.8-7.1 was needed for the algae growth (this must be noted that for different algae cultures may have different pH range). Complete culture collapse due to the disruption of many cellular processes can result from a failure to maintain an acceptable pH.

- **Temperature**: Most commonly cultured species of micro-algae tolerate temperatures between 16 and 27°C. Temperatures lower than 16°C will slow down growth, whereas those higher than 35°C, are lethal for a number of species.

- **Aeration/Mixing**: Mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients. Depending on the scale of the culture system, mixing is achieved by stirring daily by hand or vortexes. However, it should be noted that not all algal species can tolerate vigorous mixing.

- **Light: Micro-algae photosynthesize**, Light is the source of energy which drives this reaction and in this regard intensity, spectral quality and photoperiod need to be considered. Light intensity plays an important role, but the requirements vary greatly with the culture depth and

the density of the algal culture. Also, overheating due to both natural and artificial illumination should be avoided. Fluorescent tubes emitting either in the blue or the redlight spectrum should be preferred as these are the most active portions of the light spectrum for photosynthesis. The duration of artificial illumination should be minimum 14:10 of light per day, although cultivated phytoplankton develops normally under constant illumination.

Multiple Streaking:

Streaking is a technique used to isolate a pure stain from single species of microorganism. It is the rapid qualitative isolation methods. there are many different types of methods used to streak patterns, a three sector "T streak "and four- quadrant streak methods. Picking a technique is a matter of individual preference and can also depend on how large the number of microbes the sample contains. The three – phase streaking pattern is known as the T- streak. In the quadrant method, four equally sized sections are streaked.

Isolation of algal culture:

After the spreading and multiple streaking within 1 week the growth of algal sample were observed and these new developed algae colonies were transferred into the fresh culture for proper biomass production. Some of algae were isolated *like Chlorella, Nostoc, Oscillatoria, Phormedium*. For better result repeated culturing and selection was done. These algae can be further used as a renewable energy source in the form of biomass for the production of various bioproducts of microalgae.

Algal Culturing for Biomass Production:

1) **Materials:** Flask, Pipette, BG-11 Media, Algal Sample.

2) **Methods:** Take sample A and Sample B inoculate 5ml algal culture from each of them into flask. This process was done under laminar air flow to avoid the contamination. After that both flasks were kept into B.O.D Incubator for at least 10 to 15 days for proper algal growth. In between take O.D was measured with the help of UV spectrophotometer with the wavelength 680nm to check the algal growth rate

In the growth phase of algae come in decline phase then the algal biomass harvest for biochemical screening.

Serial No.	Sample	OD (680nm)
1	Bottle Sample *(chlorella*)	1.3835
2	Animal Drinking Water	0.4892

Algal Biomass Harvesting:

1) **Materials:** Pipette, Petri Plates, Falcon tube, Eppendorf tube, Centrifuge.

2) **Methods:** For harvesting the algal culturing centrifuge at 6500 rpm for 5 minutes in a falcon tube and remove the supernatant after that add 5 ml distilled water for washing and centrifuge it again for 5 minutes and after that remove the supernatant. Collect the wet biomass in the Eppendorf tube. For dry biomass pour the wet biomass in petri plate and kept in hot air oven at 40c after that collect in Eppendorf tube.

1) Carotenoid estimation:

a. Theory: Carotenoids are a class of isoprenoid synthesized by all photosynthetic organisms as well as by some non-photosynthetic bacteria and fungi with broad application in food, feed and cosmetics.

b. Materials: 2ml algal biomass, falcon tubes, 85% acetone, micropipette, centrifuge, ultrasonicates and UV-Vi's spectrophotometer.

c. Reagent preparation: 85% acetone (take 85ml acetone add 15 ml distilled water)

d. Method: Take 500 microlitre of algal biomass in a falcon tube and add 1.5ml of 85% and thawed for three to four times. After that ultrasonicate the mixture for three minutes. Centrifuge the mixture at 5000 rpm for 5 minutes and obtain the supernatant and transfer in

another falcon tube and make up the volume up to 5ml with 85% acetone. Take the absorbance at 450 nm with the help of a spectrophotometer against 85% acetone as blank.

