A Dissertation on

Collection, isolation and purification of cyanobacteria and their biochemical characterization

Submitted to the

Department of Biosciences

Integral University, Lucknow

In Partial fulfillment

For degree

of Master of Science

In Microbiology

Submitted by

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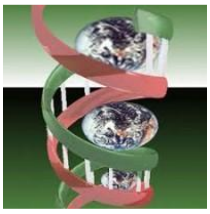
Enrollment no. 2000101416

M.Sc. Microbiology (IV Sem)



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Biotech Park in Biotechnology City, Lucknow

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CERTIFICATE

This certificate is issued to **Ms. Zia Naaz, D/o Mr. Abdul Mabood** on successful completion of an advanced Training Course/Project Work at Biotech Park, Lucknow.

Registration No./ Receipt No	:	**Offline/9520**
Sponsoring Institution	:	**Integral University**
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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 to certify that Miss. Zia Naaj a student of MSc. 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 completed this work from 2 March to 25 June 2022 at the Biotech Park, Lucknow, under the guidance of Shashank Mishra. The dissertation was a compulsory part of her M.Sc. degree.

I wish her good luck and a bright future.

Dr. Snober S Mir
Head of Department of Biosciences
Integral University, Lucknow

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DECLARATION

I, the undersigned, hereby declare that the project entitle.is a record of an original work done by me during my three months of training, from 2 March 2022 to 24 June 2022 at Biotech Park, Lucknow. The training has been carried out under the guidance of **Manish Pandey** , Biotech Park, Lucknow. I also declare that no part of this report has previously been submitted to any university or any examining body for acquiring any diploma or degree.

Zia Naaj

ACKNOWLEDGEMENT

First of all, I bow in reverence to the Almighty for blessing me with strong willpower, patience, and confidence, which helped me in completing the present work.

I would like to express my special thanks to Dr. Snober S. Mir (Head, Department of Biosciences) for allowing me to join the department laboratory and for providing all the necessary facilities ever since I started my work.I am thankful and express my sincere and deepest gratitude and deep regards to **A.K SINGH (CEO)** ,Biotech Park ,Lucknow for givimg me opportunity to pursue 120 days training dissertation in the field of analytical techniques in this reputed institute. I would like to express my sincere thanks to my training supervisor **Dr Shashank Mishra** Scientist 'C' and **Mr Manish K. Pandey** (Technical Manager) Biotech Park,for his resolute guidance, abiding interest, constructivecriticism, tremendous enthusiasm ad meticulous supervision throughout the period of my training.

Finally, I take this opportunity to extend my drop appreciation to my family and friends, for all that they meant to me during the crucial times of the completion of this training. Their unwavering faith and confidence in us have shaped our personality.

Zia Naaj

Microbiology

ABSTRACT

Microalgae have several applications in nutraceuticals , pharmaceuticals , cosmetics , biofuel production and bioremediation , along other fields . isolationand purification are extremely important for obtaining axenic cultures of microalgae from different environments and crucial for their biotechnological applications , but it is not an easy task . in view of the above , it is fundamental to know the classical and advanced techniques and examples of how scientists from around the globe have applied such methods to isolate several genera and the impact of each step on successful algal purification .this review provides a brief and simple explanation of the methodology for sampling , growth , obtention of unialgal , and posterior axenic culture , whichwill facilitate the development of novel microalgae – related discoveries and applications for new researchers .

The report provides a brief and simple explanation of the methodology for sampling, purification, isolation and growth of algae. The algae is 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 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.

ABOUT BIOTECH PARK

The biotechnology park has been setup on 8 acres of land provided by the Department of Science andtechnology, Government of Uttar Pradesh. The thrust areas identified for the initial phase of the Parkare: Health Care-Human and animalhealth care products including, immunodiagnostics, biosensors,vaccines, gene therapy, monoclonal antibodies, nutraceuticals and cosmeceuticals. Agriculture – Improvement in the quality and yield of crops, horticulture and forest tree species, biopesticides andbiofertilizers, processed food and quality enhancers; Environment Bioremediation, safe disposal ofwaste; Industrial Application-Plants as bioreactors, enzymes, chemicals and polymers and EnergyBiofuels and renewable energy sources.

Molecular biology & Analytical Facility:

The sophisticated analytical testing facility is located in an area 2280 sq. ftTheanalytical Quality. Assurance and Control Laboratory is NABL accredited and meet ISO 9000 requirements. The facilityi equipped with High Pressure Liquid Chromatography (HPLC), Nanodrop–spectrophotomete ,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 IonChromatography detectors. A wide range of metabolites or molecules can be quantitatively analysedusing 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 systemfor extraction of photochemical / lead

molecules from high value medicinal plants. Multipurposereaction cum hydrolysisand solvent recovery unit along with chromatography.

Distillation unit:

• Feature

•Improved design

•All contact parts of stainless steel

•Oilyield is more than convention

•Low oil loss

•Utilizes spent marc/agro waste as fuel

•Environmental friendlyCapacity:1000 kg fresh herbs/batchDistillation time: Onebatch 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, wasoriginally setup in November 2001, at Indian Institute of Toxicology Research (IITR), Lucknow, as anetworking site, the centre was moved to Biotech Park in 2007. Bioinformatics Centre has been setupto establish a close network with various institutio and provide information to industries regardingtechnology, facilities and expertise available with them in the area of biotechnology city.Biotechnology City Map, Biology Institutions in the City, Advanced Research Facilities, ResourcesPersons Biotechnology Products andProcesses available and being developed, Patenting ofBiotechnology Product/Processes, Biotechnology Tutorial.

Tissue culture: Tissue culture facility at Biotech Park, Lucknow is spread over 4071 sq. ft. area having the capacity toraise and multiply Banana, Potato, Jatropha seedlings. The facility has a modern Polycarbonate housefor 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 isdifficult.

CAPACITY :

10000 to 100000 plants / batch and it can produce about 2 million 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 plantscan alsobe multiplied in large scale during off season. The value crops like Geranium, Patchouli, Pyrethrumcould be saved during rainy season when the climate is hot and humid.

