Isolation and characterization of pigment producing microalgal sp.

Project report submitted in partial fulfillment of the requirement for the degree of Bachelor of Technology

In

Biotechnology

By

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UNDER THE SUPERVISION OF

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DECLARATION

I hereby declare that this project "**Isolation and characterization of pigment producing microalgal sp.**" has been done by me under the supervision of **Dr. Ashok Kumar Nadda, Assistant Professor, BT&BI**, and Jaypee University of Information Technology. I also declare that neither this project nor any part of this project has been submitted elsewhere for award of any degree or diploma.

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CERTIFICATE

This is to certify that the work which is being presented in the project report "I**solation and characterization of pigment producing microalgal sp."** in partial fulfilment of the requirements for the award of the degree of B. Tech in Biotechnology and submitted to the Department of Biotechnology And Bioinformatics, Jaypee University of Information Technology, Waknaghat is an authentic record of work carried out by Shubhankar Singh during the period from July 2022 to May 2023 under the supervision of Dr. Ashok Kumar Nadda, Department of Biotechnology And Bioinformatics, Jaypee University of Information Technology, Waknaghat.

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The above statement made is correct to the best of my knowledge.

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Shubhankar Singh (191806)

ABSTRACT

Reducing the carbon dioxide footprint of human activities requires the exploration of new production methods and product sources. Microalgae have emerged as a promising source of various products, ranging from fuels to chemicals for industrial applications. As photosynthetic organisms, algae utilize light to synthesize organic matter and it can be easily and efficiently grown, making them a sustainable alternative to terrestrial plants. This project outlines the key factors that contribute to microalgae productivity in artificial cultivation systems and identifies areas that require further research to develop photosynthetic cell factories. It also discusses the challenges of improving photosynthetic efficiency and optimizing the culture for the most optimum growth of microalgae. Additionally, the project report highlights the importance of selecting the appropriate strain of microalgae and tailoring the culture conditions for successful commercial application. Lastly it also involves the growth medium formulation, cultivation system design, and pigment extraction from the optimized culture to get the maximum yield.

Keywords: *Microalgae, Yield, Pigments, Carbon Footprint, optimization, Photosynthetic organism.*

CHAPTER-1

Introduction

New advancements that can be utilized for Carbon Dioxide (CO_2) obsession and the creation of other important items is important to decrease the CO_2 impression of human movement. Microalgae are an exceptionally promising source of various items, including synthetics and fuels. The enormous scope of microalgae can be utilized in the making of various restorative items. These may be utilized in sewage and waste water treatment methods. Since microalgae develop rapidly and use daylight energy with extraordinary proficiency, microalgae energy has been seriously explored corresponding to decreasing CO_2 outflows and growing new energy sources. Pigments derived from microalgae and cyanobacteria have attracted remarkable interest for present day applications due to their bioactive potential and their customary thing attributes. These varieties are normally sold as concentrates, to beat cleansing costs **[1].** The extraction of these combinations relies upon cell aggravation frameworks and engineered dissolvability of blends. Different cell unsettling influence frameworks have been used for conceal extraction, similar to sonication, homogenization, high-pressure, CO_2 supercritical fluid extraction, enzymatic extraction, and some other promising extraction techniques, for instance, osmotic development for electric pulses and warming. The establishment and working costs are the fundamental boundary to pigment bioprocessing, subsequently more essential and applied research is as yet expected to overcome these snags and open up the worldwide market for the microalgae and cyanobacterial industry **[2]**.

Microalgae are tiny photosynthetic organisms that are well-known for their pigments, such as chlorophylls, carotenoids, and phycobiliproteins. These pigments possess diverse biological functions and are of great interest to numerous industries, including food, cosmetics, pharmaceuticals, and biotechnology.

Marine microalgae are one of the most shifted groups of marine microorganisms, with an expected 2105 to a few million varieties, just 35,000 of which have been portrayed **[7]**. Various developed species have yielded in excess of 15,000 substances, including unsaturated fats, sterols, phenolic compounds, terpenes, catalysts, polysaccharides, alkaloids, toxins, and shades. Because of their high biodiversity and huge efficiency, microalgae address an underutilized asset with critical potential for the confinement of novel normal synthetic substances valuable

for food, medicine, or biotechnological utilities **[3].** They can be effortlessly filled in controlled conditions, dealt with like other lab microorganisms, and hereditarily changed without risking spreading, which adds to their convenience as a biotechnological biomass. Most of the strategies for extraction of lipids and pigments from microalgae depend on phytochemical procedures created on profoundly evolved plants and macroalgae. The biochemical qualities of the extracted pigments, speed, dissolvable utilisation constraints, repeatability, extraction yield, selectivity, security of the extracted pigments against synthetic modifications, aspect, cost, and convenience are the primary factors determining an extraction innovation's selection. **[3].** Conventional natural dissolvable extraction methods like maceration (dousing), permeation, counter current extraction, compelled fluid extraction, and Soxhlet are habitually referenced for the extraction of lipids and shades.

The use of catalysts such as xylanases, pectinases, or cellulases to improve pigments extractability rates has been recommended and demonstrated for better extraction of plant tissues and macroalgae **[4]**. This approach could also be useful for extracting pigments from microalgae. To prevent the chemical oxidation of pigments, there are several methods that can be used, including freezing (at -80°C or in liquid nitrogen), freeze-drying, drying, or storing in humid environments to prevent oxidation and thermal denaturation **[4]**.

Culture optimization of microalgae involves adjusting various environmental factors like light, temperature, pH, nutrient availability, and CO_2 concentration to create an ideal environment for the specific microalgae species being cultivated. This is important because different species of microalgae have varying requirements for growth and reproduction. One of the widely used methods for optimizing microalgae culture is phototrophic cultivation. This approach involves supplying the algae with light and nutrients to stimulate growth, and it is commonly carried out in enclosed bioreactors or ponds designed to provide optimal conditions for growth and reproduction. Another technique for optimizing microalgae culture is heterotrophic cultivation, which entails providing the algae with organic nutrients rather than light to promote growth. This method is often used for species of microalgae that do not require light for growth or species that grow more efficiently through this approach**[6]**.

To extract pigments from liquid solutions, strict pH control is necessary because many of these pigments can undergo chemical changes in either acidic or

alkaline conditions. Plants and microalgae can have their pigments extracted quickly and efficiently by switching from water to specific solvents such as sec-butanol. In the last decade, microwave-based extraction methods have become more common. It has been claimed that MAE (Microwave-assisted extraction) and VMAE(Vacuum microwave-assisted extraction) are efficient and rapid approaches to extract vegetable oils, plant pigments, or antioxidants from spices**[7]**. These procedures offer similar or higher extraction yields while using less solvent. MAE and VMAE provided the best recovery for all analyzed species compared to autoclaving, bead beating, sonication, or maceration in a 10% NaCl solution. For marine microalgae, they have been used to extract lipids. The extraction of astaxanthin from the red yeast Xanthophyllic is feasible when the cell walls are microwave-irradiated to destroy them and then extracted using a solvent **[6]**.

Solvent extraction is one of the most widely used methods for pigment extraction from microalgae. This technique involves using solvents such as ethanol, acetone, or hexane to extract the pigments from the biomass. Supercritical fluid extraction is another method that employs supercritical CO_2 as a solvent to extract the pigments. This method is becoming more popular due to its efficiency and eco-friendly nature.

Microwave-assisted extraction is a novel technique that employs microwaves to extract pigments from microalgae. This technique has been found to be faster and more efficient than traditional solvent extraction methods **[7]**. Enzymatic extraction is another method that uses enzymes to extract the pigments from the biomass. In summary, pigment extraction from microalgae is an essential process for obtaining high-quality pigments that are utilized in various industries. The selection of the extraction method is dependent on numerous factors, including the type of pigment being extracted, the efficiency of the method, and the environmental impact of the technique **[5]**.

Cyanobacteria are a kind of microalgae that are among the planet's earliest photosynthetic living structures and were pivotal in the development of Earth's oxygen-rich air**.** Microalgal colors are the substances responsible for light retention and energy move to the response communities, two cycles fundamental for photosynthesis. They dwell in edifices known as receiving wires, which are contained in the thylakoid films and have a receiving wire like design. In cyanobacteria (prokaryotic microalgae), this layer is situated close to and lined up with the cell

surface, rather than eukaryotic microalgae where it is tracked down inside the chloroplasts **[6].**Pigments are regularly assembled into three significant classes: chlorophylls (chl), carotenoids, and phycobiliproteins**[8]**.

Phycobiliproteins are the fundamental class of protein edifices that catch light in cyanobacteria and have red green growth. These proteins are coordinated into phycobilisome-like designs on the cytoplasmatic part of the thylakoid films**.** These microscopic organisms' photosystem II (PS II), which varies from the photosystem I (PS I), is prevalently made out of phycobilisomes **[8].** These hydrophilic proteins retain light in the noticeable reach somewhere in the range of 500nm and 650 nm in blend with chl a to boost light gathering. The four primary gatherings of phytobiliproteins are phycocyanin, phycoerythrin, phycoerythrocyanin, and allophycocyanin.

One could contend that since even plants are capable of photosynthesis, they can be employed to address the issue of CO_2 release, which will inevitably lead to the issue of global warming. In response to this question, microalgae are chosen over plants for CO_2 fixation for a number of reasons.

1. Micro algae exhibit faster growth rates and higher photosynthetic efficiencies compared to their terrestrial counterparts, making them more effective at capturing atmospheric carbon.
2. microalgae possess a wide range of antenna pigments to harvest more solar energy and a variety of CO_2 concentrating mechanisms to increase CO_2 concentration around ribulose-1,5 bisphosphate carboxylase/oxygenase.
3. Moreover, Pipe gases from modern plants have been accounted for as to be a reasonable feed of green growth which on opposite can't be handled by the plants.

Hence microalgae possess significant potential for utilizationfor the CO_2 fixation over plants. Pigments extracted from the microalgae can also be used for various function. Algal pigments have been found to possess various health benefits such as acting as antioxidants and immune boosters, anti-carcinogens, anti-inflammatory agents, neuroprotective agents, and anti-obesity agents. Additionally, they have been recognized for their ability to serve as anti-aging compounds and skin and photo-protective agents.

Problem Statement

The enormous expansion in CO_2 in the air driven by human activities is causing tremendous effects and new practical wellsprings of energy, food and materials are profoundly required. Microalgae are unicellular photosynthetic microorganism that can give an exceptionally essential commitment to this test as elective wellspring of biomass to supplement crops development. The motivation behind this work is to give a cutting-edge survey on process influences, with an emphasis on the impacts of photosynthetic biochemical cycle, microalgal species, physicochemical interaction, and hydrodynamic cycle on the proficiency of microalgal CO_2 sequestration and biomass creation **[9]**. Moreover, the viewpoints are put on a mission to act as a supportive aide for growing its central examination and significant innovations. New manageable wellsprings of energy, food and materials are required because of the gigantic ascent in air CO_2 fixation welcomed on by human action **[10].** As a substitute wellspring of biomass to enhance the improvement of harvests, Microalgae which are unicellular photosynthetic microorganisms, can give a vital commitment to this issue. Subsequently, it means quite a bit to concentrate on algal photosynthetic digestion to foster strains that are more useful and productive.

