ALGAL FLORA IN KERALA FOR WASTEWATER BIOREMEDIATION

BSC PROJECT REPORT

Submitted in partial fulfilment of the requirements for the award of the Degree of Bachelors of Science in Biotechnology by:

PAUL JOSEPH : AM.BT.U3MIB19045

JUNE 2022

UNDER THE GUIDANCE OF

DR. SANJAY PAL

School of Biotechnology

AMRITA VISHWA VIDYAPEETHAM

[Deemed to be University]

Amritapuri Campus, Clappana P.O, Kollam, Kerala – 690525

DECLARATION

I, hereby declare that this project entitled **"ALGAL FLORA IN KERALA FOR WASTEWATER BIOREMEDIATION"** which is being submitted as the BSc. Project of final semester (Semester 6, 2022) is a record of original work done by me under the guidance and supervision of Dr. Sanjay Pal. The results embodied in this project work has not formed the basis for the award of any Degree/ Diploma/ Associateship/ Fellowship or a similar title to any candidate of any University to the best of my knowledge and belief.

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Course : BSc. Biotechnology

Place : Amritapuri, Kollam, Kerala

Date : 13th June 2022

Countersigned by project guide:
Dr. Sanjay Pal

PROJECT CERTIFICATE

This is to certify that the project entitled **"ALGAL FLORA IN KERALA FOR WASTEWATER BIOREMEDIATION"** submitted to School of Biotechnology, Amrita Vishwa Vidyapeetham, Amritapuri, Kollam in partial fulfilment of the requirement for the award of the degree of Bachelor of Science in Biotechnology, by **PAUL JOSEPH (AM.BT.U3MIB19045)** is an authentic work carried out by me at Amrita School of Biotechnology under my guidanceand supervision during the period of June 2022.

The matter embodied in this dissertation has not formed the basis for the awardof any Degree/Diploma/Associateship/ Fellowship to the best of my knowledgeand belief. There is no objection to the content included in the thesis and presented as part of this work.

Signature of the Guide: Institution Seal

Name & Designation: **Dr. Sanjay Pal**

Associate Professor

Place: Amritapuri, Kollam, Kerala

Date: 13th June 2022

CERTIFICATE

This is to certify that the project entitled **"ALGAL FLORA IN KERALA FOR WASTEWATER BIOREMEDIATION"** submitted at Amrita School of Biotechnology, Amritapuri, Kollam, Kerala in partial fulfilment of the requirement for the award of the degree of **Bachelor of Science in Biotechnology** by **PAUL JOSEPH (AM.BT.U3MIB19045)** is an authentic work carried out by me at **Amrita Vishwa Vidyapeetham,** under the guidance and supervision of **Dr. Sanjay Pal** during the period of **June 2022**.

Submitted for examination held on _____13th June 2022_____

Signature of the Examiner: Signature of Dean:

(Institution seal)

Place: Amritapuri, Kollam, Kerala

Date: 13th June 2022

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I offer my prayers and salutations at the lotus feet of our divine mother Sri. Mata Amritanandamayi Devi (AMMA). I would like to express my sincere gratitude to all those who gave me guidance and service to complete my project.

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ORGANISATION PROFILE

AMRITA VISHWA VIDYAPEETHAM

Amrita Vishwa Vidyapeetham is a world-class, research-intensive university with a national and international repute as an educational institution providing higher education in an ambiance rooted in rich Indian Culture and heritage steeped in spiritual values. Sri Mata Amritanandamayi Devi, one of the foremost humanitarian leaders in the world today, is the Chancellor of the University. With seven campuses at Amaravati, Amritapuri, Bengaluru, Chennai, Coimbatore, Kochi, and Mysuru and a new upcoming campus at NCR Delhi (Faridabad) and spread over 1200+ acres with 100 lacs square feet of built-up space, Amrita is one of India's top-ranked private university. Presently, the University has a student strength of over 17000 and 1700 faculty members with 250 doctoral/ highest degree holders amongst them. The National Assessment and Accreditation Council accredited our University with 'A++' grade, the highest possible grade in India under the new system of evaluation. Amrita is the first multi- campus university to achieve this distinction. Amrita Vishwa Vidyapeetham has blossomed into a multi-disciplinary, multi-campus University that offers over 150+ undergraduate, postgraduate and doctoral programs in Engineering, Business, Medicine, Dentistry, Pharmacy, Health Sciences, Biotechnology, Microbiology, NanoSciences, Nursing, Journalism, Information Technology, Arts and Sciences, Education, Hospital Management, Visual Media Studies, Communication, Social Work, and Ayurveda providing our students with outstanding training and overall development. Needless to add, this multidisciplinary character of our University facilitates a laudable synergy within our midst, greatly enabling us all to derive maximum benefit from the expertise of each other. The faculty, many of whom are engaged in research, are committed, enthusiastic and proficient. They form an elite panel of expert practitioners and erudite professors in their respective fields. Guest lectures, seminars, and symposiums add value to studies and offer students the chance to learn from industry experts. Research plays a prominent role at Amrita Vishwa Vidyapeetham. Many centres of excellence have been started in cutting-

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A key aspect of Amrita Vishwa Vidyapeetham is its dedication to bring about change in all levels of society. In this regard, the University puts a great deal of emphasis on research and the development of innovative technology. In collaboration with many national and international organizations and institutes, Biotechnology and Life Sciences, Wireless Networks & Applications, Computer Science & Engineering, Nanotechnology and Energy, Health Sciences, Cyber Security, Artificial Intelligence etc. are some of the areas

Amrita specializes. Amrita regularly collaborates with major laboratories, industry leaders, and agencies like MHRD, TIFAC, DST, ICMR, MOES, DST, ISRO, DRDO, DBT, DIT, DRDO, Microsoft, Hewlett Packard, Media Lab Asia, Infosys, MDS Pharma, Biocon, etc. Live-in-Labs is a multidisciplinary experiential learning program of Amrita University which facilitates the research, development, and deployment of sustainable solutions for current challenges faced by rural communities in India. The program is designed to engage participants in a mutual learning and sharing experience by breaking classroom and lab barriers to implement theoretical knowledge to address real world problems. Founded by the world-renowned humanitarian, Sri Mata Amritanandamayi Devi, the multicampus University was established to provide rigorous academic instruction in an ambience rooted in India's cultural and spiritual heritage.

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Amrita School of Biotechnology, with qualified faculty including several Ph.D.'s recruited from academia and industry around the world, is perfectly poised to offer students an opportunity to develop expertise and succeed in building a career in the exciting areas of biotechnology and related fields. Our cutting-edge curricula with state-of-the-art facilities for teaching and research will provide a solid foundation in the biological sciences. With a vibrant academic environment and a unique approach to learning that involves thought-provoking discussions and constant interaction among students and faculty, the Amrita School of Biotechnology provides an ideal setting for all round development of students to achieve the goal of becoming well-trained in all aspects of Biotechnology and related fields. The school offers undergraduate, postgraduate, dual degree postgraduate programs with University of Arizona, and doctoral studies in Lifesciences. It is approved by TIFAC (Government of India) as a Centre of Relevance and Excellence (CORE) in Biomedical Technology. The school's research programme concentrates on preventive and therapeutic innovations. Dedicated faculty with extensive research experience in labs across USA and Europe, now pursuing research projects of immediate societal impact along with substantial funding from DST, DBT, ICMR, CSIR, MHRD, KSCSTEC and the Bill & Melinda Gates Foundation along with ongoing collaborations with major universities including Oxford, Cambridge (UK) and the University of California (USA) makes Amrita School of Biotechnology as the one of the top Biotechnology institutions in the country. Research focus of Amrita School of Biotechnology spanning a wide spectrum of areas including Cell Biology, Cancer Biology, Computational Neuroscience, Proteomics, Neurophysiology, Phytochemistry, Analytical Chemistry, Phage Biology, RNAi etc. Amrita School of Biotechnology was selected for the Bill & Melinda Gates Foundation-DBT-BIRAC (Biotechnology Industry Research Assistance Council) Grand Challenge India Award for the project to explore novel approaches to sanitation solutions in India. Collaboration on Tata Trust funded Project as part of the Tata Institute for Genetics and Society with University of California, San Diego to study the application of Applied Genetics

for addressing the problems of Antimicrobial Resistance (AMR) is one of the recent achievements of the school.