Advantages

•It is maintained under the guidance of trained technical staff•CompartmentalizedGlass 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 mothers perglass house.

Introduction

Algae is the live form of photosynthetic plant. Algae are chlorophyll-bearing, simple, thalloid autotrophic and microscopic organisms found in both seawater andfreshwater. They occur in a variety of other habitats –moist stones, soils and wood.Some of them also occur in association with fungi (lichen) and animals (slot bear) (Spolaore et al 2006).

Algae are useful to man in a variety of ways. At least a half of the total carbon dioxide fixation on earth is carried out by algae through photosynthesis. Being photosynthetic they increase the level of dissolved oxygen in their immediateenvironment. The first use of microalgae by human dates back 200 years to the Chinese, who used Nostoc survive during famine. Nowadays commercial application of microalgae isused to enhance the nutritional valve of food and animal feed owing to their chemical composition, they also play crucial role in aquaculture and they can also be incorporated into cosmetics. The microalgae beingthe safe non-competitive and rapidly growing organism. Also, they have the propertyof removing toxic components from wastewater treatment. Microalgae is capable of adapting new environments and it can grow at any condition ,also exhibit high growth rate. Growth curve has been developed for the study of microalgae growth in natural habitat. In the lag phase, the growth is delayed due to the presence of non-viable cells or physiological adjustments in new environments. This is followed by an exponential phase, where cells grow and divide rapidly. Microalgae possess different photosynthetic pigment of algae like chlorophyll, carotenoids and Phycobiliproteins significant commercial applications. Chlorophyll are the primary photosynthesis pigments present in different foams.Chl-a is generally used as an index of phytoplankton biomass. Green microalgae exhibit about 6.7 % of chlorophyll content. Carotenoids are naturally occurring terpenoid pigments and also being used as a pigments of microalgae and classified into phycoerythrin, phycocyanin, allophycocyanin B and apparently occur in most cyanobacteria. The recovery of microalgae biomass is considered to have a major influence on the economy of microalgae product in . When algal is in different condition there physiological, biological and chemical condition changes and we have to calculate its different parametes in acidic and basic condition .Microalgae ormicrophytes are microscopic algae invisible to the naked eye. They arephytoplankton typically found in the freshwater and marine and sediment. They are

unicellular species which column and sediment are unicellular species which exist individually , or in chains or groups.

Type : Regarding the energy and carbon sources used for their metabolism , microalgae can grow in three different ways : **autotrophy , heterotrophy , and mixotrophy .**

Microalgae play a major role in nutrient cycling and **fixing inorganic carbon** into organic molecules and expressing **oxygen** in marine **biosphere .**

Examples of prokaryotic microorganisms are Cyanobacteria (Cyanophyceae) and eukaryotic microalgae are for example **green algae** (Chlorophyta) and **diatoms** (Bacillariophyta).

CYANOBACTERIA :- Domain – Bacteria

Phylum – Cyanobacteria

Class - Cyanophyceae

Cyanobacteria alsp known as **Cyanophyta ,** are a phylum of gram negative bacteria that obtain energy via photosynthesis . The name cyanobateria refers to their color giving them their another name , **'' blue – green algae''**, though modern botanists restrict the term algae to eukaryotes and do not apply to cyanobacteria , which are prokaryotes .

Cyanobacteria ranges in size from 0.5 to 60 micrometres , which represents the largest prokaryotes organism. They are widely distributed and extremely common in fresh water , where they occur as members of both the plankton and the benthes.

Uses :A Range of mirolagae species are produced in hatcheries and are used in a variety of ways for commercial purposes , including for **human nutrition , as biofuel , in the aquaculture of other organisms, in the manufacture of pharmaceuticals in cosmetics , and as biofertiliser.**

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 nonresidual 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 the drying method depends on the scale of operation and the use for which the dried product is intend.

INSTRUMENTATION

Analytical Balance

& Make Model: - Sari torusAn analytical balance is a weighing device used to measure and weigh even minutequantities with precision (up to 5 decimal). It can weigh in gram as well as milligrams. Thedevice has slits on either side of theweighing platform to prevent entry of dust or unwantedparticles in the weighing area.

Micropipette :

Make and model: -Micropipette are utilized in the laboratory to transfer small quantities of liquid, usually downto 0.1µL they are most commonly used in chemistry, biology, forensic, pharmaceuticals, and drug discovery labs, among others. Common pipette sizes used in labs includesNot only do micropipette differ in size and volume dispensed but depending on thoseparticular aspects they also require specific pipett4e tips. Micropipettes use a disposablepipette tip to aspirate liquid, note that the tip is the only part of pipett4e that makes contactwith the solution. A new tip is utilized for every sample in order to prevent crosscontamination.The most essential aspects of pipette tips is its quality, if you are looking for a filter, lower tention, gel loading tip, make sure that the pipette tipwill perform accordingly and asprecise as your micropipette. Make sure to researchthe purity of your pipette tip.

pH Meter:

Make and model: - The instrument gives an accurate determination of pH in any solution. The instrument consistof two electrodes, one glass electrode andother calomel electrode. Glass electrode is not a bsolute in its measurements and should be repeatedly standardized with solution of known pH.

.

.Millipore Water Purification System:

Make & Model: -

It is an ultra-purification water system which provided the purest form of water for laboratoryuse. The device deploys a series of filtration & de-ionization processes to remove impuritiesand unwanted ions from water. It uses minute filtersto remove impurities and ion exchangers in 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 laboratoryapparatuses. It works on the principle of dry heat sterilization where the articles to besterilized or dried is subjected to temperature ranging from 50-300 o
C. Since it can beoperated at such high temperature, it is primarily used for glass and metal articles. A thermostat t keep temperature within set limits while a fan fitted inside the oven ensuresuniform distribution of heat.

Spectrophotometer:

Make and model: Labtronics (LT-28000) 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 nearinfrared (NIR) ranges. The absorption in the visible range directly affect ts the perceivedcolour of the chemical involved. In the region of the electromagnetic spectrum, moleculesundergo electronic transition. This technique is complementary to

fluorescence spectroscopy,in that florescence deal with transitions from the excitedstate to the ground state, whileabsorption measures transitions from the groundstate to the excited state. The Beer-LambertLaw state that the absorbance of a solution is directly proportional to the absorbing species inthe solution and the path length. Thus, for a fixed path length, UV/Vis spectroscopy can beused to determine

. the concentration of the absorber in the solution. It is necessary to knowhow to quickly the absorbance change with concentration. This can be taken from reference,or more accurately, determine from a calibration curve.