The extraction of pigments from microalgae is a significant problem since it necessitates efficient and cost-effective technologies capable of overcoming the hurdles posed by the microalgal cell wall. However, conventional pigment extraction technologies have drawbacks such as low yields, high prices, and the use of dangerous chemicals, all of which limit their scalability and sustainability. As a result, there is an urgent need to develop creative, ecologically friendly, and economically feasible ways for extracting pigment from microalgae in order to address the increasing demand for natural pigments in various industries while also ensuring their safety and sustainability.

Objectives

1. Isolation and cultivation of microalgal strain
2. Optimization of cultivation conditions
3. Pigment extraction from microalgae

Isolation and Cultivation of Microalgal Strain

- The goal is to obtain a pure culture of microalgae for future growth optimization and applications.
- Under properly controlled Conditions, the microalgal strain's growth rate, biomass yield, and biochemical makeup will be evaluated as well.

Optimization of Cultivation Conditions:

- To identify the optimal growing parameters for the microalgal strain, including light intensity, temperature, pH, and nutrient content, in order to maximise growth rate and biomass yield.

Pigment Extraction from Microalgae:

- To extract and quantify different pigments from microalgal biomass, such as chlorophyll and carotenoids.
- The extraction process will be improved to maximise pigment production and purity while using as few solvents and energy as possible.

CHAPTER 2 - LITERATURE SURVEY

The main focus of this review is on the main factors affecting the microalgae CO_2 fixation which involves light acquisition, biochemical power conversion and inorganic carbon capture and conversion [**11]**. It also covers the part where which strains is to be selected and what is the process of finalization and usage involved in it.

The ability of photosynthetic organisms to fix more CO_2 while using fewer resources is briefly described as being of utmost importance to meet key global concerns such as climate change, sustainable resource management, food and energy requirement **[12]**.microalgae have drawn the most attention among photosynthetic organisms because of a number of special traits that give them an edge over larger plants when it comes to being used in the bio-based business.

The genetic diversity, functional diversity, and metabolic flexibility produced by the microalgae are significant factors in influencing resource demand and use efficiency. The fact that cultures can easily deviate from the planned biomass quality is also a result of metabolic adaptability and maintenance in response to environmental disturbances. While choosing individuals and establishing manufacturing facilities, these elements are frequently overlooked. Consideration of such physiological complexity is the only way to produce items with consistent quality and significant quantities **[13]**. Also achieve a smooth shift to renewable energy and a circular economy, it is crucial for cell manufacturers to focus on maximizing the theoretical conversion of light energy to biomass. Several approaches can be adopted, such as selecting dependable strains and partners that produce high-value molecules naturally, implementing nutrient recycling from municipal wastewater or flue gases, characterizing cell physiological responses in different environments, optimizing molecular tools for predictable genetic manipulation, metabolic design, and computational modeling. These methods are currently being studied and are highly beneficial.

2.1 Carbon Dioxide Fixation

Algal cells found in aquatic regions compete for a very significant portion of the planet's photosynthesis. microalgal cells don't contain lignin, as opposed to earthbound plants, and photo-synthetically fixed carbon is handily reused in

biological systems through the food web. Contrasted with land plants, green growth enjoys extra benefits for modern purposes **[14]**. In contrast with land plants, they have more prominent development rates, all of their biomass is photo-synthetically dynamic, and they photosynthesize the entire year, which brings about an anticipated yield for each section of land that is almost two times as high. Moreover, they don't rival palatable plants for arable land, and on account of marine species, they don't utilize consumable water — features that are very beneficial given the expected development in worldwide populace and the following interest for food.

2.1.1 A systems approach for CO_2 fixation from flue gas by microalgae

In recent decades, the high worldwide release of CO_2 emissions has contributed to an increase of the greenhouse effect as well as other significant environmental issues. The depletion of fossil fuel reserves also continues to be a challenge for the world's energy system. In this way, both the scientific and governmental sectors have begun to show evidence of a global effort for the development of renewable fuels. Numerous studies have been devoted to the transformation of carbon sources into compounds that can be used or into sources of sustainable energy **[15].** Be that as it may, because of the contention with the food supply, the suitability of the original biofuels creation is in uncertainty.

Since they can give various feedstocks to the creation of biodiesel, bioethanol, biomethane, and biohydrogen, microalgal frameworks are a practical other option. The capacity to develop microalgae on non-arable soil is the fundamental advantage that microalgal oil offers over plant-based oils. Microalgae have additionally been recognized as reasonable bioresources for meds, live feed, and other high-esteem merchandise.

The fundamental factors governing how CO_2 is utilised by algae from flue gases have recently undergone a number of investigations in all potential directions of process development**[16].** These investigations have also revealed details regarding the composition of the flue gases, which differs between various coal-fired power plants. The latest advances in PBR plan for covered open air conditions center around the reactor's ability to help ideal light entrance, where surface to volume proportion is the principal standard, and on liquid elements, which offers an optimal light-dim (L/D) cycle to help states of high thickness culture.Conical helical tubular photo-bioreactors (CHTP) are ideal for spreading strong light over a larger photo-receiving area, as photosynthetic efficiencies are quite good . The ratio of productivities for conical

HTPs, when Chlorella was exposed to a light irradiation level of 980 mol **[16]**, suggests that photo-redistribution technology is a simple and effective way of enhancing the photosynthetic output of microalgae.

The proportion between the energy given to microalgae by light enlightenment and the energy content of the microalgae biomass delivered by photosynthesis is utilized to work out the photosynthetic effectiveness of microalgae. It should be remembered that only a small portion of the light energy, between 10 to 34 %, makes it to the surface of the reactors from the atmosphere to the ground**[17].**Because of climatic dispersing, climate, scope, reactor direction, and the obliged photosynthetic dynamic zone, energy is squandered. The cells' light saturation and the metabolism of the microalgae can then result in additional losses. When just incident light is taken into account on the reactor surface, the typical photosynthetic efficiency measured is between 0.1 and 10% **[17]**. The extra photons, up to 80% of them, are wasted as heat and harm the photosystems. A *Chlamydomonas reinhardtii* strains have been genetically altered to have a shortened light gathering antenna in order to solve this problem **[18]**.These strains are less susceptible to photoinhibition because they have less chlorophyll available for light gathering. Additionally, the self-shading effect inside photobioreactors is also reduced due to their lower light absorption per unit cell. The photo-redistribution technology improved the photosynthetic productivity in a simple photobioreactor unit system by using various cone angle types of photobioreactors.

Subsequently toward the end understanding the water science of the essential gases CO_2 and SO_2 and what it means for the whole cycle advancement augmenting the CO_2 usage from flue gas and biomass creation, separately is the significant target of this investigation, which is centered around CO_2 sequestration from vent gas **[19]**. It is important to completely study the microalgal physiology and cell digestion, flue gas organization, light illumination accessibility, and PBR configuration to understand and use their mind boggling associations for fast interaction advancement and scale-up. PBR was improved, allowing for quicker fluid circulation from the dark side to the light side. As a result, more opportunities for algal cells to experience the light/dark cycle were provided, which was advantageous for microalgal photosynthesis.

2.1.2 Enhanced CO_2 fixation in *Chlorella vulgaris*

This research suggests an innovative (Photo-bioreactor) PBR, named ASD-PBR (airfoil-shaped deflectors Photo-bioreactor), that employs static airfoil-shaped deflectors to improve the manipulation of micro-algal biomass, and subsequently, enhance the photo-biochemical conversion **[20]**. The review highlights the hydrodynamic limitations and non-uniform light distribution observed in the traditional (airlift flat-plate photo-bioreactor) AFP-PBR. The study revealed that the ASD module accelerated the flow of micro-algal suspension from the center to the sides by bubbling, increasing the exposure of micro-algal cells to the light source.

Hence the velocity of the solution along the incident light direction showed a significant increase of 114.8% in the recently proposed ASDPBR, which stands for airfoil-shaped deflectors, as compared to the control PBR, *i.e.,* airlift flat plate photo bioreactor **[21]**. Furthermore, the ASD module served as a static mixer, leading to an 11.5% increase in the mass transfer coefficient and a 21.4% decrease in the mixing time. Due to the modified hydrodynamic parameters, the maximum algal biomass output and the CO_2 fixation rate also improved by 18.3 and 10.9%, respectively **[22]**.

Chlorella vulgaris strain FACHB-31, in particular, is the strain of microalgae that was employed in this experiment and has the most pronounced speed of cell proliferation and carbon capture capability or CO_2 sequestration rate.CO_2 gas (offset with N2) was chosen as the carbon hotspot for microalgal improvement and circulated air through into PBR through a punctured gas sparger in this trial **[21]**. *Chlorella vulgaris* FACHB-31 was the microalgal strain that was initially inoculated into the PBRs.

They used ASD-PBR in which the fluid was redirected from the riser chamber to the downcomer chamber by airfoil-molded redirectors. This improved the cross-over progression of liquid along the occurrence light course in the ASD-PBR, resulting in faster liquid dissemination from the cloudy side to the brightening side. In comparison, the control PBR, i.e., airlift flat plate, had a straight up and down flow field. This provided more opportunities for algal cells to undergo the light/dark cycle, which was beneficial for microalgal photosynthesis.

They examined the impact of ASD-flow PBR on ASD spacings , in addition to flow field differences. The liquid stream direction changed equally in the ASD-PBR with 0 mm ASD spacings and the ASD-PBR with 5 mm ASD spacings. The stream field on the airfoil-formed redirectors appeared slightly different because bubbles

couldn't pass directly from the riser chamber to the fluid surface without a gap between them. However, increasing the airfoil-formed diverter spacing to 10 mm allowed more air pockets to pass through the airfoil-shaped redirectors, creating a flow field similar to that in the control PBR**[22]**. The plates and airfoil-shaped deflectors in the ASD-vertical PBR created a gap, causing more liquid to converge from the top chamber and downcomer chambers to the riser chamber. This was due to the airfoil-shaped deflectors blocking fluid from flowing vertically between the vertical plate and the sidewall.

2.1.3 Improving CO_2 fixation with microalgae by bubble breakage

The primary focus of this research work was on the up-down chute baffles utilised in raceway ponds, where the aeration gas was broken into smaller bubbles with improved local solution velocity to promote CO_2 fixation with microalgae **[23] .**The estimation of air pocket creation and residence periods, which were impacted by paddle wheel speed, aerator hole breadth, gas stream rate, and arrangement profundity, was finished utilizing a rapid photography framework and online precise pH tests. To build the development pace of the microalgae and the adequacy of CO_2 obsession, bewilders were explored in the field of shut miniature green growth reactors.

The study results from the enclosed microalgae reactor illustrated the significance of baffles in generating vortex flow fields and increasing the mass transfer coefficient between gas and solution.**[24]** An up-down chute baffle was developed to enhance the flashing light effect in the raceway pond. The use of this baffle reduced the mixing time required and improved the mass transfer coefficient by 41% and 25% respectively, resulting in a 32.6% rise in biomass yield.