SYNOPSIS

Most of the wastewater treatment is based on about 100 years old activated sludge technology where aeration (activation) of the wastewater promotes aerobic metabolism mediated fast growth of microbial population with a tendency to settle down, called activated sludge. The removal of sludge leads to nutrient depletion in the wastewater, a major objective of wastewater treatment. Subsequently the pathogen load is reduced by physico-chemical treatment such ultraviolet radiation or chlorination/ozonation. All these processes have a large ecological and economic footprint & hence need to be improved. Algae mediated bioremediation has a great potential in that respect: they grow fast, assimilate carbon dioxide, increase oxygen concentration in water during photosynthesis and thereby promote rapid microbial growth and thereby nutrient removal from wastewater. Algal biomass is potentially rich in metabolites which can be valorised for industry, agriculture or aquaculture. Several algal species have been explored for the wastewater treatment & valorisation, but very few or none in our local geo-climatic context. Exploration of local algal flora naturally growing in local wastewater is likely to add onto the great scope of algal bioremediation of wastewater.

Hence we collected different algal species growing in local wastewater around the university campus in Amritapuri and some other places in Kerala. We identified them by their habitat, morphometric features using taxonomic keys available in the literature. Few strains were reported in “Algal Identification” (an algal interest groups in social media) for feedback. To explore their valorisation potential, we also characterised the growth rate, protein and lipid content of a few species. Previously, our school has discovered one such filamentous algae which had very high antioxidant activity with a potential to be used as sun protection factor (SPF) in cosmetic & food products. DNA isolation method was optimised adopting the CTAB method. Ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCo) large subunit (rbcL) gene primers were used to amplify the DNA for barcoding the algal strains by Polymerase Chain Reaction (PCR). The amplified DNA is being sent for

sequencing to confirm the identity of the algal species which showed the morphometric characteristics and habitat of ***Chaetomorpha minima***. Altogether, 29 algal strains were identified by morphometric measurements and habitats. Growth rate was compared among three strains and lipid content was measured in ***Chaetomorpha*** *species.*

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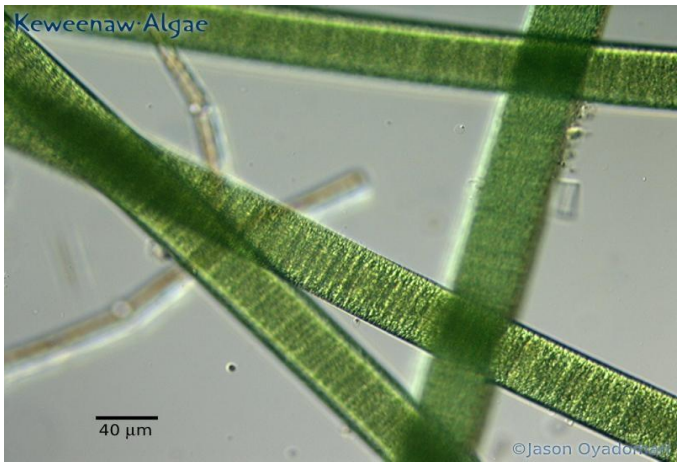
CHAPTER 1
INTRODUCTION

Algae diverse photosynthetic eukaryotes. Microalgae such as Chlorella, Prototheca, and diatoms as well as larger multicellular forms like the gigantic kelp, which can grow up to 50 metres (160 feet) in length, are also found in this area. Most aquatic and autotrophic plants lack stomata, xylem, and phloem cells and tissues, which are found in terrestrial plants. Largest and most intricate freshwater algae are the Charophyta, a group of green algae that includes Spirogyra and stonewort's. Seaweeds are the largest and most intricate marine algae.



Green algae have primary chloroplasts that originate from endosymbiotic cyanobacteria. Diatoms and brown algae have secondary chloroplasts that were formed by endosymbiotic red algae. Reproductive mechanisms for algal species range from basic asexual cell division to more complicated sexual reproduction.

Bryophyte phyllids (leaf-like structures), nonvascular plants' rhizoids, and the roots, leaves, and other organs of tracheophytes are all absent from algae (vascular plants). Both photosynthesis and organic carbon intake are used by the majority of these organisms, whether they are phototrophic or not (i.e. mixotrophic).Photosynthesis in algae produces oxygen, unlike other photosynthetic bacteria like purple and green sulphur bacteria, which produce carbon dioxide as a waste product. Many industrial and traditional uses for algae have emerged as a result of the wide variety of algae types. Calf feed, bioremediation or pollution management, the production of algal fuels or other chemicals for industrial activities, and medicinal and scientific research are some of the additional applications. Assessments in 2020 suggest that algal

applications could play a large role in carbon sequestration while also providing attractive value added items to global economies. Phycology, or algology, is the modern science of studying marine and freshwater algae.

Wastewater treatment is the removal of pollutants such as heavy metals and organic carbon from wastewater that is done using physical, chemical, and biological processes. The bacterial oxidation of carbon compounds (organic) and the nitrification stage of the removal of biological nitrogen are both driven by vigorous aeration in conventional wastewater treatment methods. Aeration is energy-intensive and will take more than half of the total energy expenditure of the wastewater treatment facility. While the oxidation of organic carbon directly emits CO2 into the atmosphere, the energy consumed for aeration indirectly emits CO2. N2O, a strong greenhouse gas, is also generated as a by-product of biological nitrogen removal. In addition, conventional wastewater treatment rarely recovers useable nitrogen and phosphorus.

Algae-based wastewater treatment has been considered eco-friendlier strategy for removing and recovering nitrogen and phosphorus. As photosynthetic creatures, algae create oxygen and absorb CO2. This can maintain a beneficial cycle between bacteria and algae, in which bacteria use algal-produced oxygen to oxidise organic carbon and algae take bacteria-produced CO2. By introducing algae into wastewater treatment operations, the amount of aerationrequired and the accompanying CO2 emissions can be minimised. Algae absorb nitrogen, phosphorus, and photosynthetically fixed carbon throughout their growth. This can minimise the amount of nitrogen and phosphate removed by bacteria, as well as the aeration and N2O emissions that come with it. Furthermore, the captured algal biomass can be converted into usable items likeanimal feed, slow-release fertiliser, or biofuel, thereby converting waste into resources.