2)Chlorophyll Estimation:

Theory: Chlorophyll A and its derivatives also have profound antioxidant properties. Chlorophyll is one of the valuable bioactive compounds that can be extracted from microalgae. Chlorophyll A is important for evaluation of growth and photosynthetic rate. Chlorophyll gives green colour to the plants and algae because it reflects the green wavelength found in sunlight while absorbing all the other colour.

Materials: 2ml algal biomass, falcon tube, test tube, 90% methanol, micropipette, centrifuge, ultrasonicate, UV-Vi's spectrophotometer.

Reagent preparation: 90% methanol (for 25ml take 22ml methanol and 2.5 ml distilled water).

Method: Take 500 microliter of algal biomass in a test tube and add 4ml of 90% methanol and then mix the solution with the help of a vortex mixer. Cover the mouth of the test tube with aluminium foil. Then keep the mixture in the ultrasonicate for 180sec and after that keep in a water bath for 30 min in 60c. The kept it in the sonicate for 180sec. After that add 1ml of 90% mixture in the solution. Centrifuge at 5000 rpm for 5 min and remove the supernatant. Take the absorbance at 645 nm, 665 nm and 663 nm with the help of a spectrophotometer.

Serial No.	**Sample**	**Wavelength 645nm**	**663nm**	**665nm**
1	A1	0.0730	0.1166	0.1178
2	C	0.0977	0.1252	0.1270
3	B	0.0781	0.0874	0.0882
4	F1	0.2500	0.3128	0.3145

5	E	0.0902	0.1305	0.1327

Phycobiliprotein:

1) **Theory:** Phycobiliprotein are a family of highly soluble and reasonably stable fluorescent proteins. These proteins contain covalently linked tetrapyrrole groups that play a biological role in collecting light and through fluorescence energy transfer conveying it to a special pair of chlorophyll molecules located in the photosynthetic reaction centre and also serve for nitrogen storage of cyanobacteria. **Phycocyanin, Phycoerythrin, Allophycocyanin** are the phycobiliprotein.
2) **Materials:** falcon tube, test tube, centrifuge, UV-Vi's spectrophotometer, phosphate buffer (pH=6.8).
3) **Reagent preparation:** Buffer preparation (Dissolve 0.43 gm of dipotassium hydrogen phosphate (K_2HPO_4) and 0.3gm of potassium dihydrogen phosphate (KH_2PO_4) in 50ml distilled water separately and set the pH to 6.8 by adding KH_2PO_4 to K_2HPO_4.
4) **Method:** Centrifuge 10ml of homogenized algal suspension at 6500 rpm for 10 minutes then subject the pellet to repeated freezing and thawing in 5ml of 0.05m phosphate buffer. Centrifuge the suspension to remove cell debris yielding a blue coloured supernatant. Take the absorbance at 562 nm, 615nm, 652 nm with the help of spectrophotometer.
5) **Formula:**

Serial No.	**Sample**	**Wavelength 562nm**	**615nm**	**652nm**
1	B	0.2196	0.2124	0.2089
2	C	0.2491	0.2422	0.2364
3	E	0.2205	0.2118	0.2074

4	F1	0.2602	0.2502	0.2439
5	A1	0.1314	0.1285	0.1267

1. **Carbohydrate:**
2. **Theory:** A carbohydrate shell or a protective carbohydrate coat is a common cyanobacterial tactic for self-defence against both the environment and other organisms. These layers provide mechanical protection and define the cell shape. Carbohydrates are first hydrolysed into simple sugars using dilute HCL acid medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 620 nm.
3. **Materials:** falcon tube, test tube, centrifuge, UV-Vi's spectrophotometer, anthrone reagent.
4. **Reagent preparation:** Anthrone reagent (add 0.1g of anthrone reagent in 85% H_2SO_4 (conc.) and kept in cool and dark place).
5. **Method:** Prepare the 2.5 normal HCL and standard of glucose 10, 20,40, 60,80 mg/litre. Take 2mg dry biomass in the test tube and add 1.6% of 2.5 normal HCL in the test tube and keep in a water bath at 60-70c for 2 hrs. After that add sodium carbonate in the test tube till the effervescence is not seen. Add distilled water to make the volume up to 5ml. Centrifuge at 4000rpm for 3-5 min. Take 1 ml supernatant in a fresh test tube. Take 1ml in the test tube from standard and add 2ml of anthrone reagent in supernatant and in each test tube of standard. Keep all the test tube at 90^oc for 15 min it turns green in colour then check the absorbance at 620 nm. For blank take 1ml of distilled water in the test tube add 3 ml of anthrone reagent.
6. **Reagent preparation:** Anthrone reagent (add 0.1g of anthrone reagent in 85% H_2SO_4 (conc.) and kept in cool and dark place).