Autoclave :-

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

BOD Incubator

Make and model: YSL440Serial no.:08A0268Incubator is an insulated and enclosed device that provide an optimal condition of temperature, humidity, and theother environmental condition requirement for growth of organisms. An incubatoris 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 theheating cycle, the thermosta heats the incubator, and during no-heating cycle, the heating is stopped, and the incubatoror cooled by radiating heat to the surrounding. Insulation from out screateseate an isolated condition inside the cabinet, which allow the microbes grow effectively. Similarly,other parameters like humidity and airflow are also maintained through differ mechanism that create an environment similar to the natural environment of the organism Similaryl,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 likea shaking incubator also available, which allows for the continuous movement of the culturerequired for cell aeration and solubility studies.

Centrifuge:

Make and Model:

A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis (spinsit 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 displacedand 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 sedimentsuspended solids, or to separate immiscible liquids

.•High speed centrifuge-provide very high accelerations to separate fine particles down to thenano-scale, and molecules of different masses

.•Large centrifuge-used to stimulate high gravity or ac acceleration environments

.•Medium sized centrifuge-used in washing machine

.•Gascentrifuge-used for isotope separation, such as to enrich nuclear fuel forfissile isotopes.

Hot Plate with Magnetic Stirrer

Make & Model:

Remi 5MLH PLUSA 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 ofthe rotating magnetic field. It is preferred over mechanical stirrers since it is not prone to wear& tear and doesn'tinterfere with the flow of liquid .

Vortex mixer:

Make and Model: -

CM 101It is a relatively simpler device which is used to decrease the time required to dissolve asubstance in a liquid by creating a small vortex within the container of the liquid. The deviceconsists of a rubber end which moves in a circular motion at very high speeds. When thecontainer of a liquid comes in contact with it, the motion is transferred to it .

Laminar Air Flow Cabinet : - A Laminar air flow cabinet or tissue culture hoodis 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 the useris not protected from the sample . The cabinet is usually made stainless steel with no gaps or joints where spores might collected .Such hoods exist in both horizontaland vertical configurations, and there are many different types of cabinets with a variety of airflow patterns and acceptable uses. Laminar air flow cabinet may have a UV – C germicidal lamp t sterilize the interior and contents before usage to prevent contamination of the experiment . Germicidal lamps are usually kept on for15 minutes to sterilize the interior before the cabinet is used . The light must be switched off when the cabinet is being used,to limit exposure to skin and eyes as stray UV emission can cause cancer and cataracts.

MATERIALS AND METHODS

Sample Collection: -Algal samples werecollected from soil and water at Biotech park area where we have collected4 samples from. 1 Water canal (sample 1), 2. From water pump(sample 2) and, 3. From tank (tank 1) and 4. Tank 2 upper portion of tank (tank 2) , 5 . another from bottle sample .3 more samples from places,Animal drinking , Wall sample Home drainage , Road side sample .

Sample pictures :

Microscopy: -The algal cells were observed under optical microscope for their morphological

features and other cellular details .

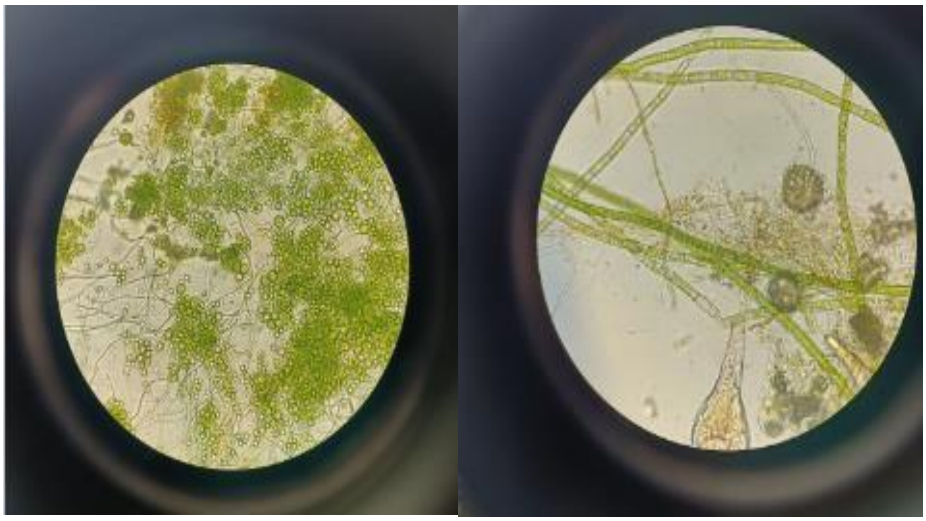
1. sample i.e, water canal we observed abundance of diatoms .

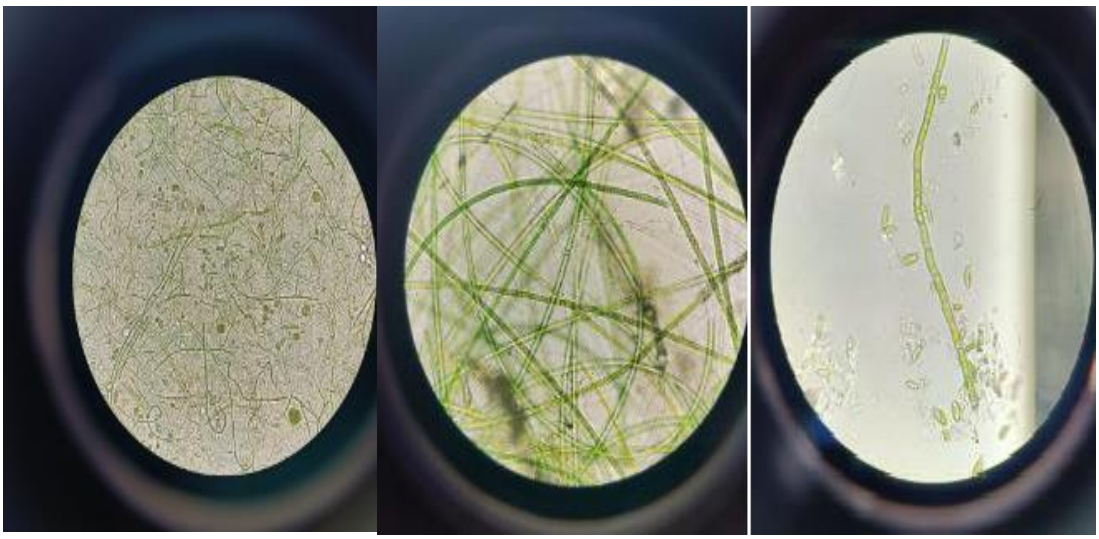
2. sample i.e, water pump – we observed *phormidium* , *oscillatoria* , and *chlorella.*