Aside from evolutionary classifications, algae can be classified as microalgae or macro algae based on their size. Microalgae are multicellular and visible without a microscope, whereas microalgae are unicellular and cannot be seen without a microscope. Microalgae have traditionally been preferred above

macro algae for wastewater treatment. High-rate algal ponds (HRAPs) and raceway ponds can also be used to cultivate microalgae in the open air. Commercial uses for microalgae farming include the production of biodiesel and bioethanol, along with wastewater treatment. The cost of production of microalgae now limits their economic viability to high-value pigments and nutraceuticals, despite the fact that they have the potential to create commodity fuels and feed. HRAP has economic and environmental advantages over commercial algal ponds in wastewater treatment. HRAPs' main aim is to remove these nutrients from wastewater, whereas nitrogen and phosphorus supplies are a cost to commercial organisations. Furthermore, the availability of organic carbon and bacterial CO2 production in wastewater reduces the requirement for a CO2 supply from outside. Algal pond wastewater treatment falls somewhere between more expensive but intensive activated sludge systems (with effective nitrogen removal) and less expensive but less land efficient and less effective nitrogen removal in facultative lagoon-based wastewater treatment in terms of infrastructure costs and land use.

Microalga HRAPs are technically possible for wastewater treatment, but extracting the microscopic microalga cells remains a significant challenge. Microalgae are naturally difficult to collect due to their tiny cell size, near-neutral buoyancy, and negative surface charge. As a result, in a commercial operation, harvesting microalgae can account for more than 90% of equipment expenditures and 20–30% of overall production costs. In HRAPs, zooplankton grazing on microalgae influences biomass production and nutrient removal efficiency consistently. Alternative culture approaches and photo bioreactors (PBR) have been investigated for wastewater treatment applications to improve system performance. For wastewater treatment, many configurations such as submerged membrane PBR, tubular PBR, and rotating algal biofilm reactors have been examined. However, these systems have drawbacks like difficult construction, high energy consumption, and pollution.

Macro algae, particularly filamentous aquatic algae, offer major benefits over microalgae for wastewater treatment, including harvest ability and improved

predation resistance. Algae's macroscopic size makes biomass collection easier, which reduces the cost and complexity of implementing an algae-based sewer system. Furthermore, the capacity to physically maintain filamentous algae while washing off bacteria and microalgae enables significant culture enrichment as well as the maintenance of a single culture of the desired strain. The ability to increase the filamentous algae's solids retention time (SRT), above the wastewater's hydraulic residence time (HRT) allows stable operations despite flow changes, which can be especially problematic for microalgae during high rain events[3].

Algae remove nutrients from effluent streams from wastewater treatment plants (WWTPs). As perceptions regarding algae change, there is increased interest in algae as a more energy-efficient and less expensive alternative to activated sludge treatments. The large subunit of the carbon dioxide-fixing enzyme RuBisCO is coded for by the rbcL gene, which is used for phylogenetic categorization. The rbcL gene-based phylogeny is conserved across all autotrophic species and provides more resolution than the 18S rRNA gene-based phylogeny. Furthermore, Carbon fixation activities can be assessed using rbcL mRNA levels, allowing diversity studies and functional characterizations to be combined with the same gene[4].

rbcL is a group-specific barcode. Because it codes for a portion of the essential photosynthesis enzyme ribulose bisphosphate carboxylase (RuBisCo), the rbcL gene is a good barcode. One region of its DNA sequence differs greatly from species to species, making it perfect for DNA barcoding. Over the last decade, several plant barcoding regions have been established, but rbcL is particularly well suited to emerging technology[5]. The rbcL gene region was selected to specifically amplify microalgal DNA and prevent the amplification of gene sequences from non-photosynthetic microorganisms. The rbcL gene present in chlorophytes encodes the major subunit of the CO2 fixing enzyme RuBisCO, which is exclusively found in green plants and autotrophic algae, with prokaryotic and eukaryotic bacteria-contaminated in its absence [6].

In biology and biochemistry, a lipid is a large biomolecule that is soluble in nonpolar solvents. Non-polar solvents are often hydrocarbons that are used to dissolve other naturally occurring hydrocarbon lipid molecules that do not dissolve (or dissolve slowly) in water. Lipids serve as structural components of cell membranes, storing energy and signalling[6]. Lipids generated from algae are an appealing potential source of biofuel. However, lipid buildup in algae is a stress response that is intimately linked to growth arrest, limiting output significantly.

Phytoremediation is a way to clean up dangerous contaminants in the soil, air, and water by using living plants. It is proposed as a low-cost, plant-based way to clean up the environment that takes advantage of plants' ability to concentrate elements and compounds from the environment and clean up different compounds. The effect of making things better is very different. Heavy metals that are toxic can't be broken down, but organic pollutants can, and most phytoremediation focuses on them. Several field tests showed that plants can be used to clean up the environment[7] .

Organic pollutants (pesticides, PCBs, DDT, etc.) and heavy metals (Cd, Pb, Se, As, etc.) accumulating in aquatic systems can have serious consequences for the environment and species, affecting the stability of many aquatic ecosystems and posing health concerns to animals and people. Intensive anthropogenic activity is to blame for the build-up of these contaminants. Phytoremediation methods, which remove toxins from the environment using algae or aquatic plants, can assist in solving some of these issues[8].

Chaetomorpha minima

Thallus filamentous, unbranched, uniseriate, epiphytic, yellowish-green to dark green, to 5(–10) mm in height, cylindrical, occasionally slightly constricted at cell walls, (10–)22.5–30(–40) μm in diameter. Cells (20–)45–80 μm length (2–4 diameters long). Basal cell 12.5–15 μm in diameter, to 55 μm long, terminates into tiny disc or lobed disc-like holdfast. Growing on firm substratum, or epiphytically on Bostrychia tenella, Hypnea charoides, Sargassum ilicifolium (Xiaodong Hai), Cladophora vagabunda (Luhuitou) (Luhuitou).

Distribution :- North, Central, and South America, Atlantic islands, Caribbean islands, western Atlantic, Africa, Indian Ocean islands (Maldives), Pacific islands

CHAPTER 2
LITERATURE

2.1 CLASSIFICATION

Classification is the process of categorising organisms based on their evolutionary or structural links. According to the International Code of Botanical Nomenclature (ICBN), plants are classified into Division, Class, Order, Family, Genus, and Species (Sharma 2011). The ICBN recommends the following categories for algae:

DIVISION: Phyta E.g. Chlorophyta
SUB–DIVISION: Phytina

CLASS: Phyceae E.g. Chlorophyceae
SUBCLASS: Phycidae

ORDER: Ales
SUBORDER: Inales

FAMILY: Aceae
SUBFAMILY: Oideae

R. E Lee proposed the latest classification of algae. Lee separated algae into two groups: Prokaryota and Eukaryota, which were further subdivided. Cyanophyta is the only division of Prokaryota, while Eukaryota is further subdivided based on the type of chloroplast membrane that it has..

- **GROUP I: PROKARYOTA**

  Division I : *Cyanophyta*
  Class I : *Cyanophyceae*
  Pigments : Chlorophyll a; phycobilliproteins

- **GROUP II: EUKARYOTA** (The Two Membranes of the Chloroplast Envelope Surround the Chloroplast)

  Division I: *Glaucophyta*
  Endosymbiotic Cyanobacteria perform photosynthesis; represent an intermediate position in the evolution of chloroplasts.
  Division II: *Rhodophyta*
  Flagellated cells absent; storage product is Floridian starch; pigments: chlorophyll a, phycobilliproteins
  Division III: *Chlorophyta*
  Storage product - Chlorophyll-a and b: starch inside the chloroplast

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- **GROUP III: EUKARYOTA** (One Membrane of the Chloroplast Endoplasmic Reticulum Envelope Surrounds the Chloroplast)

  Division I: *Euglenophyta* (euglenoids)
  Pigments: chlorophyll a and b, one flagellum with a spiralled row

  Division II: *Dinophyta* (dinoflagellates)

- ***GROUP IV: EUKARYOTA*** (Two Membranes of the Chloroplast Endoplasmic Reticulum Envelope Surrounds the Chloroplast)

Division I: *Cryptophyta* (cryptophytes)

Between the inner and outer membranes of the chloroplast endoplasmic reticulum, there is a nucleomorph ; pigments: chlorophyll a and c, phycobiliproteins.