The aim of this study was to improve aeration bubble trajectory and optimize bubble generation by measuring bubble creation and residence periods using a high-speed photography (HSP) system and online precise pH probes. A vortex flow field was created by the up-down chute baffle, **[25]** leading to an increase in bubble residence time as observed in the results obtained.

The *Chlorella* mutant PY-ZU1 was grown in the raceway pond at 24 degrees Celsius under continuous lighting using Brostol's solution as the principal source of algal biomass. The study also included the utilisation of *Nannochloropsis salina* and *Spirulina platensis*.

The stream bearing was adjusted by the up-down chute baffles **.**In this manner, the center and back bits of the up-down chute baffles made both clockwise and anticlockwise vortex streams. Because of the expanded flat speed of the nearby arrangement, bubble creation time diminished. At the point when the principal current went through the descending piece of the up-down chute astound, the nearby arrangement speed upstream of the gas aerator rose. At the point when the up-down chute confuse was utilized with a gas stream pace of 0.03 vvm, bubble home time expanded by 27%. Little air pockets have a high mass exchange coefficient and a huge explicit surface region **[26]**. Such air pockets, which are additionally moving rapidly at the lower part of the raceway lake, will rapidly separate the oxygen delivered by the green growth and permit them to multiply more rapidly.As a result, when the up-down chute baffle was introduced to the raceway pond, the biomass yield rose by around 29%.

2.2 Pigments extraction

Out of the millions of species belonging to marine microalgae, which constitute the ocean's microorganisms, only 35,000 have been identified **[7]**. Through meticulous screening of cultivated species, approximately 15,000 chemicals such as fatty acids, sterols, phenolic compounds, terpenes, enzymes, polysaccharides, alkaloids, poisons, and pigments have been isolated and chemically analyzed. The high biodiversity and immense productivity of microalgae make them a largely unexplored resource with significant potential for obtaining novel natural chemicals of interest in the fields of food, health, or biotechnology **[27]**. Microalgae's suitability as a biotechnological biomass is another factor that makes them interesting. They can be easily cultivated under controlled conditions, handled like other laboratory microbes, and genetically modified without posing any risk of spread.

This study has provided a description of Many methods for preventing the chemical oxidation of pigments have been suggested.To prevent oxidation and warm denaturation, tests can be frozen (80 °C, fluid nitrogen), freeze-dried, dessicated, or put away in water-fume soaked conditions**[28].** For the cyanobacterial blue shade phycocyanin, maceration in fluid nitrogen was trailed by cradle extraction, which remembered color precipitation for half ammonium sulfate. To desalt color arrangements, notwithstanding, extra dialysis and gel fifiltration chromatography techniques are required. Since numerous porphyrin colors can go through compound

changes in either acidic or antacid conditions, extraction in watery arrangements requires tough pH control. Chlorophylls, for instance, can be epimerized, dephytylated, and demetallized

The rapid degradation of porphyrin pigments can be attributed to the presence of chlorophyllases in microalgae extracts. In order to extract pigments from plants and microalgae quickly and efficiently, some solvents such as sec-butanol can be used as a substitute for water. Furthermore, this solvent can also be used to concentrate pigments extracted with acetone. To extract carotenoids from *Dunaliella salina* without damaging the cells, Hejazi and colleagues developed a biocompatible method**[29]**. There are two more cell disruption techniques based on mechanical or osmotic shock: bead pounding and ultrasound-assisted extraction. Mechanical treatments, especially when using an ultrasonic sharp probe, can be extremely harsh and lead to thermal denaturation of the target molecules. Osmotic shock may not be effective for frustulated species or armoured dinoflagellates.

Acetone was utilized as the model extraction dissolvable in this study since it eliminates most of photosynthetic colors in a large number of polarities and $CH3)_2CO$ 90% is prompted for phytoplankton shade examination**[28]**. To restrict chl a hydrolysis by the thylakoid-bound protein chlorophyllase, which is available in most of diatom species and chlorophyceae and is enacted during extraction even before dissolvable extraction and is still to some degree dynamic in 90% $CH3)_2CO$**[28]**, shade extraction was done in 100 percent $CH3)_2CO$. Chlorophyll movement was recently observed to be low in CC, with 0-5% chlorophyllide A made as a level of all out chl a, and high in DT, with 12-79% chlorophyllide A framed, when shade extraction was done in half $CH3)_2CO$ at 20 C**[30].**

Although selectivity was lacking, the high extraction yields achieved in acetone enabled a precise comparison of the methods' effectiveness. Selective solubilization of apolar pigments (chlorophylls, carotenoids) or polar pigments (xanthophylls, phycobiliproteins) can be achieved by performing MAE(Microwave assisted extraction) and VMAE(Vaccum microwave assisted extraction)**[8]**in different solvents, increasing the process' selectivity for a particular pigment. In addition, MAE and VMAE can be conducted using combinations of solvents that are permissible by law for extracting food-grade pigments (e.g., carotenoids) or in apolar solvents like hexane combined with weflflon.

This paper contends that customary methodologies are similarly viable regarding yields, speed, and security of separated atoms from substance change as microwave-helped processes for shade extraction from species without frustule and thick external exopolysaccharide envelope. Apparently in numerous species, freeze-drying is adequate to debilitate the cell film and give colors admittance to dissolvable solubilization.

2.2.1 Biotechnological aspects of microalgae pigments

This literature focuses on biotechnological aspects of micro-algal pigments including medical products, cosmetic products, industrial products like food, healthcare etc.

Since microalgae have great productivity and are simple to extract under regulated conditions, using them in cosmetics and cosmeceuticals is an intriguing technique to meet the increase in demand for new natural components from environmentally sustainable biomass.**[31]** Since it is challenging to include the entire microalgae biomass into cosmetic formulations, innovative extraction and purification techniques have been developed to investigate its potential in cosmetics**.** The development of numerous cosmetic products incorporating microalgae is aided by scientific studies on the biological functions of microalgae extracts or their constituents, such as rheology modifiers like several specified polysaccharides. In addition to astaxanthin and its esters, which have excellent antioxidant qualities to suppress tyrosinase-induced hyperpigmentation, other substances may activate biochemical pathways on the biopeptides to stimulate collagen.

Medium and culture conditions: Chlorella sp. was subcultured in Half Strength Chu 10 media, while Chroococcus sp., Jaaginema pseudogeminata, Chroococcus sp., and Achrochaete sp. were subcultured in MN+ medium.The way of life were kept at a temperature of 25 2 °C, 1.5 Klux, and a 12:12 h photoperiod.

2.3 Extraction methods

At their individual fixed stages, every one of the way of life were reaped and lyophilized.Each customary extraction method utilized lyophilized algal cells, and every extraction was completed multiple times.Following extraction, the absorbance of the supernatants for carotenoids and chlorophyll a was estimated at 663 and 470 nm, separately.Based on the proposed formulae, the color content was assessed**.[32]**

2.3.1 Direct extraction 10 mL of $CH3_2CO$ was added to 30 mg of dried cells, which were then centrifuged at 8,000 rpm for 10 minutes in the wake of being put in a rotational shaker for 24 hours.At the fitting frequencies, the absorbance of the supernatant was estimated.

2.3.2 Preheated solvent method: 10 mL of warmed $CH3_2CO$ was added to 30 mg of lyophilized cells, and the mixture was then centrifuged at 8,000 rpm for 10 minutes after 15 minutes on a rotating shaker.Their absorbance was calculated after collecting the supernatant **[33].**

2.3.3 Mechanical grinding method: In a mortar and pestle, 30 milligramme of lyophilized cells were placed along with a small amount of glass powder.First, 2 mL of acetone was added, and then the mixture was ground for 2-3 minutes.The mixture of cells and solvent was centrifuged, and the supernatant was gathered in a separate tube.Until the particle was colourless, the process was repeated, and the collected supernatants were combined.It measured the absorbance.

2.3.4 Heating method: In a bubbling cylinder, 30 miligram of lyophilized cells and 10 mL of $CH3_2CO$ were added.For three hours, the dissolvable cell combination was held at 50°C in a water shower that was physically shaken once like clockwork.Subsequent to cooling and centrifuging the items, the supernatant was accumulated.At the pertinent frequencies, the supernatant's absorbance was estimated**.**

Therefore, such atoms might be available in a few businesses' restorative details, like emollients, sunscreens, and hair care items as well as hostile to maturing and regenerative creams.**[34]** However, very few goods are sold on a global scale. As a result, biopigments made from microalgae biomass are advantageous alternatives with the potential for a new market because of their quick growth and high pigment concentration, which allows for a wide range of colours with little to no danger of skin allergies.

The colour of biomass is a natural pigment created from the photosynthetic process of microalgae. Carotenoids, chlorphylls, and phycobiliproteins are the three classes of chemical compounds that make up photosynthetic pigments. Thus, autotrophic organisms like plants, algae, and cyanobacteria use them to harvest sun

energy for photosynthesis, which converts it into chemical energy. Controlled environmental conditions during cultivation could increase the production of lipids, proteins, and pigments in microalgae, which are primarily described in specialised literature.**[35]** The most well-known species in literature, including Dunaliella, Chlorella, Haematococcus, and Arthrospira, are associated to the extraction and use of microalgae chemicals for cosmetics. microalgae pigments could be used in cosmetics, which is one of the many medicinal uses that some microalgae chemicals have been shown to have**.**

2.4 Techniques for isolation and purification of microalgae-

microalgae from natural habitats can be sampled and isolated according to recognized protocols. Algal strains vary in how simple they are to grow in a lab, depending on their various environments. Creating culture conditions that closely resemble a microalgae's natural environment is an important step in the isolation process.

Temperature, pH, and salinity are physiologic factors that microalgae are sensitive to. Some microalgal species need particular nutrients to flourish, such as silica for diatoms, while others can only grow in the presence of bacteria or other algae. Another difficult stage is purification of cultures to produce monocultures of algae. A variety of approaches are used in the separation of a pure culture from its natural habitat, laboratory cultivation, and maintenance of isolated cultures.

2.4.1 Sampling

One of the most important steps in separating microalgae from their natural habitat is the gathering of microalgal samples. Microalgae can be tracked down in different territories and ecological conditions, including soil, ice, natural aquifers, new and bitter water, streams, seas, dams, saline waterways, rocks, and soil**.[36]** For the collection of microalgae, proper sampling methodology, sampling season, habitat evaluation, and sample preservation are crucial. Syringe sampling, brushing, scraping, the inverted petri dish method, and more sampling procedures are available.To recreate these conditions in the lab, it is important to record biotic elements like microorganisms and any contenders at the examining site, as well as abiotic boundaries like light, water temperature, disintegrated O2 and CO_2, supplement fixation, pH, and saltiness**.**

2.4.2 Isolation of single micro-algal cells -

The process of picking a single microalga cell out of the sample with a micropipette or glass capillary while it is being observed under a microscope is known as isolation. These individual cells are then put into sterile water droplets or other suitable medium. This method calls for precision and skill. The cell may be harmed by shear stress brought on by capillary or micropipette tips. Numerous signs of damage include flagellates ceasing to swim, a change in the way that diatoms reflect light due to damaged frustules, and protoplasm leakage. To perform this purification method successfully, caution is required**.[37]** For marine samples in particular, ultrapure droplets are necessary to discriminate between microalgal cells and other particles.