3. sample i.e, tank 1 – *phormidium, chlorella , diatoms, scenedesmus* .

4. sample i.e, tank 2 – abundance of*oscillatoria* .

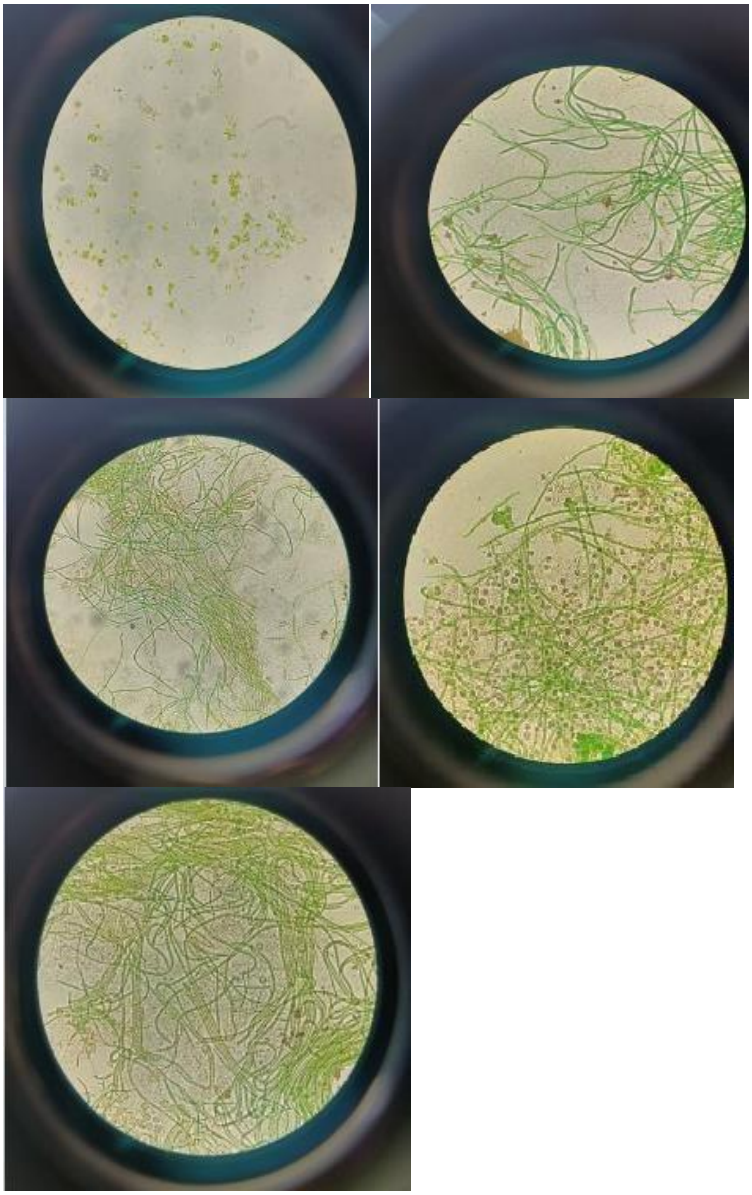
sample i.e, bottle sample – abundance of *chlorella vulgar*





New samples –

1. Animal drinking water – Diatoms , abundance of *green filamentous*, andabundance of *neochlrosis* , less amount of *Nostoc* .
2. Wall sample – abundance of N*ostoc*, oscillitoria , and *filamentous algae* .
3. Home drainage- *oscillitoria* and *phormedium*, less amount of *diatoms*.



MEDIA PREPARATION

There are different types of media used for the culturing of algae. **BG 11 media** :- In the conical flask we put distilled water after that the all Chemical composition were weight according to their compositions serial wise ,then the chemical compositionwere put into the volumetric flask and shake well , Weigh the BG-11 composition accurately and dissolve in 1litre distilled water and and maintain the PH of the media( ) and after that autoclave the media to avoid the contamination. (BG11 broth is a universal medium for the cultivation and maintainance of blue green algae). Sterilize by autoclaving at 121 ° C for 15 psi pressure for 30 to 45 minutes . Cool the medium to room temperature. For maintenance of blue green algae exposure for period of 24 hours a day is optimal . The media was appeared off white to cream solution . Cultural characteristics observed after an incubation at20 -25 °C for 1 week .Label a clean glass 500 ml ,autoclavable bottle with media name ,date ,and initial.Add distilled water to the bottle accordingly.Add 3.3g of media with agar powder to the same bottle (For 250 ml).Add a stir bar (optional) .Stir or shake until fully mixed and check that there are no lumps.Add a piece of autoclave tape to the cap or bottle and loosen the cap a half – turn. Ifusing a container with no cap , then cover loosely with aluminum foil.

BG 11 Composition :-

COMPONDS	1 LITRE
$NaNO_3$	1.500gm
K_2HPO_4	0.040gm
$MgSO_4.7H_2O$	0.075gm
$CaCl_2.2H_2O$	0.036gm
Citric Acid	0.006gm
Ferric ammonium Citrate	0.006gm

EDTA(Disodium salt)	0.01gm
$NaCO_3$	0.020gm
Trace Metal Mix AS	1.000 ml
Agar	10.000gm
Distilled water	1000ml

BOD Culture – In conical flask 100ml media was poured and sample were addedafter that flask were put into the incubator for one week.