Division II: ***Prymnesiophyta*** (haptophytes)

Class I: *Prymnesiophyceae*

- Flagella: two, whiplash
- Pigments: chlorophyll a, C1, C2, fucoxanthin
- Storage product: chrysolaminarin is present in cytoplasmic vesicles. Scales are typically found on cells

Class II: *Synurophyceae*

- Chloroplast endoplasmic reticulum lacking.
- Chlorophyll C 1 absent.
- Plant body - bilaterally symmetrical

Class III: *Dictyophyceae*

- Chlorophyll C1
- Tentacles/ rhizopodia present

Class IV: *Pelagophyceae*

- cells - unicellular; appear as indistinct protoplasm

Class V: *Bacillariophyceae*

- Cell-wall - silicified.
- Pigments: chlorophyll a, C1 and C2; fucoxanthin;
- Flagella: absent
- Storage product: chrysolaminarin

Class VI: *Raphidophyceae*

- Pigments: chlorophyll a and c
- Flagella: two, anterior tinsel, posterior whiplash.

Class VII: *Xanthophyceae*

- There is an eyespot on the chloroplast.

- Chlorophyll a and e are present.
- Flagella: anterior tinsel, posterior whiplash

Class VIII: *Eustigmatophyceae*

- Pigments: β carotene, chlorophyll a, violaxanthin vaucherioxanthin, pyrenoids
- Present polygonal,
- Lacking in zoospores;
- Unicellular; terrestrial and freshwater.

Class IX: *Phaeophyceae*

- Fucoxanthin, chlorophyll a, c, and carotene are the most common pigments.
- Alginic acid, fucinic acid, and cellulose make up the cell wall.
- Pyrenoids: present.
- Flagella: present.
- Reserve food material: laminarin (polysaccharide), manitol (alcohol), fat.
- Pyrenoids: present
- One sort of zoospore is tinsel type
- Sexual reproduction: Isogamy to Oogamy[2]

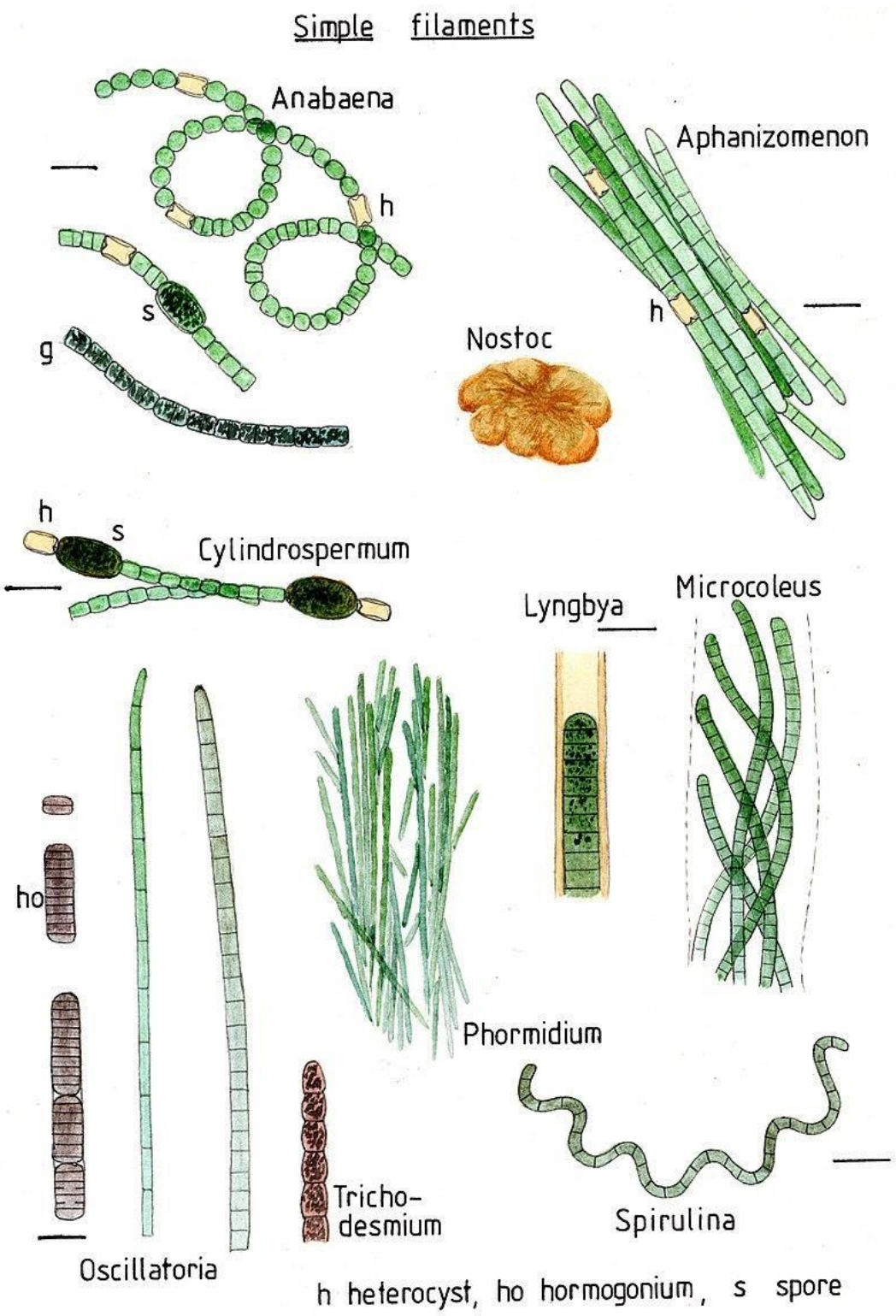

Figure 1 : Simple cyanobacterial filaments Nostocales, Oscillatoriales and Spirulinales