2.4.3 Media used for its growth -

The dietary needs of microalgae vary based on their cellular physiology and natural habitat. To achieve single-species isolation, enrichment cultures can be used with selection pressure. Specific nutritional media, soil extracts, nutrients like nitrate and phosphate, and trace metals are examples of substances that are frequently utilised as enrichment agents.

An approach that is frequently used to produce cultures free of microorganisms is pH adjustment .**[37]**The medium may also be supplemented with organic materials like yeast extract and casein from different fruit and vegetable juices. It's possible that one or more nutrients needed for the growth of microalgae are insufficient in natural settings. Nutrients are recycled or supplied by other creatures' physiological processes in the natural world. Sampling hinders the recycling of nutrients, which can lead to microalgae death.

2.4.4 Microalgae screening and strain selection criteria -

The choice of microalgal strains is crucial for the effective and profitable production of preferred goods. The ideal use or item for which microalgae are being utilized decides the screening standards. Various boundaries, including development physiology, resistance to biotic and abiotic upgrades, metabolite combination, wholesome necessities, and so on are considered for the determination and screening of microalgal strains. Determining the microalgae's proteins, lipids, and carbohydrates is a step in the screening process for metabolite synthesis. Tolerance for high CO_2

concentrations is a crucial screening requirement for CO_2 sequestration. Robustness, stability, and predator susceptibility are taken into account for mass culture [**38].** Various chromatographic techniques, such as thin-layer chromatography, liquid and gas chromatography, along with spectrophotometric techniques like near infrared, Fourier transform infrared, and nuclear magnetic resonance spectroscopy, are employed to screen microalgae for metabolite synthesis. Due to the diverse nature of microalgae, it is essential to develop efficient screening methods that can evaluate several phenotypic traits at once.

2.5 How to measure the growth achieved by the micro-algal sample -

Due to their tiny cells, measuring microalgal growth accurately is challenging. However, there are a number of methods available to calculate the kinetics of microalgal growth. Microalgal growth is typically measured in terms of biomass, cell count, or the amount of pigments and proteins produced over a certain time frame. Some of these techniques are simple, while others call for complicated equipment.

Therefore, the scale and accessible laboratory facilities determine which growth measuring approach is used. Spectrophotometry, gravimetric biomass calculation, cell enumeration using counting chambers, and flow cytometry are standard methods for measuring microalgal growth **[38].** The density of the culture and the amount of chlorophyll affect spectrophotometric measurements of microalgal growth. Although it is a quick and simple procedure, accuracy may be impacted by suspended and dissolved solid particles.

The growth of microalgae was observed within the visible region of the electromagnetic spectrum, specifically at a wavelength of 400–700 mm. To improve the accuracy of measurements, gravimetric or counting techniques can be used in conjunction with this method to create calibration curves.

The evaluation of microalgal growth gravimetrically relies on the weight of the dry or wet biomass of cells **[39]**. To obtain the cell biomass, it is first concentrated through centrifugation, and the weight of the resulting pellet is determined. In the case of dry biomass measurement, the separated pellets are dried before being weighed.

The cells are scrubbed before to gravimetric assessment since other suspended particles and media parts could weaken the precision of development estimation. Another specification technique includes minute counting of microalgal cells put on particular slides with chambers. However, counting can be inaccurate if cells or other

contaminants that resemble microalgal cells are present .The automated method of flow cytometry analyses the fluorescence and light scattering of microalgal cells.

The human counting errors associated with the counting chamber approach are significantly reduced by this extremely complex methodology. However, one disadvantage of flow cytometry is the requirement for expensive, high-tech equipment.

2.5.1 Different types of culturing techniques for microalgae

There are numerous methods for growing microalgae. The chosen micro-algal strain and its application heavily influence the method choice. The fundamental aspects of cultivating microalgae include selecting appropriate culture media, establishing a suitable cultivation system, and maintaining optimal abiotic and biotic cultivation parameters.

2.5.2 Open Ponds and Raceways

The oldest method of growing microalgae dates back to 1950 and involves the use of natural ponds or lagoons. Later, this method was changed to employ artificial raceways, but it still functioned similarly to a natural system. These microalgal culture systems can be broadly divided into artificial ponds and natural open ponds**[40].** The raceway ponds pond cultivation system can be either an open type or a closed type . Raceway ponds are essentially length- and depth-variable, 1- to 100-cm-deep, shallow concrete pits lined with thick plastic sheets. For the smooth distribution of sunshine, nutrients, and algal culture, paddle wheels are included in the raceway system.

Microalgae cultivation in open ponds or raceway ponds has a number of benefits, including scalability, simplicity of design, large production capacities, and cheaper operating and maintenance expenses.

However,the principal downsides of open lake frameworks incorporate microbial pollution from microscopic organisms, phycophases, parasites, zooplanktons, and the development of unfortunate algal species, evaporative misfortunes, low air CO_2 dissemination, the requirement for a huge land region, unfortunate efficiency, the limitation to a couple of strains, and outrageous weather patterns (downpour, temperature, and light powers).

Thermal stratification or unequal distribution of nutrients **[41]** can occur when there is a prolonged power outage or if the paddle wheels in a raceway malfunction or break. However, closed raceways are more resistant to contamination and environmental disruptions compared to open racetrack ponds.

2.5.2 Photo-bioreactors -

To overcome the issues with open pond systems, closed Photobioreactor (PBR) or Lumostat systems have been developed. PBRs are made of glass, Plexiglas, or transparent PVC and equipped with internal or exterior illumination, controlled gas exchange, media mixing, and circulation. Depending on the strain, final biomass application, and scalability, PBR sizes and types may vary. In these reactors, critical factors affecting algae growth, such as light type, intensity, duration, temperature, mixing, gas exchange, CO_2, and evaporation loss, can be efficiently regulated **[42]**. Due to reduced susceptibility to contamination from bacteria and other sources, axenic algae monocultures can be more easily cultivated in closed PBRs.

The design of PBRs is versatile as they can be used both indoors and outdoors and can be customized to meet specific needs and growth conditions. However, the main disadvantage of these systems is the significant capital expense required for installation and maintenance. Closed bioreactors come in various types, such as flat panel (flat plate), vertical/inclined tubular, helical, airlift, horizontal/serpentine tube airlift, bubble column, membrane, or hybrid PBRs.

2.5.3 Factor's affecting microalgal growth -

- Photoperiod quality :- Growth, nutrient uptake, and all metabolic processes in photoautotrophic organisms are directly influenced by the matching light quality, its intensity, and photoperiod. This relationship can be illustrated by a light response curve or photosynthesis.Algal cultures must have access to light because, according algal cells' capacity for photosynthetic activity rises as light intensity rises up to a certain point known as the light saturation/threshold**[43]**. Higher intensities harm the chloroplasts' light receptors, which causes photoinhibition and thus lower biomass synthesis. Algal cells produce more biomass and contain more carotenes after being exposed to high levels of radiation over extended periods of time, but light-harvesting pigments like chlorophyll diminish. Regarding the white, blue, green, and red wavelengths, photosynthetic and metabolic reactions differ and are species-specific

- Temperature affecting :- According to, changes in metabolic cycles, compound energy, the compliance of fundamental designs, and so on, as well as species strength, all straightforwardly influence the development of microalgae. Contingent upon other ecological variables, the temperature-subordinate

development of green growth might be outstanding or straight. Algal cells can adapt to a wide range of temperatures and exhibit a variety of physiological and biochemical reactions, however this varies according on the species**[44].** The microalgae can be roughly split into two types based on their resistance to temperature changes: eurythermal, which can withstand large temperature swings, and steno-thermal, which can withstand small temperature shifts . In general, higher temperatures are linked to accelerated photosynthetic rates and high nutrient uptake.

- Ph of the used media for growth:- The absorption of essential nutrients, such as nitrate and phosphate, is significantly impacted by the pH level of the algal culture media. pH affects various processes, including the speciation of inorganic carbon sources like carbon dioxide ionization, natural metabolites precipitation of phosphates, solvency, and accessibility of minor components. Additionally, since different algal species are pH-sensitive and have preferences for different inorganic carbon sources, pH also regulates the dominance of various species in mixed populations.
- Carbon dioxide Sequestration rate and oxygen exchange :- Algal cells contain about 45–50% carbon and need to continuously absorb carbon to grow. By sparging gas with CO_2, carbon can be produced **[45]**.Dissolved CO_2 is transformed into carbonic acid during gas exchange, which is then used by algal cells for photosynthesis. Due to increased oxygen accumulation, reactive oxygen species, and photorespiration, the high oxygen level causes irreversible photo-oxidative damage to the photosynthetic equipment.

2.5.3 Contamination - Micro-algal culture contamination is a major challenge in the cultivation of algae. Open pond systems are particularly susceptible to bacterial and chemical contamination. Additionally, algae have a tendency to amplify the levels of various chemical contaminants present in the growing media **[46]**. Studies have identified exudates and metabolites generated by algae, such as the polyunsaturated fatty acid, linoleic acid, and monounsaturated oleic acid, that can act as chemical pollutants and inhibit growth.

CHAPTER-3

MATERIALS AND METHODS

Materials used - Distilled water, Autoclaved tips (1000µ, 200µ, 20µ), Pipettes (1000µ, 200µ, 20µ), Conical flasks (1000ml, 500ml, 250ml), Measuring cylinders, Beakers, Spatula, Glass slide, Cover slips, Micro-plate, Hemocytometer, Tissue paper, Falcon tubes, Petri plates, Inoculating loop.

Instruments used - Centrifuge, Weighing balance, shaking incubator, Thermo-multiscan, Laminar air flow, -80°C freezer, 4°C freezer, Lypholizer, Vortex machine, Ph metre, Microscope (40x, 100x).

Chemicals used - Ethanol, Nutrient agar, BG 11 media salt.