Note: shake in every 1 hour.

Culturing conditions:

To check the pH of the nutrient broth medium conical flask was taken in which500 ml BG-11 media was added The pH obtained was 6.8 .

Culturing algal samples:

1. Primarily, 500ml of culture medium i..e BG-11 was prepared in a 1000ml conicalflask then 4 test tube were taken of 55 ml capacity in which 5 ml medium was poured in each test tube and then inoculated with 1 ml of collected samples.
2. After inoculation, 55ml flask were kept in BOD incubator for 12 days for propergrowth of algae.
3. Specific photoperiods was maintained during the 12 days for proper growth ofalgae.

- **Pouring of media and plating**

  Agar is derived from red algae which is a polysaccharides. The agar powder is first dissolvedin a boiling liquid, and then cooled to form a gelatinous solid matrix. As microbes cannotdigest agar, this material is used commonly in laboratories to hold the nutrients that is needed.An autoclave is a high- pressure apparatus that is used in laboratoriesto sterilize equipment, instruments, glassware, growth media, liquids and biohazardous waste.The autoclave applies high pressure (15 psi) and saturated steam at 121oC (250oF) for 30-40minutes to kill microbes and spores. After the media has gone through this cycle, it is sterile.Cool to 60-65oC before adding any antibiotics and pouring into sterile Petri dishes.

Sterilizing the workspace :

To avoid contamination of the media and plates , a lab coat and gloves , and use a disinfecting agent or wipes to wipe down all surfaces.This includestabletops and edges , gloves , scissors, permanent markers , etc .Make sure to clean your gloves if they have touched another surface that is not disinfected (e.g , if you touched face , chair , arm etc.), Once the area is disinfected , bring out the sterile petri dishes . Keep the sterile dishes closed

.

Pouring the Plates

- Once the media is cooled to 60° C , the liquid solution is ready to bepoured.
- Uncap the media bottle and hold in your dominant hand **. Note** : oncethe bottle is opened , do not talk . Talking will allow few bacteria from your mouth to become airborne and may contaminate the media .
- The cap can be held in the same hand (between fingers) as yourbottle or can be placed on a disinfected surface .
- Grab a plate with your other hand and slide it towards the edge ofthe table, while keeping it closed .
- Poured the media into the bottom of the plate until it just covers thesurface . Do not over fill.
- Close the lid and allow to cool . The media will solidify . It will take 10 to15 minutes .
- Label the plates with the type of media , date produced , and name of theindividual that produced the stack.
- Stored sealed stacks in the refrigerator until use .
- Make sure all this process is done under laminar flow to prevent contamination.

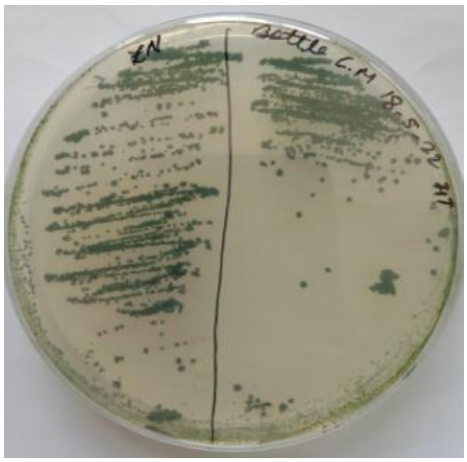

- **Spreading**

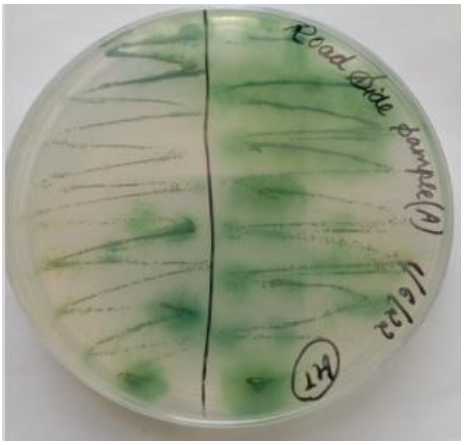
  Spread plates are simply microbes spread on a media plate. Microbes are ina solution, and can be diluted. They are then transferred to a petri dish with media specific for the growth of the microbe of interest. The solution is poured in the from of droplets through pipette then spread uniformly by spreader, spreading the microbe-containing liquid evenly on the plate. The spreader is sterilized and used to spread the microbe-containing liquid uniformly on the plate. After spreading the plates are kept in BOD for 7-10 days for growth.


Streaking

In microbiology, streaking is a technique used to isolate a pure strain from a singlespecies ofmicroorganism. Samples can then be taken from the resulting colonies and a microbiologicalculture can be grown on a new plate so that the organism can be identified,studied, or tested.The streaking is done using a sterile tool, such as a cotton swab or commonly an inoculationloop. This is dipped in an inoculum such as a broth or patient specimen containing manyspecies of bacteria.The sample is spread across one quadrant of a Petri dish containing agrowth medium, usually an agar plate which has been sterilized. Choice of which grow medium is used depends on which microorganism is being cultured or selected for. It isagain kept in BOD for growth for 7-10 days.

Importances of streaking : Streak plate technique is used to grow algae on a growth media surface so that individual algae colonies are isolated andsampled. 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 surfacegrows microorganisms which are all the genetically same, the culture is thenconsidered as a pure culture. The commonly used petri dishes are of hundred millimetre diameter. The agar surface of the plate should be dry without any moisture such as condensation drops. In the streaking procedure, a sterile loop or swab is used to obtain an uncontaminated microbial culture. The process is called "picking colonies" when it is done from an agar plate with isolated colonies and is transferred to a new agar or gelatin plate using a sterile loop or needle. The inoculating loop or needle is then streaked over an agar surface. On the initial region of the streak, many microorganisms are deposited resulting in confluent growth or the growth of culture over the entire surface of the streaked area. There are two most commonly used streak patterns, a three sector "T streak " and a four quadrant streak methods.