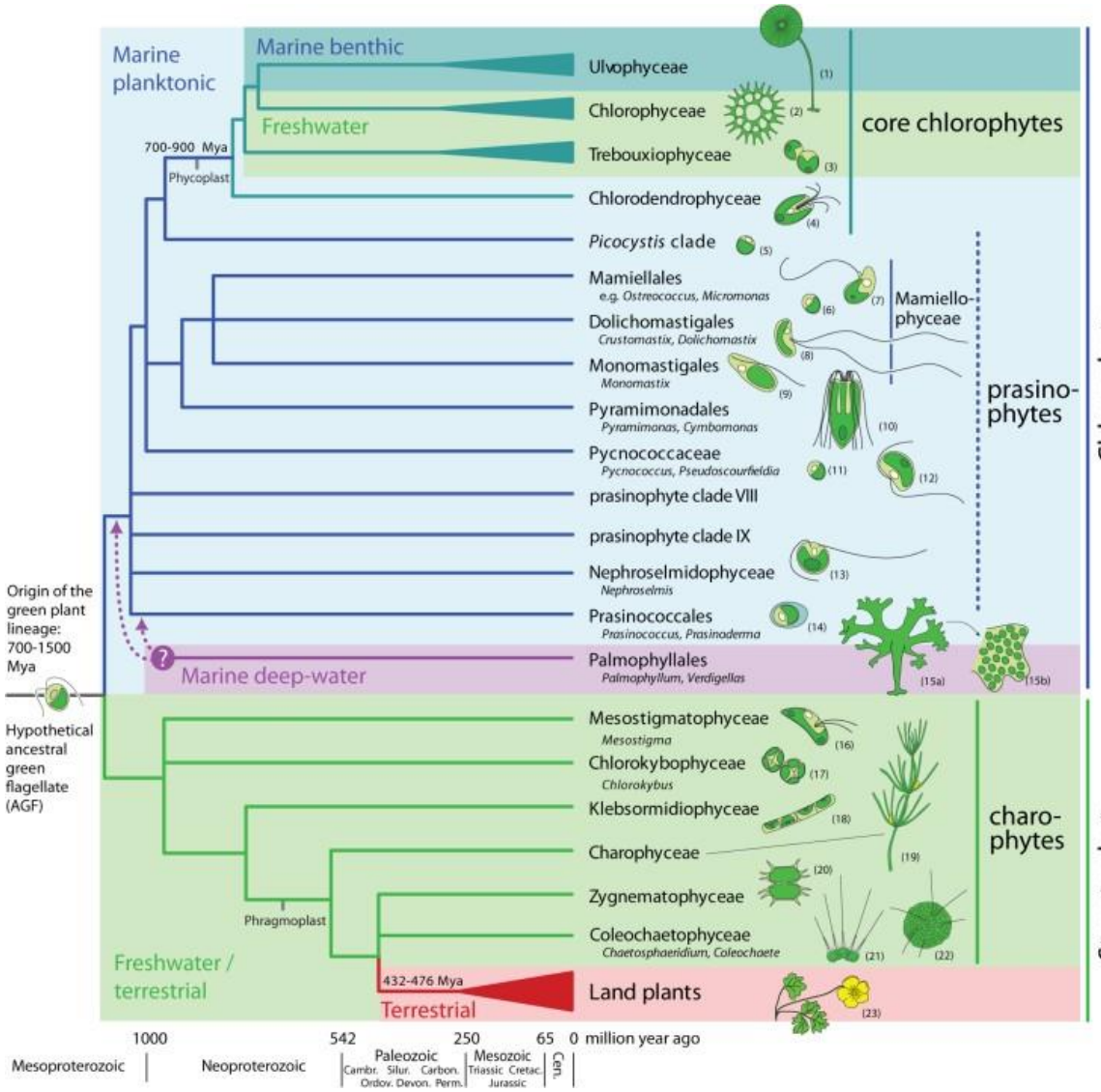

Figure 2 : Phylogeny of major green algae under present study

2.2 AQUAPONICS

The rising global population, demands for increased food production and the resources like land, water, and nutritional supplies are becoming scarcer by the day, there is a pressing need to discover alternative sustainable and reliable methods to provide food. Aquaponics is a hybrid system that combines aquaculture and hydroponics, with the fish metabolic waste being used as nutrients by the plants[9].

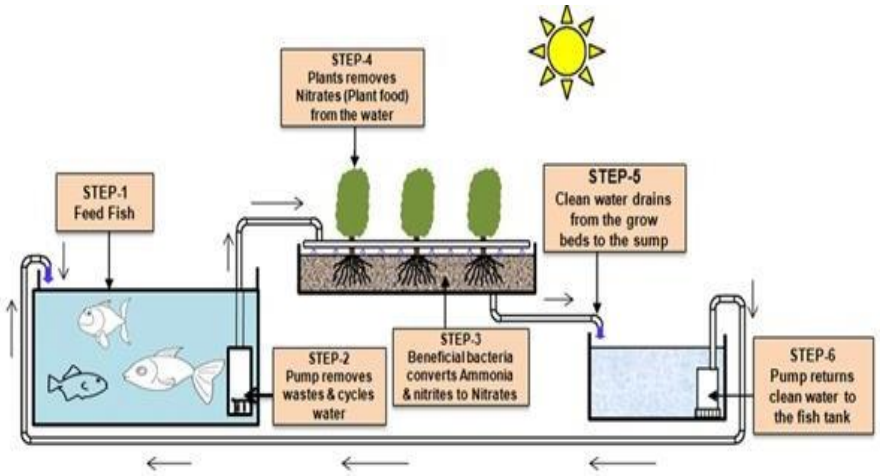

Figure 3 : **Diagrammatic Representation of Aquaponics**[10]

The water from the aquaculture is rich in nutrients and these have to be circulated from time to time so as to facilitate the aeration. Fishes mainly produce nitrogen rich waste, that if accumulated can be harmful even for the fishes, removing this nitrogen from water and using it as a fertiliser for plants can be effective[11]. The main nutrient conversion that occurs in this system is the conversion of ammonia (NH3+) to nitrate (NO3-), via nitrifying bacteria[9]. This technique can also help eliminate the dissolved N and P from aquaculture systems as these are taken up by the plants, through their roots and are incorporated into plant tissues. The wastewater from aquaculture, which is rich in nutrients, is filtered and then introduced into a hydroponic system, where the plant roots and bacteria are fertilised by the effluent. After that, the water is recirculated within the tanks used for the breeding of aquatic organisms. Due to the fact that it is a soilless agricultural system, it has the ability to produce organic crops, which would help to balance out the effects of an increasing

global population and the possible effects that climate change could have on food production.

Aquaponics serves as a model for sustainable food production by following certain principles:

1. The waste products of one biological system serve as a source nutrient for a second biological system.
2. The integration of fish and plants increases diversity and can yield multiple products.
3. Water is reused through biological filtration and recirculation.
4. Local food production provides access to healthy food[12]

Basic components of an aquaponics system:

a. Fish Tank
b. Bio filter
c. Grow Bed
d. Sedimentation Tank

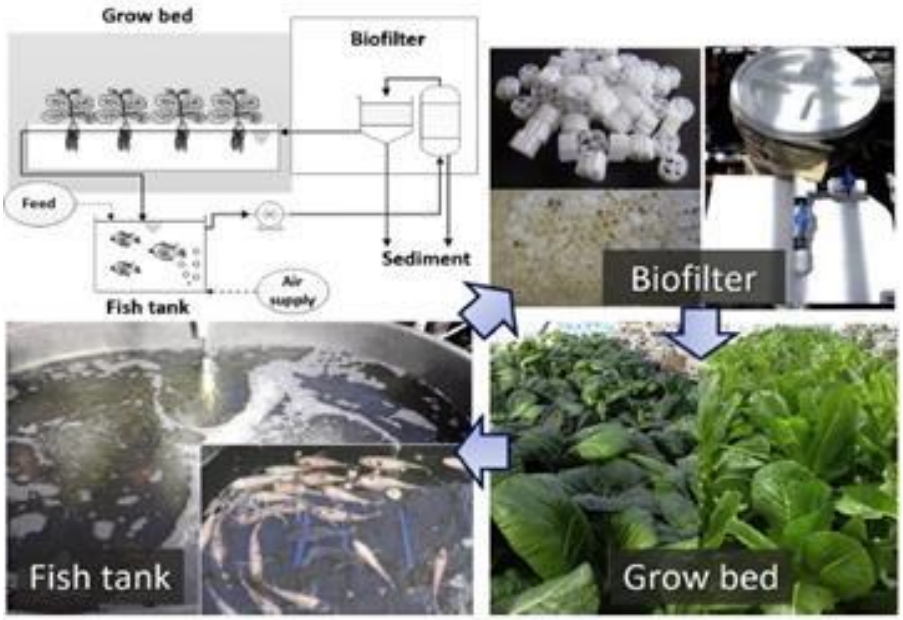

Figure 4 : Parts of Aquaponics[12]

The sedimentation tank performs the function of capturing suspended solid waste, such as fish faeces and biomass (microbes) from the aquaculture effluent.