RESULT AND DISSCUSION:

1. CHLOROPHYLL CONTENT

In chlorophyll there are two main chlorophyll A and chlorophyll B. In chlorophyll a being a green/yellow with maximum absorbance from 642 to 663 nm. Table 1 shows the result and after apply the following formula:

In the study it was found chlorophyll A content in pH 6 more than pH 8. Chlorophyll B content in pH 6 more than ph8. It shows pH6 growth condition more suitable than pH 8**.**

- **CAROTENOID CONTENT:**

  Carotenoid is also a fat-soluble molecule and can be extracted from thylakoid membranes with organic solvents such as methanol. The maximum absorbance at 450 nm. The table 2 shows the result and after apply the following formula:
  In this study it was found carotenoid content in pH 6 more than pH 8. It shows pH 6 growth condition could be more suitable than pH 8.

D= OD at 450 nm
V= Volume of extract (after centrifuge)
F= Dilution factor
2500= Average extinction of coefficient at pigment is 2500
D= FINAL VOLUME/INITIAL VOLUME
Final volume= 5ml
PH=6 initial volume = 2.1
PH=8 initial volume = 2.3
PH=6 D= 5ml/2.1ml = 2.38
(0.3715*2.1*2.38) *10/2500 = 0.0074mg/ml
PH=8 D= 5ml/2.3 = 2.17
(0.3529*2.3*2.17) *10/2500 = 0.0070mg/ml

Table- 1

SAMPLE	**450nm**
PH=6	**0.3715**
PH=8	**0.3529**

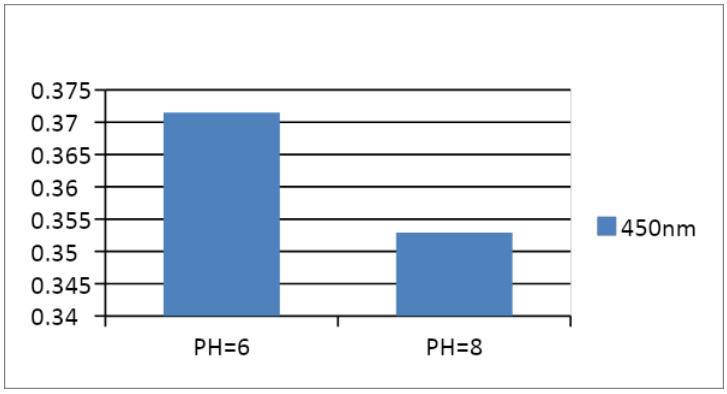


Fig 2

- **PHYCOBILIN CONTENT:**

The phycobiliproteins are present as phycobilisomes anchored on the thylakoid membrane lie adjacent to the photosynthetic reaction centre of PS II in microalgae. Extraction was done using a phosphate buffer. The major absorbance is 650nm-655nm and 610 nm-620nm. Phycoerythrin is a red coloured phycobiliprotein with absorption maxima range 562 nm. The following equations are proposed for correct calculations of pigment concentrations. The table 3 shows the result and after apply the following formula:

PC = (OD615– 0.476 * OD652)/5.34
APC = (OD652 – 0.208*OD615)/5.09
PE = (OD 562 – (2.41*PC) – (0.849*APC))/9.62
For PH=6 (PC) = (0.1085 – 0.4761*0.0937)/5.34
= 0.1001mg/ml
(APC) = (0.0937 – 0.208*0.1085)/5.09
= 0.0892mg/ml
(PL) = [0.1102 – (2.41*0.1001) – (0.849*0.0892)]
= (- 0.2067)
For PH=8 (PC) = (0.1633 – 0.4761*0.1017)/5.34
= 0.1148
(APC) = (0.10187 – 0.4761*0.1633)/5.09
= 0.0864
(PL) = [0.1314 – (2.41*0.1148) – (0.849*0.0864)]
= (-0.8776)