Isolation of algal sample :

After 5 days, the algae growth could be observed. The developed algal colonies were transferred to culture tubes to obtain pure culture. The desired algae which were Chlorella, Scenedesmus, nostocs, and phormediumwere isolated.

Cultivation:

For cultivation of the algae, suitable conditions are required. These conditions aregiven 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 begrown. 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.

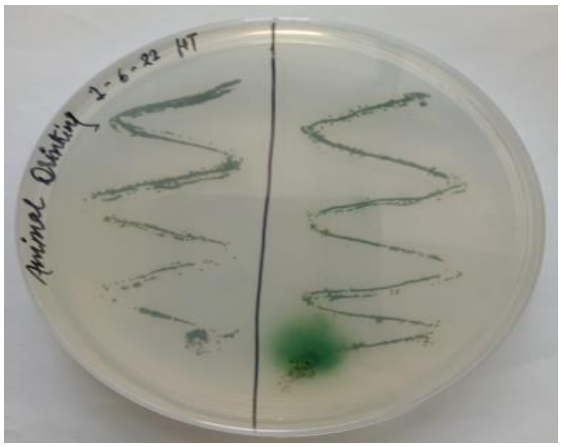
Temperature: Most commonly cultured species of micro-algae tolerate temperatures between 16 and 27°C. Temperatures lower than 16°C will slow downgrowth, whereas those higher than 35°C, are lethal for a number of species.

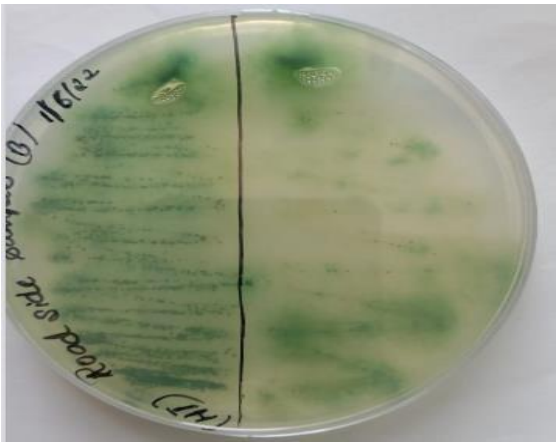
Aeration/Mixing: Mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients.Depending on the scale of the culture system, mixing is achieved by stirring daily by hand or vortexes. However, it should be noted that 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 tobe 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 red light 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 underconstant illumination.

Streaking / multistreaking (sub-culturing) :

Spreading and Streaking: The common microbiological techniques of isolation by spreading, streaking and pouring agar plate were used to obtain the unialgal cultureof the desired species of algae. The algal plates were put in BOD incubator under suitableconditions of temperature and artificial illumination (photoperiod of 14:10 at 20°C of temperature). The growth can be observed in 5-6 days.





Isolation of algal culture :

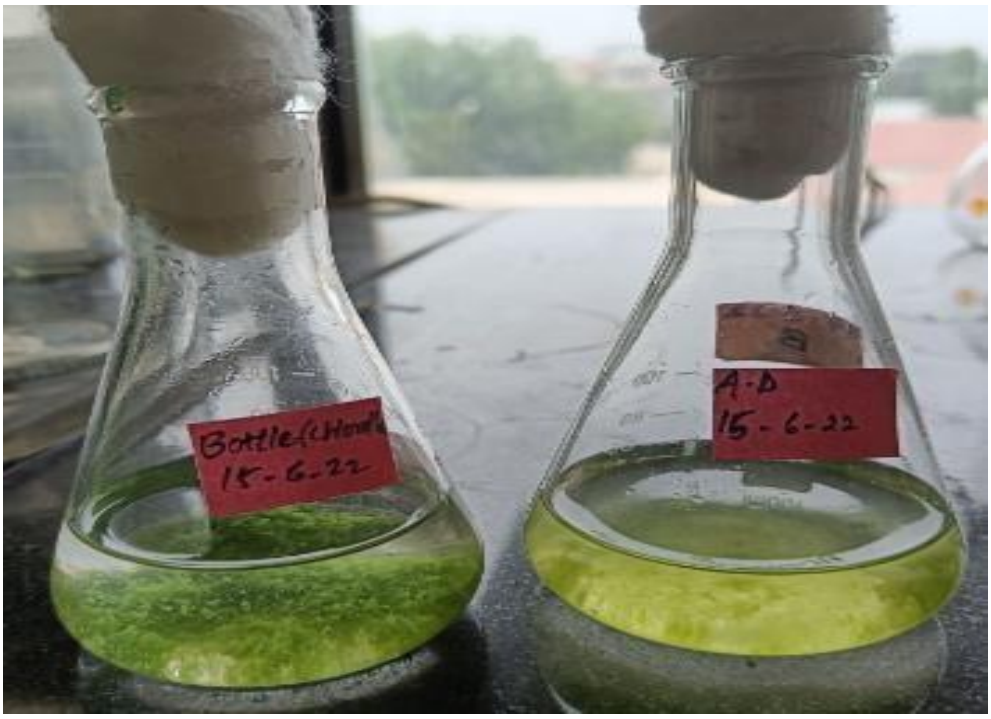
After5 days, the algae growth could be observed. The developed algal colonies were transferred to fresh culture tubes to obtain pure culture.

The algae which was Chlorella ,phormedium and Scenedesmus were isolated.By repeated selection and culturing of the least contaminated cultures, each specieswas eventually separated. As a result, the unialgal, bacterial free culture of Chlorella , phormedium andScenedesmus was obtained. These algae can be further used as a renewable energy source in the form of biomass.

Algal culturing :

1.1 Materials: 2 flask, pipette, media BG-11, algal sample.

1.2 Method:SAMPLE A and SAMPLE B in each sample inoculatedthe 5mL of algal culture in laminar air flow to avoid the contamination. After inoculation cultured flask were kept in BOD incubator for 15 days for algal growth but in between OD was measured with the help of UV spectrophotometer with the wavelength of 680 nm to check the algal growth rate.