Hydroponic plants are supplied with fertilisers that are injected into irrigation water on a periodic basis to maintain moist roots and provide a constant supply of nutrients. Some of the most common species of fishes that have been used in aquaponics are: tilapia (Oreochromis niloticus), catfish (Clarias gariepinus), carp (Cyprinus carpio), trout (Oncorhynchus mykiss), and pacu (Piaractus mesopotamicus), these are mainly used due to their high tolerance to suspended solids, nitrite levels above 44.67 mg L–1 and low concentrations of oxygen, O^{12}. In the hydroponic system, the most common type of crops cultivated are the leafy vegetables, due to their high capacity to withstand and grow in high N concentrations, their shorter growth period, their relatively low nutrients requirements and their high demand. Some of the parameters used in assessing composition of aquaculture wastewater include:

1. pH
2. Temperature
3. Odour
4. Organic Matter
5. Biochemical Oxygen Demand
6. Chemical Oxygen Demand
7. Oil and grease content
8. Nitrogen and Phosphorous Content

Biochemical Oxygen Demand (BOD)

It represents the amount of oxygen used up by bacteria and microorganisms as they decompose organic matter under aerobic conditions at a specific temperature.

Chemical oxygen demand (COD)

It is a test that helps measures the amount of oxygen required to chemically oxidise the organic material and inorganic nutrients, such as Ammonia or Nitrate, present in water.

2.3 ALGAE AS FISH FOOD

Fishmeal is very extensively used in feeds for fish as well as other animals. A recent global survey estimated aquaculture[13] consumption of fishmeal at 3724 thousand tonnes in 2006. A recent global survey found that aquaculture used 3,724 thousand tonnes of fishmeal in 2006. Now, it's becoming clearer that continuing to use this natural resource in this way won't be good for the environment or the economy in the long run.

Conventional land-based crops, particularly grains and oilseeds, have been favoured as replacements due to their low costs. These conventional crops have also proven to be successful for some applications when they were utilised as substitutes for a portion of the fishmeal. However, despite the fact that these plant-based alternatives are capable of supporting adequate growth, they can nonetheless cause major alterations in the nutritional content of the fish that are produced. Both macro algae (also known as "seaweeds") and microalgae (such as phytoplankton) are types of algae that could potentially be used in place of fishmeal. One of the most important aspects to take into account is the fact that algae are the foundation of the aquatic food chains that are responsible for the production of the food resources that fish have evolved to ingest [14].

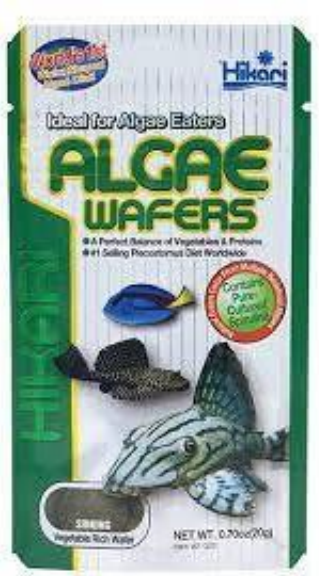

Figure 5 : Algal Fish

Fishmeal contains high-quality proteins containing all the essential amino acids. One of the drawbacks of crop plant proteins that are commonly used in fish feeds is that they are deficient in certain

amino acids such as lysine, methionine, threonine, and tryptophan. The analysis of the amino acid content of numerous algae has found that although there is significant variation, they generally contain all the essential amino acids.

Taurine is usually an essential nutrient for carnivorous animals, including some fish, but it is not found in any land plants. However, although taurine has been much less often investigated than amino acids, it has been reported in significant

quantities in macroalgae such as ***Laminaria***, ***Undaria***, and ***Porphyra*** as well as certain microalgae, for example, the green flagellate ***Tetraselmis***, the red unicellular algae ***Porphyridium***, the dinoflagellate ***Oxyrrhis*** and the diatom ***Nitzschia.***

A few algae are used as sources of pigments in fish feeds. Haematococcus is used to produce astaxanthin, which is responsible for the pink colour of the flesh of salmon. Spirulina is used as a source of other carotenoids that fishes such as ornamental koi can convert to astaxanthin and other brightly coloured pigments. Dunaliella produces large amounts of beta-carotene.

Algae have been recognised as an obvious alternative source of 'fish oil' fatty acids for use in fish feeds especially eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (ARA).

Algae play a key part in aquaculture. The incorporation of microalgae into fish culture tanks housing larvae results in a number of advantageous outcomes. These outcomes include the prevention of larval fish from colliding with the sides of the tanks, an increase in the rate at which predators consume zooplankton, an increase in the nutritional value of zooplankton, and an improvement in the digestive and immune capabilities of the larvae. Some marine fish have biochemical compositions that are well-matched to the biochemical compositions of particular marine microalgae, which in turn meet their nutritional requirements.

According to a number of studies, the addition of even minute quantities of algae to fish food can have a beneficial impact on the growth performance of the fish as well as the efficiency with which they utilise the feed.

To fully take advantage of the enormous potential given by such a varied set of organisms, it will be necessary to do additional research on a variety of algae.

2.4 HIGH RATE ALGAL POND [HRAP]:

HRAP is the open pond system that is usually set up outside to keep natural light coming in. They are shallow and can be used to remove algal species from wastewater. Advantages include: low investment, easy maintenance, smaller energy input, and also very low levels of hydrodynamic stress on the algae.

While designing algal ponds, the first aim is to find an area with minimal impact of rainfall or flood[15].

CO2 can boost algal production and give an increased recovery of nutrients from HRAPs. Controlling the pond water Ph below 8 with high CO2 can help eliminate ammonia[16]. Operational depths of HRAPs range between 0.2 and 0.8m. Paddlewheels with a specific speed is used for gentle mixing on the surface of water. Increasing the amount of algal biomass makes it easier for algae and bacteria to break down organic waste, which makes wastewater treatment more effective[17]. Depending on the soil conditions the bottom of the pond can be lined or unlined and CO2 can be given to a gas sparging pit to create turbulent flow. The ideal temperature for algal development varies by algal species, but is usually between 28- and 35-degree Celsius. When nutrients or light are limited, algae's ideal temperature varies when algae are treated to a quick change in temperature. Maintaining ideal colonial algal species in HRAPs is vital for dependable algal output. It is most probable that HRAPs will be used to control the greenhouse gas and may increase energy output[16].