In this study it was found that phycobiliprotein content in pH 8 is more than pH 6 because of the elevations in stress level and production of pigments. It shows pH 8 growth conditions could be more suitable than pH 6. (Fig 3)

Table-3

SAMPLE	562 nm	615 nm	652 nm
PH=6	0.1102	0.1035	0.0937
PH=8	0.1314	0.1633	0.1017

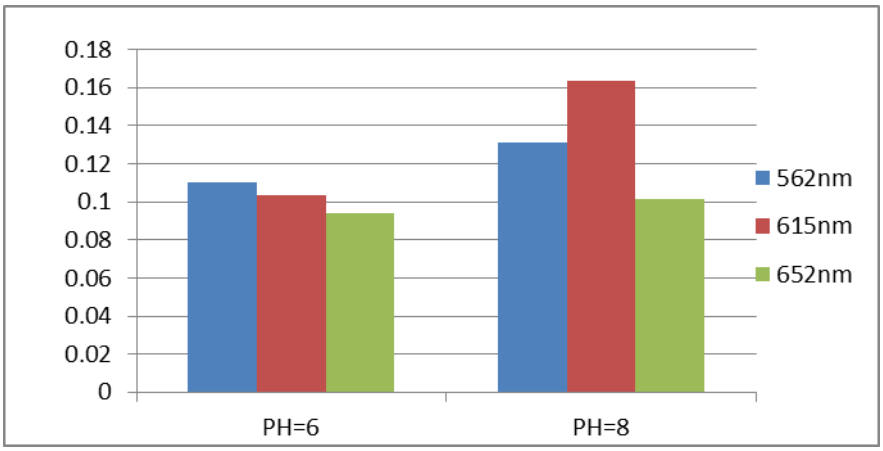


Fig 3

1. CARBOHYDRATE CONTENT:

Carbohydrates are first hydrolysed into simple sugars using dilute HCL acid medium glucose is dehydrated to hydroxymethyl furfural. The major absorbance at 620 nm. Carbohydrate one of the energy biomolecules that assimilate during the photosynthesis process. In this study it was found carbohydrate content in pH 8 more than pH 6. It shows pH 8 growth conditions could be more suitable than pH 6.

Table -5

SAMPLE	620 nm
PH=6	0.3523
PH=8	0.9954

Carbohydrate calibration table:

STANDARDS	620 nm
10ppm	0.6346
20ppm	0.8286
40ppm	1.1379
60ppm	1.1749
80ppm	1.5822

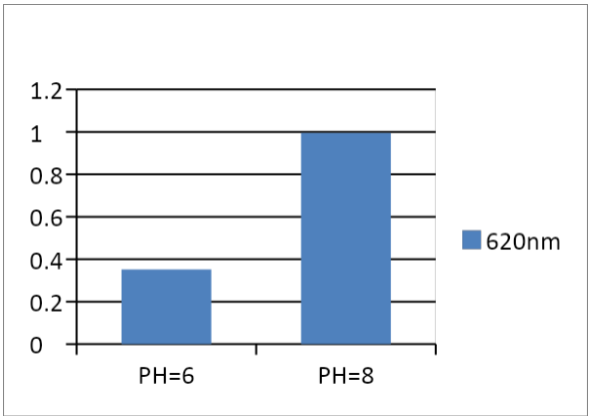


Fig 4 carbohydrate

CONCLUSION

Microalgae are industrially valuable microorganism, especially in the production of biodiesel feedstock. However, there are several biological and culture system limitations that result in low productivity of microalgae cultures. Hence there is an urgent need to enhance the yield and productivity of microalgae mass cultures. With the advancement of molecular biology and engineering, these limitations can now be better understood and overcome through approaches such as genetic engineering and culture system design. the limitations in productivity of microalgae and the drawbacks of bioprocessing technologies render the fully utilization of microalgae biomass to be impractical. Therefore, more work needs to be done to further improve the existing technology. For instance, more advanced culturing technique should be developed to increase the productivity of microalgae, and novel biotechnology such as gene editing can be attempted to increase the output of bioactive compound from the microalgae strain.

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