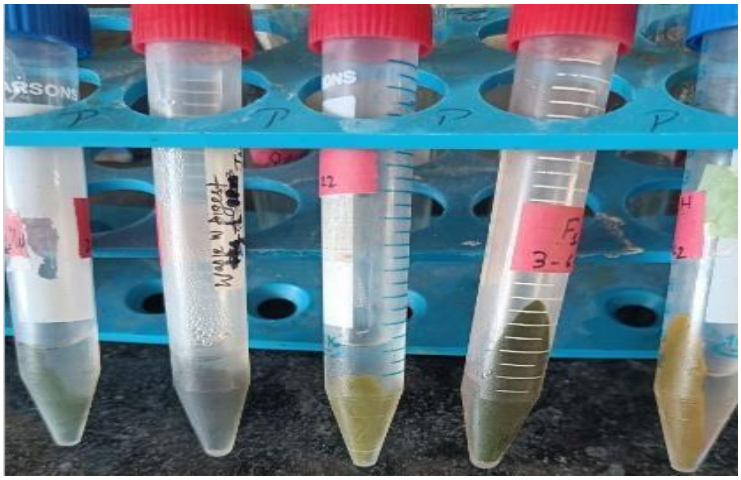
In the growth phase of algae come in decline phase we harvest the biomass forbiochemical screening.

SERIAL NO.	SAMPLE	OD(680nm)
1	Bottle sample	1.3835
2	Animal drinking sample	0.4892

Algal biomass harvesting:

2.1Materials:pipette, petri plate, falcon tube, eppendrof tube, centrifuge. **2.2Methods:** for harvesting the algal culturing centrifuge at 6500rpm for 5 min in falcon tube and remove the supernatant after that add the 5 ml distilled water for washing and centrifuge it again for 5 min and after that remove the supernatant. Collect the wet biomass in eppendrof tube. For dry biomass pour the wet biomass in petri plate and kept in hot air oven at 40c after that collect in eppendrof tube .

Result:



Wet Biomass of different cynobacteria

Carotenoid estimation:

1.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.

1.Materials: 2ml algal biomass, falcon tubes, 85% acetone, micropipette, centrifuge, ultrasonicator and UV-Vis spectrophotometer.

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

3.Method: Take 500 microlitre of algal biomass in a falcon tube and add 1.5ml of 85% acetone and then mix the solution with the help of a vortex mixer. The mixture is then frozen and thawed for three to four times. After that ultrasonicate the mixture for three minutes. Centrifuge the mixture at 5000rpm for 5 min and obtain the supernatant and transfer in another falcon tube and make up the volume upto 5ml with 85% acetone. Take the absorbance at 450nm with the help of a spectrophotometer against 85%acetone as blank.

1. **Formula :**

OD*V*DF*10/2500mg/ml

OD- Absorbance at 450nmV-

Volume of extract

DF- Dilution factor

Dilution factor= total volume/volume of supernatant

2.Chlorophyll estimation:

2. 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 growthand 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.

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

4. **Reagent preparation:** 90% methanol (for 50ml take 45ml methanol and 5ml distilled water)

5. **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 keepin a water bath for 30 min in 60c. ThenI keptit in the sonicate for 180sec. After that add 1ml of 90% mixture in the solution. Centrifuge at 5000rpm for 5 min and remove the supernatant. Take the absorbance at645nm, 665 nm and 663 nm with the help of a spectrophotometer.

6. **Formula:**

Chlorophyll A = OD_{66}*13.9µ/mL
Chlorophyll B = (22.9*OD_{645})*(4.68*OD_{663})µ/mL

3.Phycobiliprotein:

3. **Theory:** Phycobiliprotein are a family of highly soluble and reasonably stable fluorescent proteins. These proteins contain covalently linked tetrapyrrolegroups 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.

4. **Materials:** falcon tube, test tube, centrifuge, UV-Vis spectrophotometer, phosphate buffer (ph=6.8)

5. **Reagent preparation:** Buffer preparation (Dissolve 0.43gm 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

6. **Method:** Centrifuge 10ml of homogenized algal suspension at 6500rpm for 10 min 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

7.Formula:

$PC = (OD_{615} – 0.476 * OD_{652})/5.34$
$APC = (OD_{652} – 0.208*OD_{615})/5.09$
$PE = (OD_{562} – (2.41*PC) – (0.849*APC))/9.62$

4.Carbohydrate:

6.1 Theory: A carbohydrate shell or a protective carbohydrate coat is a common cyanobacterial tactic for self-defence against both the environment and otherorganisms. These layers provide mechanical protection and define the cell shape.Carbohydrates are first hydrolysed into simple sugars using dilute HCL acidmedium glucose is dehydrated to hydroxymethyl furfural. This compound formswith anthrone a green coloured product with an absorption maximum at 620 nm **6.2Materials:** falcon tube, test tube, centrifuge, UV-Vis spectrophotometer,anthrone reagent

6.3 Reagent preparation: Anthrone reagent (add 0.1g of anthrone reagent in 85%H_2SO_4 (conc.) and kept in cool and dark place)

6.4 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 keepin 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 distilledwater to make the volume up to 5ml. Centrifuge at 4000rpm for 3-5 min. Take 1ml 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 add3 ml of anthrone reagent.

Result and discussion

- **CHLOROPHYLL CONTENT:**

In chlorophyll there are two main chlorophyll A and chlorophyll B. Inchlorophyll a being a blue/green pigment with maximum absorbance to 665 nm and chlorophyll b being a green/yellow with maximum absorbance from 642 to 663 nm. Table 1 shows the result and after apply the following formula:

Chlorophyll A = OD66*13.9µ/mL

Chlorophyll B = (22.9*OD645)*(4.68*OD663) µg/mLFor PH=6

(chl. A) = (0.2036*13.9)

=2.83004µg/ml

PH=6 (chl.B) = (22.9*0.1137) – (4.68*0.2008)

= 1.663986µg/ml

For PH=8 (chl.A) = (0.1315*13.9)

= 1.87789µg/ml

PH=8 (chl.B) = (22.9*0.0907) – (4.68*0.1338)

= 1.45526µg/ml

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 pH8.