- Chemicals used for B.B.M.(Bold Basal Media) in following ratio for 100ml ofmedia as shown in table 1**[47]**

Table 1 -

Volume for media	**Chemical salts**	**Concentration(g) per 10ml of distill water**
1 ml	Sodium nitrate	0.25g
1 ml	Magnesium sulfate heptahydrate	0.0075g
1 ml	Sodium chloride	0.025g
1 ml	Dipotassium hydrogen phosphate	0.075g
1 ml	Potassium dihydrogen phosphate	0.175g
1 ml	Calcium chloride Dihydrate	0.025g
0.1 ml	Zinc sulphate heptahydrate	0.0882g
	Manganese chloride tetrahydrate	0.0144g
	Molybdenum trioxide	0.0071g
	Copper sulphate pentahydrate	0.0157g

	Cobalt(II) Nitrate Hexahydrate	0.0049g
0.1 ml	Boric acid	0.114g
0.1 ml	EDTA disodium salt dihydrated	0.5g
	Potassium hydroxide	0.31g
0.1 ml	Ferrous sulphate heptahydrate	0.0498g
	Concentrated sulphuric acid	0.1ml

- Chemicals used for T.A.P.(tri acetate Phosphate) media in following ratio for 100ml of media as shown in table 2 **[48]**

Table 2 -

Volume for media	**Chemical components**	**Concentration(g) per 10ml of distill water**
0.242g	Tris base	
2.5ml	Ammonium chloride	0.15g
	Magnesium sulphate heptahydrate	0.04g
	Calcium chloride dihydrate	0.02g
0.1 ml	Dipotassium hydrogen phosphate	2.88g
	Potassium dihydrogen phosphate	1.44g
0.1 ml	Disodium EDTA	0.5g
	Zinc sulphate heptahydrate	0.22g
	Boric acid	0.114g
	Manganese chloride tetrahydrate	0.05g
	Ferrous sulphate	0.05g

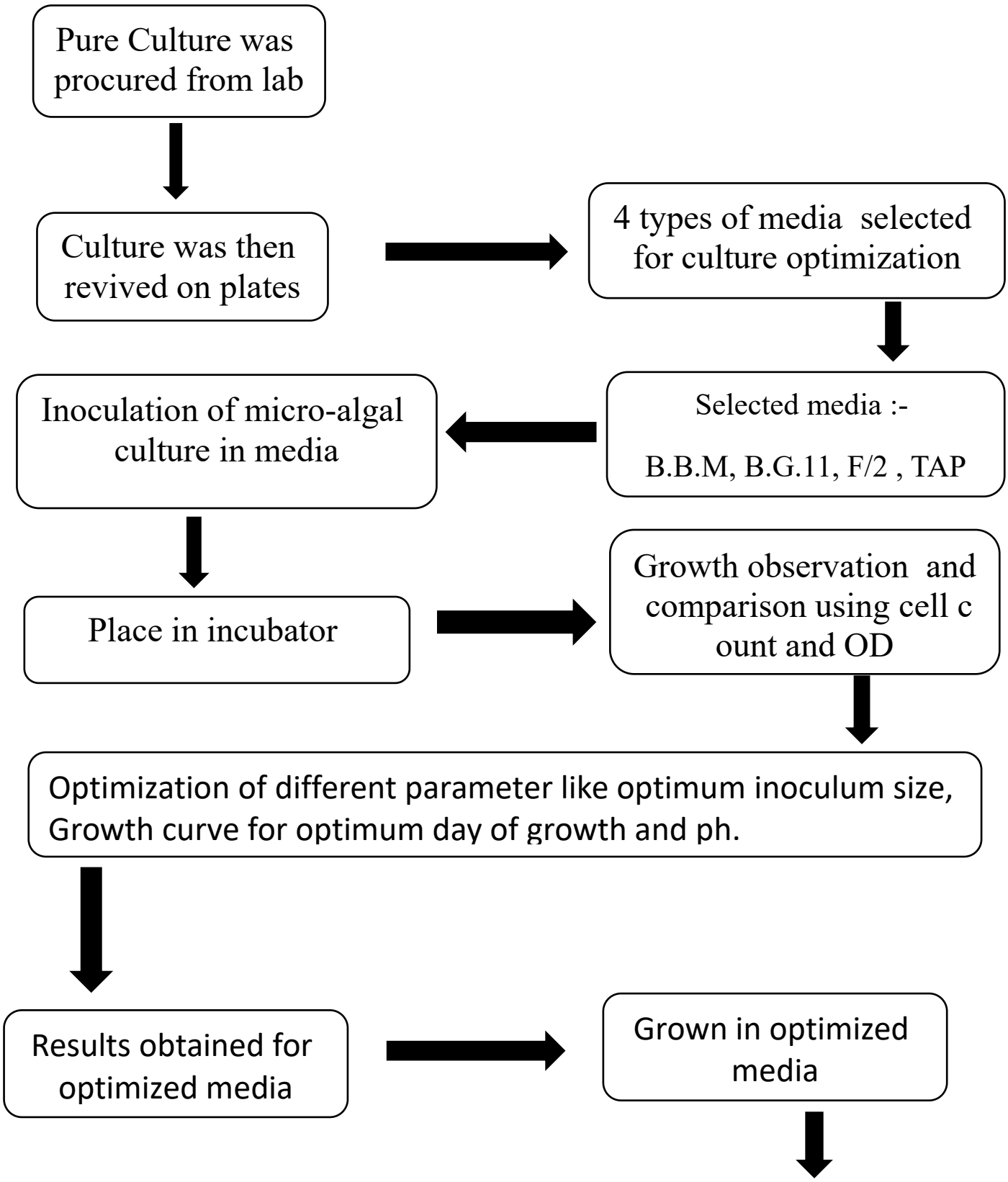
	heptahydrate	
	Cobalt(II) chloride	0.016g
	Copper sulphate pentahydrate	0.016g
	Ammonium Phosphate	0.011g
0.1 ml	Acetic acid	

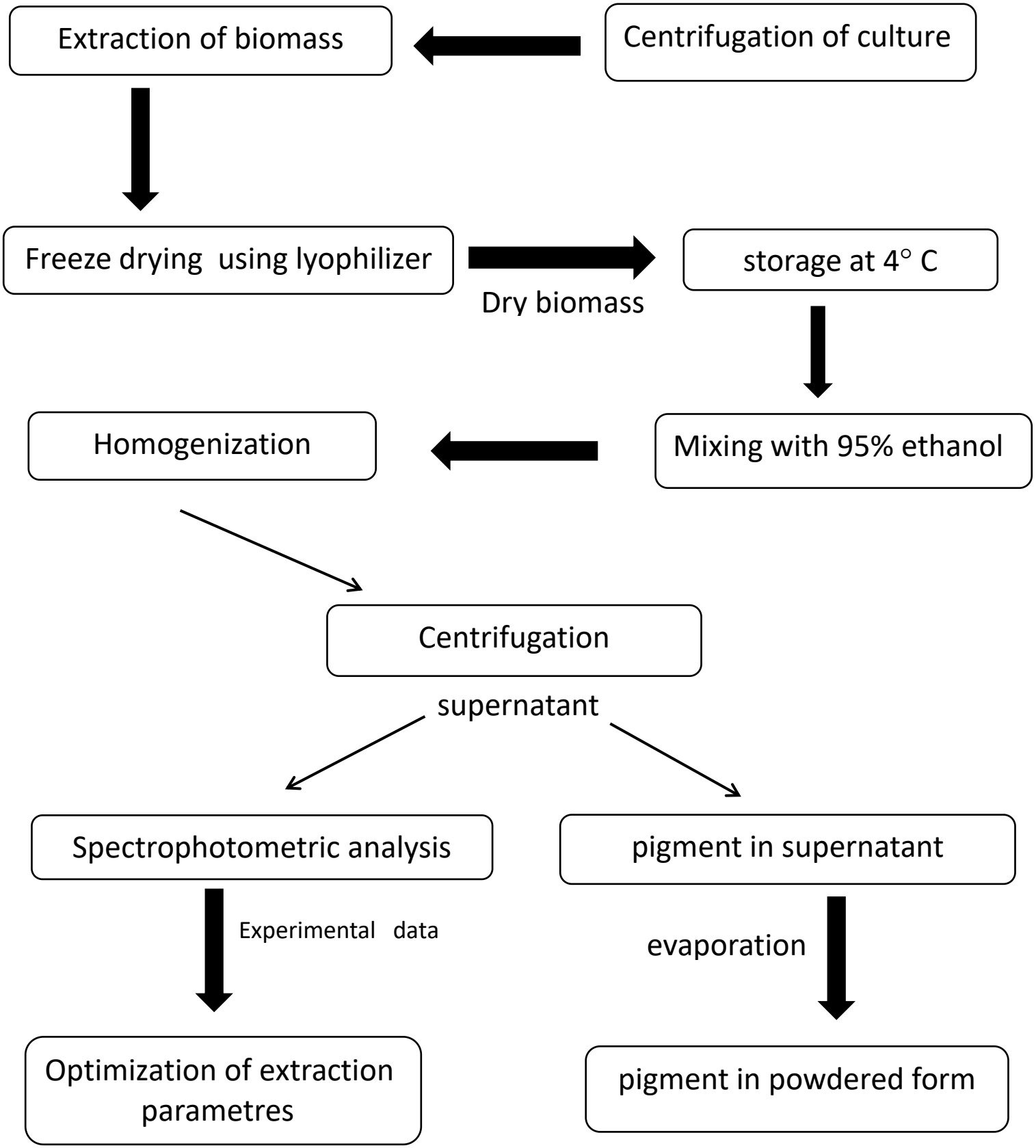
- Chemicals used for F/2 media in following ratio for 100ml of media as shown in table 3 **[49]**

Table 3 -

Volume for media	Chemical components	Concentration(g) per 10ml of distill water
100µl	Sodium nitrate	0.75g
100µl	Monosodium phosphate monohydrate	0.05g
100µl	Sodium meta-silicate	0.3g
100µl	Disodium EDTA	
	Copper sulphate pentahydrate	0.098g
	Sodium molybdate dyhydrate	0.063g
	Zinc sulphate heptahydrate	0.22g
	Iron(III) chloride hexahydrate	
	Cobalt chloride Hexahydrate	0.1g
	Manganese chloride tetrahydrate	1.8g
50µl	Cobalmin	10mg
	Biotin	10mg
	Thiamine HCL	200mg/in total media

Methodology- This is a flow chart explaining the methodology followed for isolation of microalgae from mixed culture, Media optimization for best growth and Pigment extraction.




Extraction of biomass
Centrifugation of culture
Freeze drying using lyophilizer
Dry biomass
storage at 4° C
Homogenization
Mixing with 95% ethanol
Centrifugation
supernatant
Spectrophotometric analysis
pigment in supernatant
Experimental data
evaporation
Optimization of extraction parametres
pigment in powdered form

Method Followed For Isolation of microalgae from mixed culture [37]

- **Step 1** 400 ml of B.G. 11 media was madeas shown in figure 2(a). Divided in 2 sections for agar plating in figure 2(b) and suspended culture.