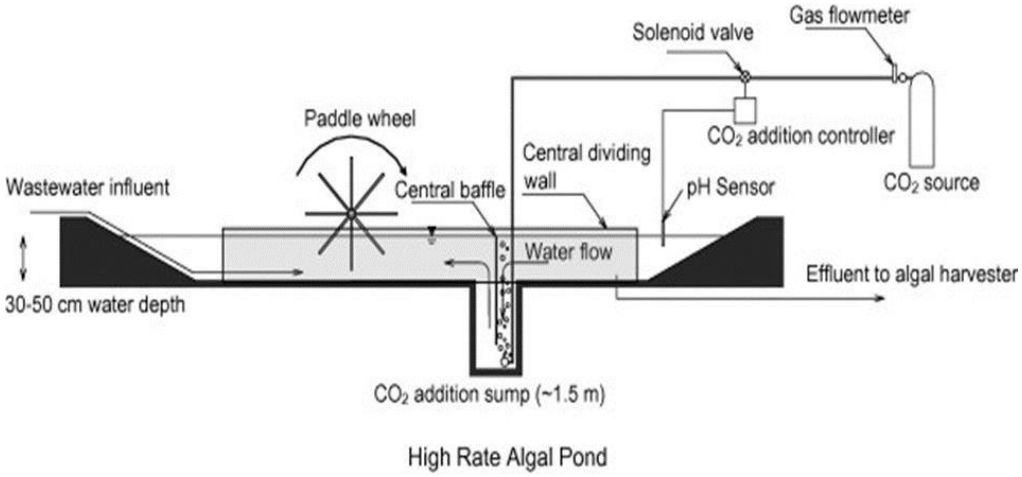

Figure 6 : High Rate Algal Pond[18]

2.5 BIOREACTOR DESIGN FOR ALGAE CULTIVATION

A bioreactor performs reactions involving living creatures or the biochemically active chemicals that are derived from living organisms. Microalgae have found widespread use in a variety of industries, including aquaculture, medicines, health goods, and the generation of biodiesel. They are utilised in the treatment of wastewater in order to eliminate pollutants such as phosphorus, nitrogen, and heavy metals. Microalgae can be grown in open-system photobioreactors (PBRs) like a raceway pond or closed-system photobioreactors like tubular, bubble-column, airlift, flat-panel, and so on. In terms of maintaining the culture environment and obtaining higher algal productivity, a closed PBR outperforms open ponds. A device used to cultivate micro or macro algae is called an algal bioreactor. Algae can be used to produce biomass (as in a seaweed cultivator), clean wastewater, fix CO2, and filter aquariums and ponds (as in an algae scrubber) Algae bioreactors may be used to make biofuels like biodiesel and bioethanol, as well as animal feed to reduce NOX and CO2 emissions from power plants' flue gases[20].

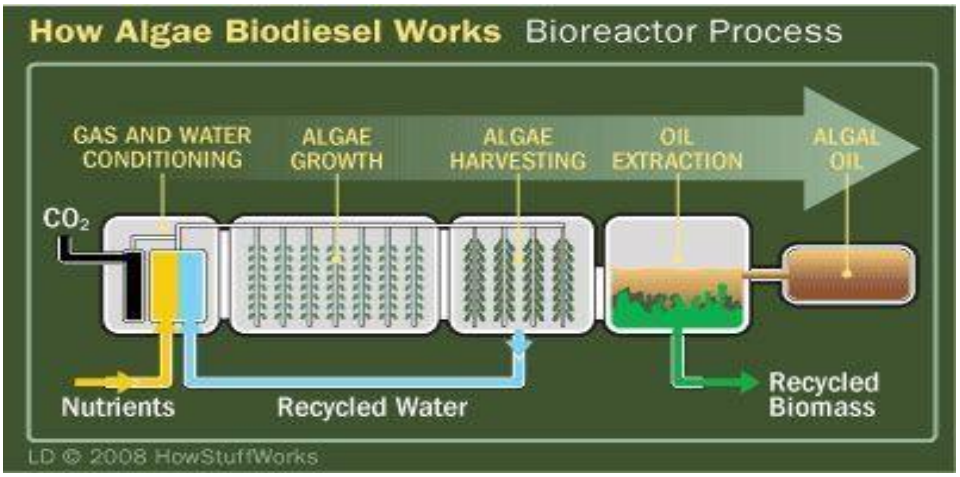

Figure 7: Working of Algae Biodiesel[19]

The photosynthetic interaction of chlorophyll-containing algae with dissolved carbon dioxide and solar energy is the basis for this sort of bio-reactor. For algae access, carbon dioxide is introduced into the reactor fluid. Transparent material should be used to construct the bioreactor..

Due to its huge surface area/volume ratio, low light/dark cycle, simple structure, and easy scalability, the flat-plate photo bioreactor (FPPBR) is widely used for microalgae culture[20]. The inner structural parameters of a FPPBR are optimised by following ways: Novel, wavy baffles were introduced in FPPBR in order to improve the light gradient mixing. Horizontal baffles were introduced in FPPBR to improve light utilisation efficiency. The new baffle reactor enhancedbiomass production by 1.7 times over the same-sized bubble column.[20]. In most laboratories and pilot facilities, small-scale bioreactors are frequently utilised and work well. When cultivation is shifted to large-scale bioreactors, particularly those with lengthy light paths, the results are consistently dismal, with low growth rates and low biomass production. When increasing bioreactor dimensions, especially light route length, consider large-scale photo bioreactors. Micro-algae growth relies heavily on the right combination. Mixingcan alter not only the mass transfer of medium nutrients and carbon dioxide intoalgae cells, but it can also speed up algae development by evenly spreading radiation over all cells in the culture and lowering diffusion barriers around algalcells[20]. Due to increased mixing the algal production per unit reactor also increased. So it is very much important to investigate the mixing performance when we are designing a bioreactor. Regular light/dark cycle mixing pushes algae cells in and out of the photic zone, enhancing light use and algae development. The photosynthesis efficiency of cells increases. and dark areas at the appropriate frequency. Now lots of research is going on intensifying lightor dark cycle mixing to increase the algal growth and improving bioreactor performance.

Figure 8: Algal Bioreactors[21]

Computational fluid dynamics (CFD) is used to build and optimise chemical industrial reactors due to its low effort, low cost, and rapid design period. CFD visualises the flow field and calculates hydrodynamic parameters to investigate flow and mixing performance.

In outdoor cultivation, only the sun-facing side of the PBR receives light. Bioreactor design centred on single-sided lighting. Due to light absorption and dispersion, there is a bright zone and a dark zone further from the light source in the reactor. Long light paths caused inadequate lighting in the dark zone.

In bioreactors lacking baffles, algal growth was delayed by a lack of light in the dark zone. The optimised bioreactor's horizontal baffles created an excellent light/dark cycle, allowing algal cells to migrate between dark and light zones to absorb more light energy and produce more biomass. Horizontal barriers restricted rising bubbles, boosting mass transfer to algal cells, such as CO2. Optimised bioreactor mixing improved algal growth. By placing baffles, uniform flow, mixing, and algal growth between zones might be obtained.

The algae grew exponentially in the traditional bioreactor before being gradually slowed and the reason for this could be related to the minor difference. Algal growth was accelerated by favourable lighting conditions. In outdoor cultivation, only the sun-facing side of the PBR receives light. Bioreactor design centred on single-sided lighting. Due to light absorption and dispersion, there is a bright zone and a dark zone further from the light source in the reactor. Long light paths caused inadequate lighting in the dark zone.