TABLE: 1

SAMPLE	645nm (chl.B)	663 (chl.B)	665 (chl.A)
Sample A	**0.0730**	**0.1166**	**0.1178**
Sample B	**0.0977**	**0.1252**	**0.1270**
Sample C	**0.0781**	**0.0874**	**0.0882**
Sample D	**0.2500**	**0.3128**	**0.3145**
Sample E	**0.0902**	**0.1305**	**0.1327**

● CAROTENOID CONTENT:

Carotenoid is also a fat soluble molecule and can be extracted from thylakoid membranes with organic solvents such as methanol. Themaximum absorbance at 450 nm . The table 2 shows the result and after apply the following formula:

OD*V*DF*10/2500mg/mlD=

OD at 450 nm

V= Volume of extract (after centrifuge)F=

Dilution factor

2500= Average extinction of coefficient at pigment is 2500D= 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/mlPH=8

D= 5ml/2.3 = 2.17 (0.3529*2.3*2.17)*10/2500 = 0.0070mg/ml

In this study it was found carotenoid content in pH 6.8 . It shows pH 6.8growth condition could be more suitable than pH 8.

Table 2

SAMPLE	562nm	615 nm	652 nm
Sample A	0.2196	0.2124	0.2089
Sample B	0.2491	0.2422	0.2364
Sample C	0.2205	0.2118	0.2072
Sample D	0.2602	0.2502	0.2439
Sample E	0.1314	0.1285	0.1267

● 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)/5.34APC =

(OD652 – 0.208*OD615)/5.09

PE = (OD 562 – (2.41*PC) – (0.849*APC))/9.62For

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

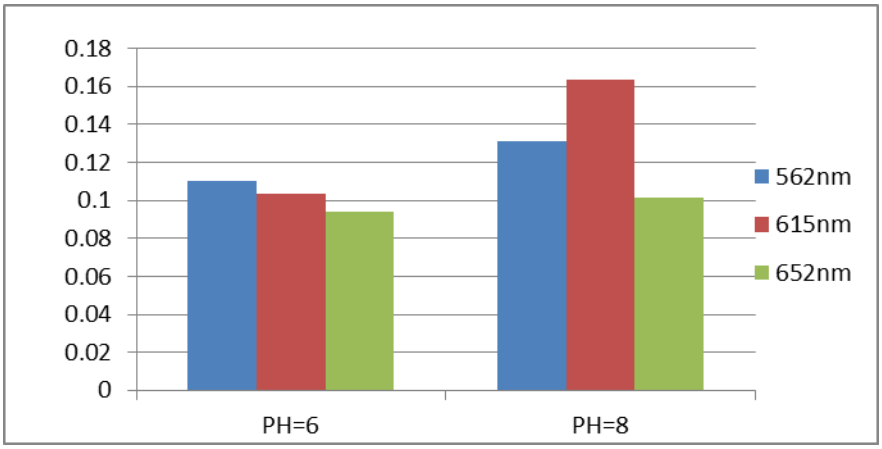


Fig-3

- **CARBOHYDRATE CONTENT:**

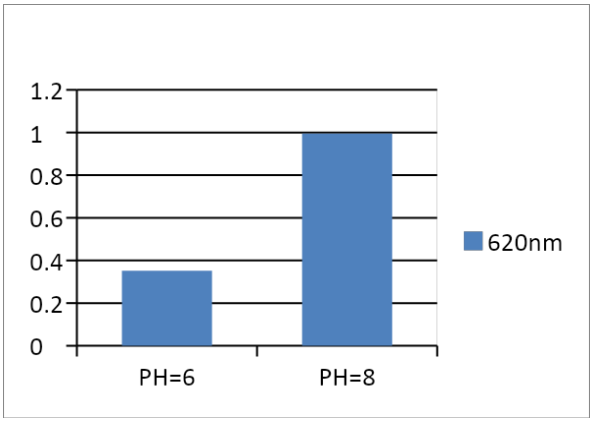
Carbohydrates are first hydrolysed into simple sugars using dilute HCL acidmedium glucose is dehydrated to hydroxymethyl furfural. The major absorbance at 620 nm. Carbohydrate one of the energy biomolecules that assimilate during the photosynthesis process. In this study it was found carbohydrate content in pH 8 more than pH 6. It shows pH 8 growth conditions could be more suitable than pH 6.(table - 5)

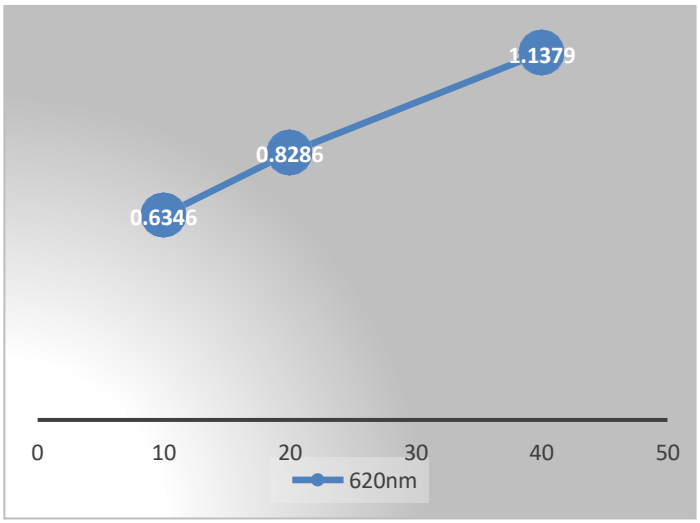
Table -4

SAMPLE	620 nm
PH=6	0.3523
PH=8	0.9954

Table 5: Carbohydrate calibration table

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





Carbohydrate calibration graph

Conclusions

It is well established that microalgae have tremendous potential as a source of biofuel, food and high value bio-compounds. However, the limitations in productivity of microalgae and the drawbacks of bioprocessing technologies renderthe fully utilization of microalgae biomass to be impractical. Therefore, more workneeds 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 compounds from the microalgae strain. Besides,the ILs studied should be examined for their toxicity before application. In addition, computer simulation can be used to further explore the combination of various cation and anion of ILs to maximize the extraction capacity of desired compounds from microalgae.

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