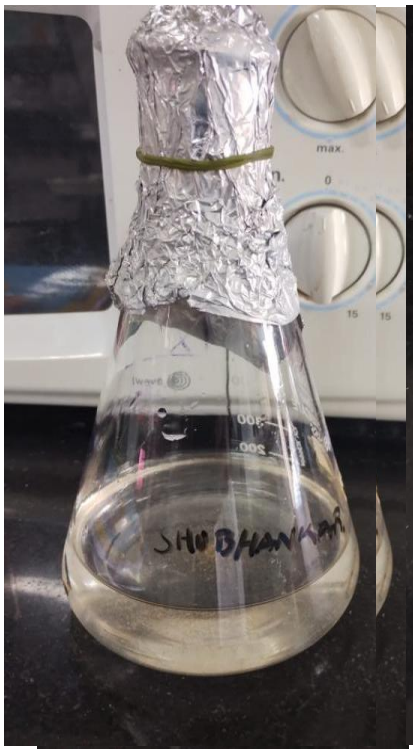


Figure 2(a) B.G. 11

Figure 2(b) :- Agar plates

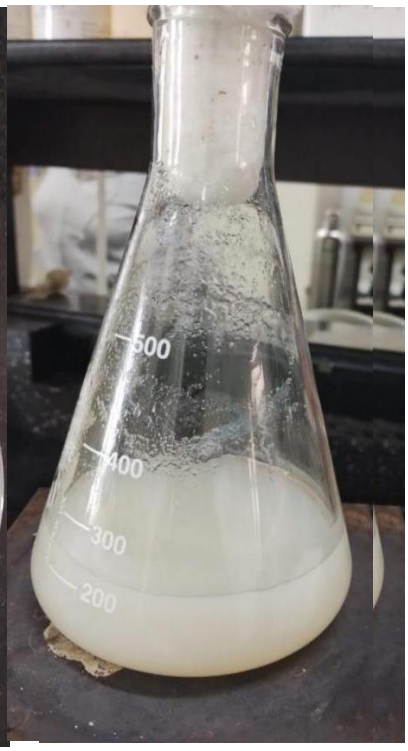


Figure 2(c) :- Agar media

- **Step 2**- Mixed culture was revived on agar plates and the growth of culture was observed after 8 daysas shown in figure 3(a) and 3(b) .

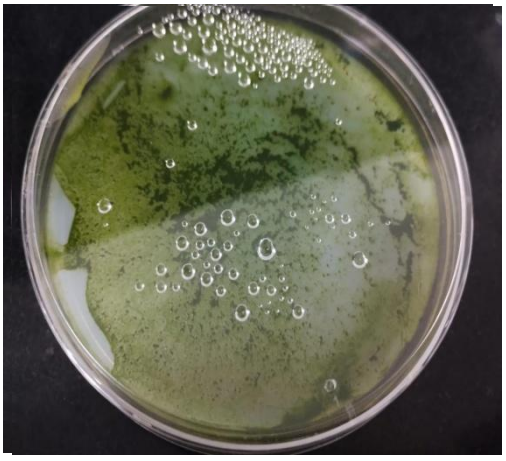


Figure 3(a) :- Mixed culture grown on agar plate 1

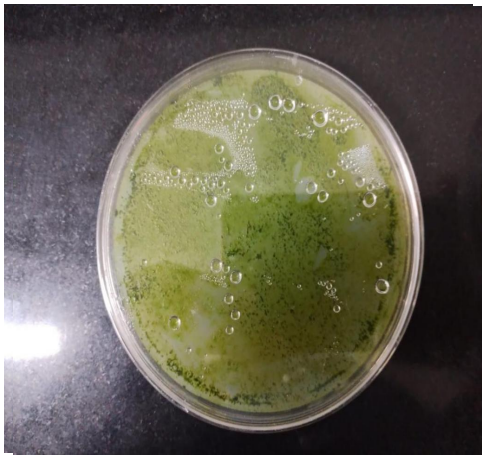


Figure 3(b) :- Mixed culture grown on agar plate 2

- **Step 3**- From mixed culture further streaking was done to isolate pure colony of microalgae.

Method Followed for Culture/Media Optimization

STEP 1 4 types of media were prepared**[50]**

- B.G. 11 Media
- B.B.M. (Bold basal media)
- F/2 Media
- T.A.P. Media (tris acetate phosphate)

All media were prepared in quantity of 150 ml following the concentration mentioned above in chemicals used . As shown in (Figure 4) F/2 media was prepared Following the composition metioned in (Table 3) .

Figure 4 :- F/2 media preparation

- **STEP 2** All media i.e. B.B.M. , T.A.P. , F/2 , B.G.11 Were inoculated with 3 different microalgal strains A,B, and C (as shown in figure 5) which were procured from lab .

Figure 5 :- 3 microalgal strains

- **STEP 3** Growth was observed (as shown in figure 6(a)) and 3 different parameters absorbance, haemocytometre cell count (as shown in figure 6(b)) and bio mass weight were taken for observation and comparison .

Figure 6(a) :- Observed green growth in 4 medias

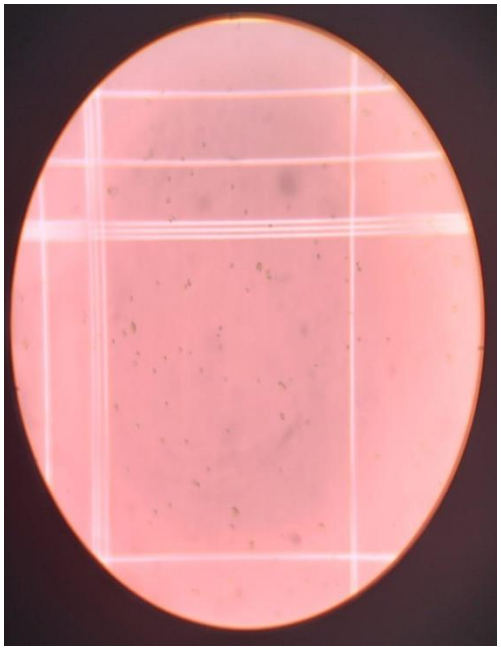


Figure 6(b) - Haemocytometre cell count

- **STEP 4** Culture was Freeze/dried (as shown in figure 7) (lypholized) and biomass was extracted for biomass weighing parameter .

Figure 7 :- Freeze dried cells

Method followed to make the growth curve on the optimized media

- **STEP 1** Optimized media was inoculated with strain A culture and cell count and absorbance readings were noted after every 24 hours at 600nm**[38].**
- **STEP 2** To make the growth curve absorbance at 600nm after 24 hours on thermo-multiscan and cell count by haemocytometre were noted on day 1.
- **STEP 3** Same procedure was repeated on day 2, day 3, day 4 etc untill the growth of microalgal culture started receding (I.e. decreasing).
- **STEP 4** The day when the cell count was the maximum was noted for further parameter.

Method followed to measure the best inoculum size [43]

- **STEP 1** 500ml of optimized media was made following the concentration mentioned above in chemicals used and it was divided in 5 flasks 100 ml each **[43].**
- **STEP 2** It was then inoculated with Strain A of microalagal culture each with different concentration .

Figure 8 :- Growth in B.B.M. with 5 different concentration of

- **STEP 3** Starting from 0.5µl in flask 1 to 1ml, 1.5ml, 2ml, 2.5 ml in flask 5 respectively (as shown in figure 8) .
- **STEP 4** Cell count was noted at day 7 to find out the maximum growth at particular inoculum size.

Method followed for the pigment extraction

- **STEP 1** Optimized culture was selected (as shown in figure 8) and used for pigment extraction process **[32]**.

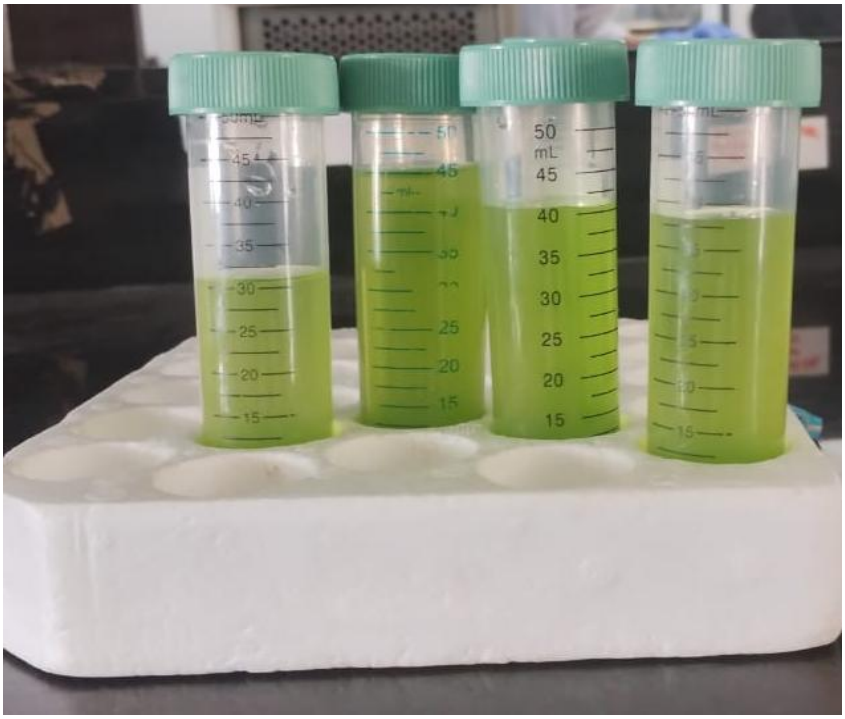


Figure 8 - Optimized culture

- **STEP 2** Biomass was extracted through centrifugation at 7500rpm for 15 minutes in the form of a pellet (as shown in figure 9) .

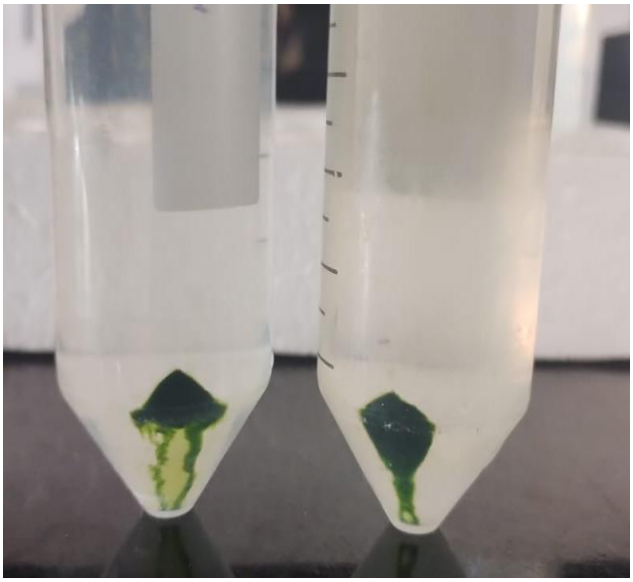


Figure 9 - Biomass in pellet

- **STEP 3 :** Biomass was then dissolved with 10ml of 95% ethanol (as shown in figure 10) and was centrifuged at 7500 rpm for 15 minutes .

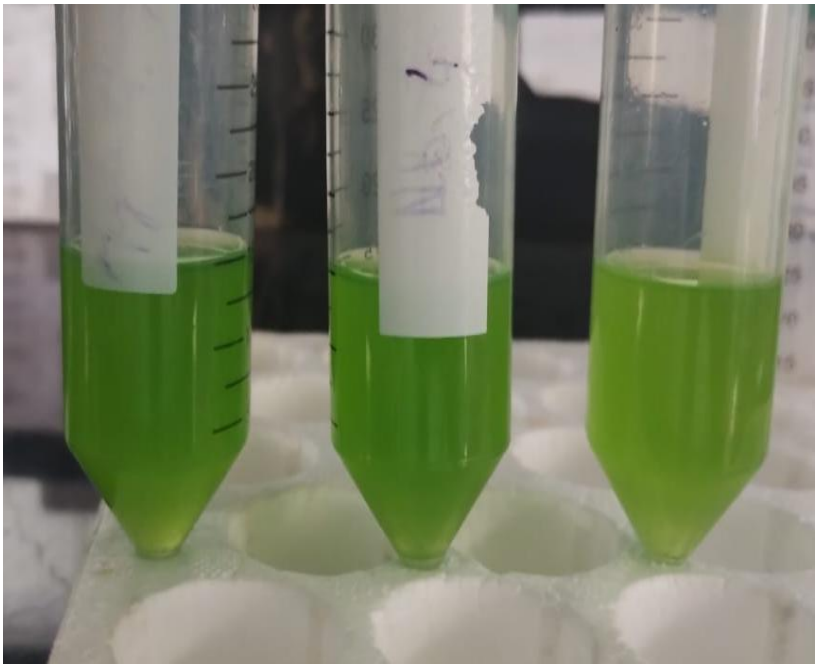


Figure 10 biomass dissolved in etahnol

- **STEP 4** After centrifugation pigments was extracted in supernatant(ethanol) (as shown in figure 11) Absorbance at 665 nm, 649 nm and 470 nm was measured to obtain the yield of the pigments extracted.

CHAPTER-4

RESULTS AN DISCUSSION -

Results for isolation of pure colony of microalgae -

- **Step 1** After the streaking the plates from mixed culture and incubating the plates for 7 to 8 days following growth was seen (as shown in figure 12(a)) .

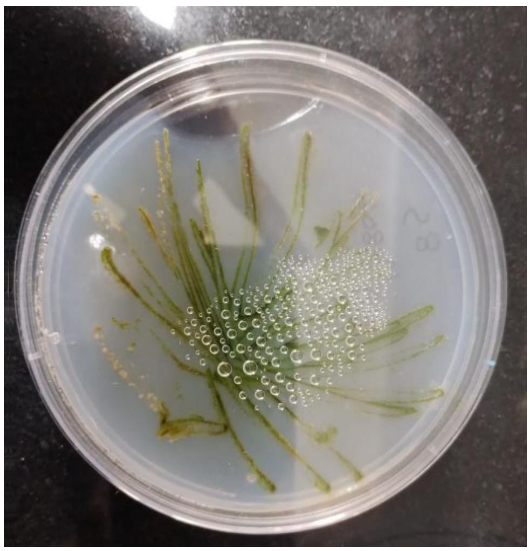


Figure 12 (a) :- Streaked plates

Figure 12 (b) :- All three Streaked plates with different concentration of mixed culture

- **STEP 2** After the growth was seen the microalgae was then seen under microscope using 100x lens (as shown in figure 13).

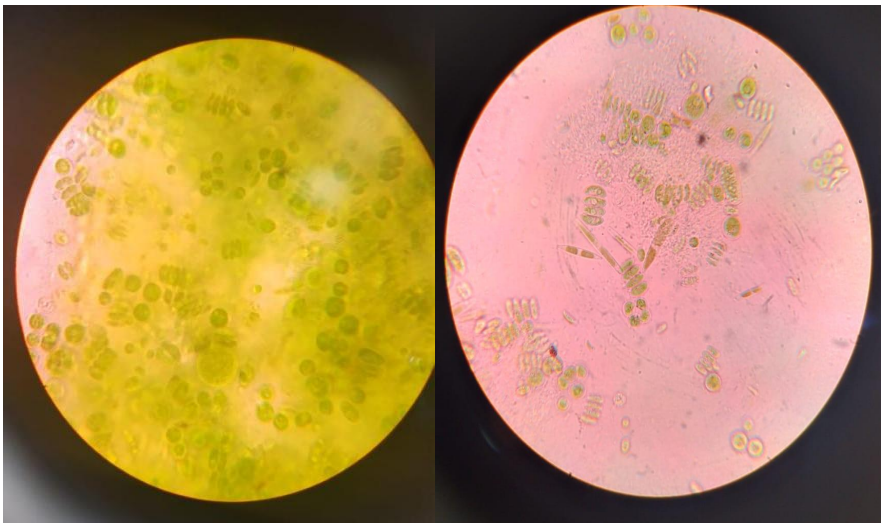
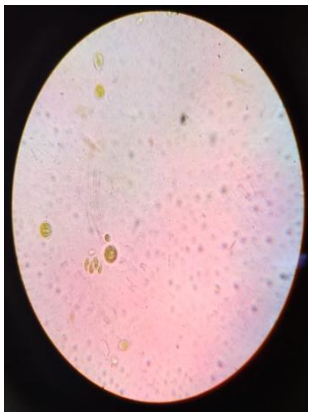


Figure 13 Mixed culture Cells under microscope (100x lens)

- **STEP 3** Streaking was repeated so as to isolate the different cells (as shown in figure 14(a) streaking was repeated) from each other .



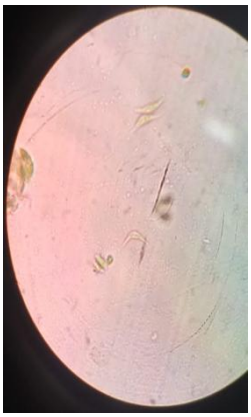


Figure 14 (a) :- Cells under microscope (100x

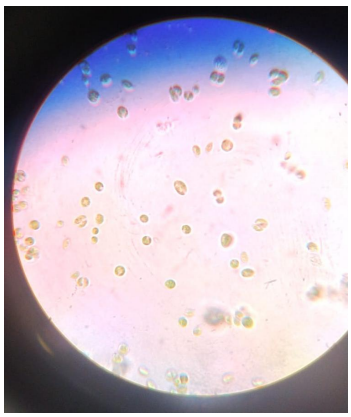


Figure 14(b) - Strain A under microscope (100x lens)

After the pure culture was procured from lab strain A (as shown in figure 14(b) was selected for optimization .

Results obtained from media optimization

STEP 1 After about 7-8 days when growth was observed in all media (as shown in figure 15) all the micro-algal cultures were then taken out and readings were noted with different parameters like Absorbance at 600 nm, Haemocytometre cell count, and Biomass weigh .

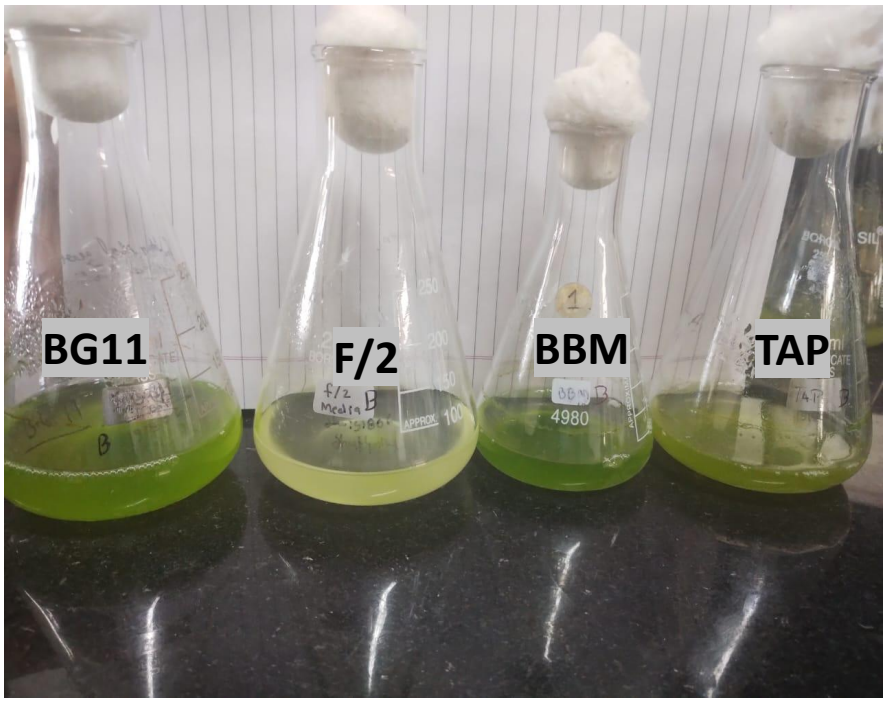


Figure 15 - Growth observed in B.G.11,B.B.M.,T.A.P.,F/2 media

Out of all the three Strains A,B and C for strain A was selected which showed best growth for B.B.M.following the parametres.

- **Absorbance** - readings at 600 nm for Strain A culture shows B.B.M. with the highest cell density (as shown in table 4).

Table 4 -

Media	**B.G.11**	**B.B.M.**	**T.A.P.**	**F/2**
Absorbance at 620	0.23	0.26	0.24	0.18
Control	0.05	0.051	0.062	0.063

- **Haemocytometre Cell count** -After counting cells it shows that Bold basal media has the highest cell count (as shown in table 5) out of all medias .

Table 5 -

B.G.11	**B.B.M.**	**T.A.P.**	**F/2**
88×10^5 cells/ml	126×10^5 cells/ml	16×10^5 cells/ml	30.25×10^5cells/ml

- **Biomass weight -** After freeze drying the culture and extracting the biomass was weighed (as shown in table 6) and B.B.M. gave the highest biomass .

Table 6 -

B.G.11	B.B.M.	T.A.P.	F/2
9.59 mg	16 mg	7 .45 mg	2 mg

- These three parameters gave the result for the most optimum media for growth now for further optimization i selected bold basal media and made the growth curve to get the day at which growth is at maximum.

Discussion -

Comparing my results with the results taken from the references it has been seen that their optimized media was B.G.11 with is 0.2 g of dried cell biomass in 1000ml of bg 11 media**[51]** in comparison I got 16 mg of dried cell biomass in 100 ml of Bold basal media at the same time their cell count for B.G.11 media is 148

Results obtained from growth curve

- Growth Curve was made by noting cell count after every 24 hours of inoculation until cell count starts decreasing .
- Amount of cells inoculated at day 0 in all 4 medias :- 170 x 10^5 cells/ml
- Following is the cell count starting from day 1 to day 8 (as shown in table 7) .

Table 7 -

Days	B.B.M. cells/ml	B.G.11 cells/ml	T.A.P. cells/ml	F/2 cells/ml
Day 1	0.5×10^5	0.3×10^5	0.2×10^5	0.1×10^5
Day 2	17.8×10^5	5.075×10^5	1.775×10^5	5.325×10^5
Day 3	30.25×10^5	24.75×10^5	2.4×10^5	13.325×10^5
Day 4	59.75×10^5	37.5×10^5	2.2×10^5	14.44×10^5
Day 5	87.25×10^5	54.5×10^5	2.346×10^5	15.5×10^5
Day 6	147.5×10^5	93×10^5	2×10^5	16.75×10^5
Day 7	155.25×10^5	123×10^5	_____	35.25×10^5
Day 8	117.75×10^5	107.4×10^5	_____	24.25×10^5

- After observing the table growth for bold basal media is the best on every day and on day 7 it shows the maximum growth .
- Below are the graph showing the growth curve (as shown in figure 16) in line form and bar graph form . In the graph Y- axis shows the Cell count in the unit of 10^5 cells/ml and X- axis shows the days .

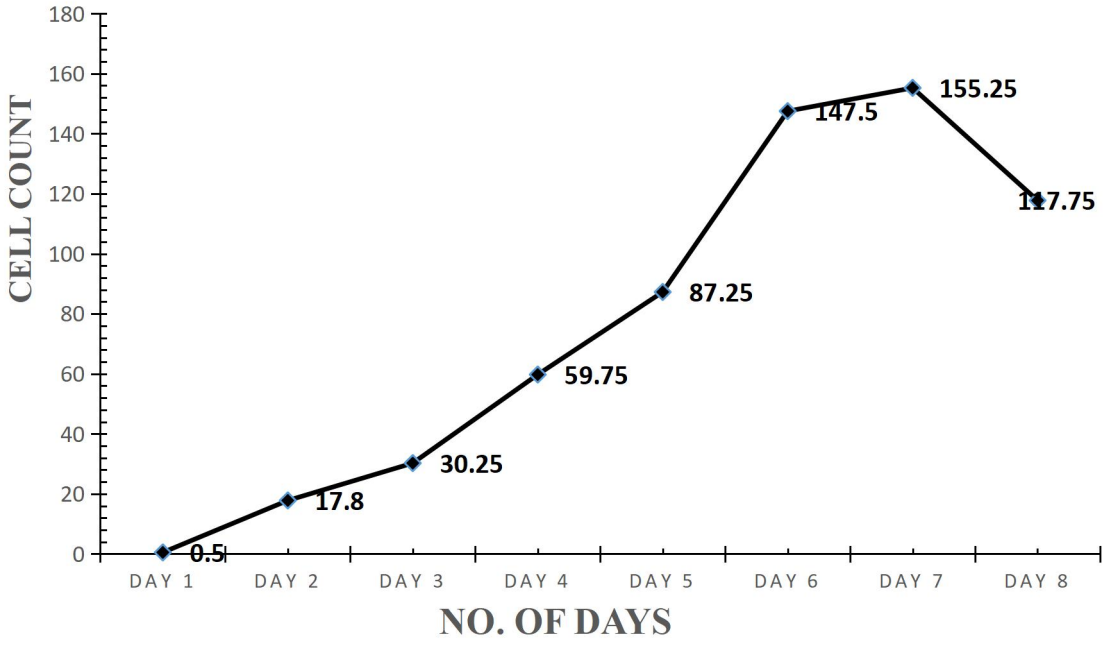


Figure 16 :- Graph shows the growth curve following 8 days using cell count

Results obtained from optimization of inoculum size :-

- Cell count at day 7 for all the inoculum(0.5ml, 1ml, 1.5ml, 2ml, 2.5ml) size for Bold basal media as shown in **figure 17** 2ml inoculum size gave the best growth.

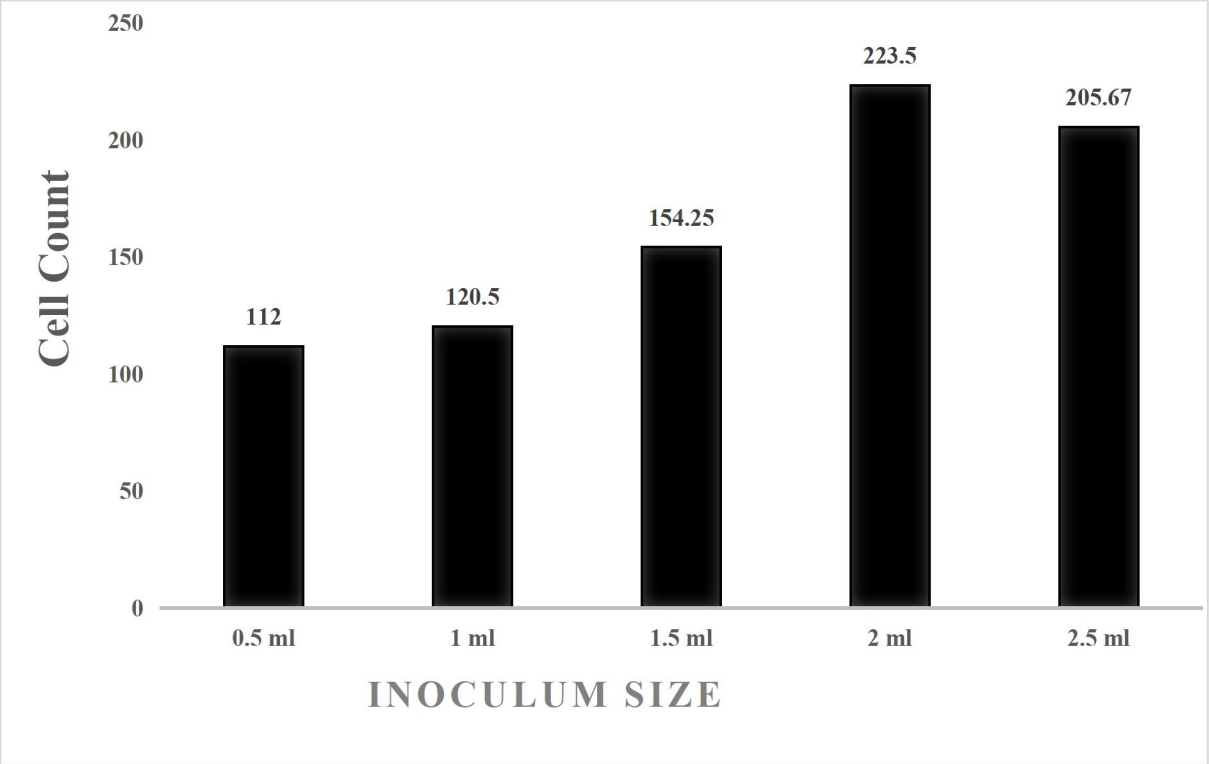


Figure 17 :- Graph shows best inoculum size out of 5

- Hence the inoculum size 2 ml gives the maximum growth for bold basal media .

Results obtained from pigment extraction

- Reading obtained at wavelenght at 470nm, 649nm, and 665nm are 0.2414, 0.1239, 0.1579 respectively as shown in **table 8** .

Table 8 -

470nm	**649nm**	**665nm**
0.24	0.12	0.16

To obtain the yield for Chl a , Chl b and Total Carotenoids Lichtenthaler equation is used :-

- Chlorophyll a (µg/ml) = (13.95 x Absorbance at 664nm) – (6.88 x Absorbance at 649nm)
- Chlorophyll b (µg/ml) = (24.96 x Absorbance at 649nm) – (7.32 x Absorbance at 665nm).
- Total carotenoids (µg/ml) =

((1000 x Absorbance at 470nm) -2.05 x Chl_a - 114.8 x Chl_b) / 245

After calculating the yield for Chl a = 0.97µg/ml , Chl b = 1.01 µg/ml and Total carotenoids = 0.2545 µg/ml

Discussion -

Comparing the results with the reference their total concentration for Chl a is 1.32 µg/ml in B.G. 11 media **[30]** compared to mine which is 0.97µg/ml in B.B.M. For Chl b it is 1.88µg/ml compared to mine which is 1.01 µg/ml and total carotenoi content is 0.3428 µg/ml compared to 0.2545 µg/ml in B.B.M. **[31].** By comparing this we can say the the optimized culture in B.G.11 gave more yield in comparison to my optimized yield in B.B.M. but as the species is not accurately known can be the reason and knowing the exact strain will further help in the optimization process by narrowing down the media of interest and other parameters .

CHAPTER -5

CONCLUSION

Microalgae have recently been used as important sources to extract bioactive chemicals that unquestionably exhibit biological activity and may have health advantages when consumed. As was said in the introduction, microalgae create a vast variety of bioactive pigments. These pigments are used in a wide range of sectors, from food colouring to health-improving goods. Algae are unique among all photosynthetic organisms in that they can exist in freshwater, marine water, and wastewater and range in size from macro to microscopic. These algae have a greater potential to provide innovative and bioactive compounds than conventional biomass sources, which can be used to produce energy, manure, remove toxins, provide nutrition, provide shade, and other bioactive compounds.

Given the experiment performed for the optimization of culture we can come to following conclusion of results.

- The strain A of microalgal culture give best growth in bold basal media out of the 4 medias selected .
- According to growth curve strain A gives maximum growth on day 7 in B.B.M
- According to inoculum size parameter strain A gives best growth in 2ml inoculum size on day 7 in B.B.M.

Extracting the pigments from optimized culture the yield came out to be Chl a = 0.97µg/ml , Chl b = 1.01 µg/ml and Total carotenoids = 0.2545 µg/ml respectively for given pigments .

In the past ten years, there has been a significant increase in awareness about the use of microalgae biomass as a source of colours as well as lipids, polyunsaturated unsaturated fats, nutrients, carbs, cell reinforcements, and so forth. At the moment, many businesses, including those involved in the production of drugs, cosmetics, and nutraceuticals, are utilising microalgae biomass as a sustainable and controllable resource.Regular colours are more reasonable when the detrimental effects of manufactured tones and tints are taken into account. The use of microalgae biomass as a source of colours, lipids, polyunsaturated unsaturated fats, nutrients, carbohydrates, cell reinforcements, and other supplements has gained significant public attention in recent years.

The use of microalgae biomass as a source of colours, lipids, polyunsaturated unsaturated fats, nutrients, carbohydrates, cell reinforcements, and other supplements has gained significant public attention in recent years.

Microalgae biomass is currently being utilised as an unlimited and cost-effective resource by a wide range of businesses, including those in the pharmaceutical, personal care, and nutraceutical sectors. When you consider the unfavourable outcomes of manufactured kinds and hues, normal shades are best. microalgae colours are increasingly being requested as food additives, feed, supplements, and colours due to their essentially higher bioactive potential compared to other natural colours.Additionally, its use as a nutraceutical, cosmeceutical, and pharmaceutical has recently attracted attention. These shades find more extended uses as an anticancer because to their high oxidative enemy potential. Microalgae colors have incredible variety and restricted shades have been portrayed.

FUTURE PLAN

Increase in the population has created a large number of ecological and environmental issues. Excessive increase in the population has been coupled with the excessive increase in the machinery and vehicles and hence the emissions of CO_2 in the environment which has led to the problems like global warming and climate change. Hence our target is to design and construct an experiment in which by the use of microalgae we would be able convert CO_2 into some less harmful or useful products.

Apart from this microalgae are great source of variety of pigments which can be extracted out and be taken in medical as well as commercial usage. Hence our target would be to establish a protocol by experimentation which would be less extensive yet efficient in pigment extraction.

After obtaining the pigments, we would conduct variety of studies using spectrophotometer to calculate the yield of the pigments extracted and other techniques like chromatography to purify and quantify them.

After successfully extracting the pigments, we will try to extract pigments from specific algae to form various nutraceutical and medicinal products

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