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2.6 WASTEWATER TREATMENT USING FILAMENTOUS ALGAE

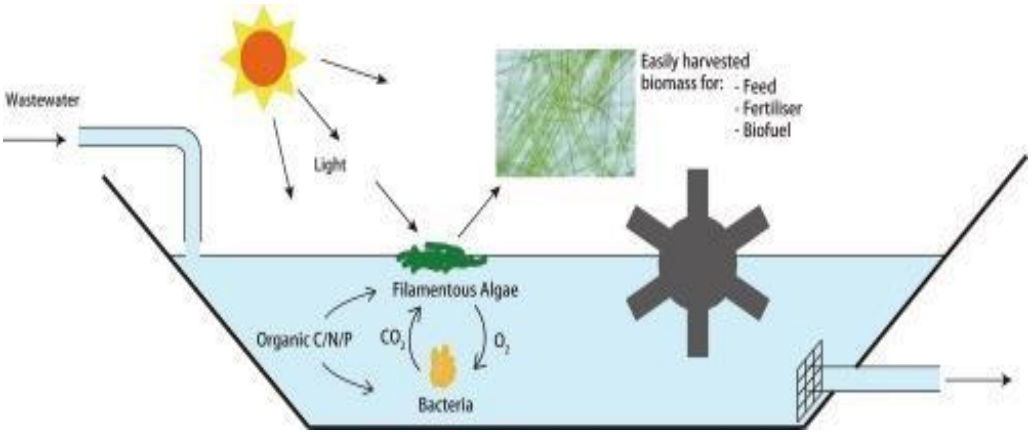


Figure 9: Wastewater treatment using filamentous algae [3]

Wastewater treatment requires the removal of organic carbon, nitrogen, phosphorus, heavy metals, and other pollutants. This removal can be accomplished using a combination of physical, chemical, and biological processes. The aeration of large volumes of wastewater is an energy-intensive operation that can account for much more than 50 percent of a wastewater treatment plant's overall energy expenditures[3].

Algae-based wastewater treatment has been recommended as a more eco-friendly method, especially for the removal and recovery of nitrogen and phosphorus. Algae have been shown to be effective in both of these processes. Microalgae are an ideal source of biomass due to their accessibility, production rate, yield per unit area, and capacity to address future challenges without competing for agricultural land. In comparison to many other sources of biomass, microalgae are an outstanding source[23]. However, the production and recovery of

The production of microalgae has been hampered by a number of issues, including but not limited to national approaches and support, species, culture methods, growing environments, isolation techniques, and many more. Among these are high production costs because of the involved separation process, which have resulted in an increase in the price of the finished product. Both microalgae and macro algae are types of algae that are classified according to the size of their cells. macro algae are multicellular and have individual

organisms that can be seen without the use of a microscope, in contrast to microalgae, which are microscopic and typically consist of a single cell. Microalgae have traditionally been preferred over macro algae for use in treatment of wastewater.

Microalgae have traditionally been preferred over macroalgae for use in treatment of wastewater. Growing microalgae in the open air as suspended cells in systems such as high-rate algal ponds (HRAP) or raceway ponds is possible[3].

Figure 10: Growth of Algae in Wastewater

The cultivation of microalgae is used for a variety of commercial purposes, including the production of biodiesel and bioethanol, in addition to the treatment of wastewater. Although microalga HRAPs are technically feasible for applications involving the treatment of wastewater, the harvesting of the small microalga cells continues to be a significant challenge. Due to the small cell size, close to neutral buoyancy, and negative surface charge of microalgae, harvesting them is challenging. As a result, the cost of harvesting microalgae can account for more than 90 percent of the equipment costs and between 20 and 30 percent of the total production costs in an operational facility[3].

There are primarily two ways to use freshwater filamentous algae for the treatment of wastewater:

1. Algal Turf Scrubbers (ATS)
2. Raceway Pond Monocultures

Algal Turf Scrubbers (ATS)

This technology is based on attachment of complex communities of micro-organisms, also known as periphyton, that is made up of microbes that are capable of self-seeding and consist of filamentous algae. These attach themselves to a screen and in order for the organisms that make up the periphyton to have access to the nutrients, this screen is submerged in wastewater that is moving slowly. After that, the biomass can be physically extracted using a scraper or a vacuum, and it might later be put to use in applications involving biofuel[3].

Figure 11: Periphyton on the surface of rock[26]

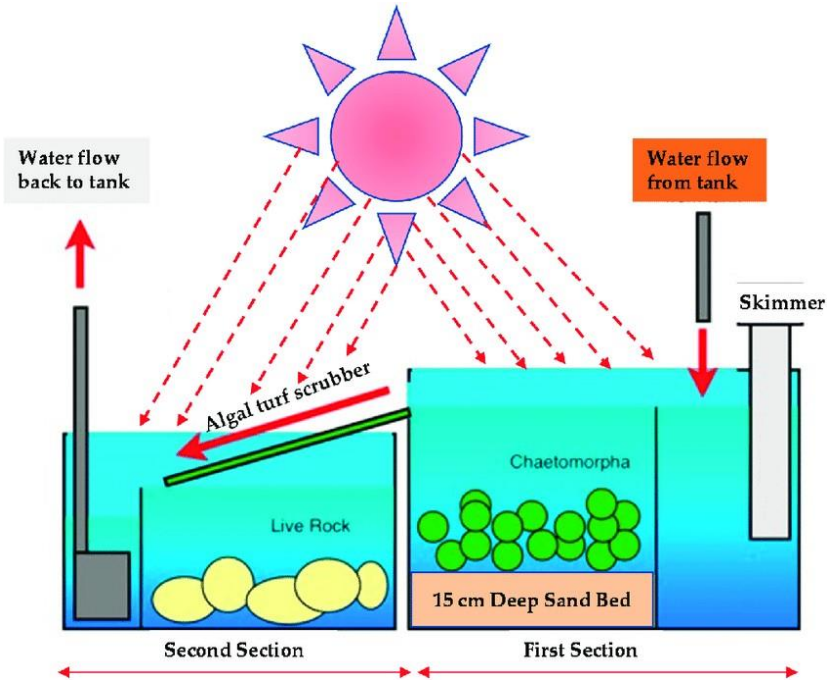


Figure12: Algal Turf Scrubber Cultivation System[27]

ATS is capable of treating a wide variety of contaminated water, such as[3]:

1. Agricultural Runoff
2. Aquaculture Wastewater
3. Dairy And
4. Swine Manure Effluent
5. Primary Domestic Wastewater
6. Secondary Effluent With
7. High Biomass Productivity

Raceway Pond Monocultures

This wastewater treatment method is based on the development of monocultures of filamentous algae in algal ponds. This technique has a significant benefit over ATS in that it permits the harvesting of selected species of grown macro algae, allowing for the control of biomass quality for potential downstream uses. As potential wastewater species, species from the genera Oedogonium, Cladophora, Spirogyra, Klebsormidium, and Stigeoclonium have been recognised. applications for treatment[3]

2.7 BIOREMEDIATION

Bioremediation is one of the most promising technical approaches to the problem of hazardous waste. It uses microorganisms like bacteria, fungus or algae to convert harmful chemicals into molecules that are either less toxic or not poisonous at all. A change of this kind by biological means is preferable to one that uses direct chemical or physical therapy. Microorganisms directly break down pollutants rather than passively moving them from one medium to another. They utilise metabolic degradation pathways and may be used in situ, which minimises the amount of disruption caused at the cleaning site. As a result, microbes have the potential to serve as efficient, cost-effective, and non-disruptive instruments for the removal of toxic substances[28]. The principle of bioremediation is the process of dissolving or transferring contaminants. Microorganisms are supplied because they have the enzymes that speed up the degradation process. Microorganisms degrade contaminants based on organisms, type of contaminant, geological and chemical conditions at the polluted site[29.]

Classification of bioremediation:

1) Ex-situ bioremediation

Ex-situ bioremediation is a method that uses processes like composting, bioreactors, etc. to remove pollutants from a contaminated site.The contaminated water or soil must be relocated to an area with better environmental circumstances. The kind of pollutant, the degree of pollution, the cost of treatment, the location, and the geological region of the site all influence the choice of treatment method.[29]

2) In- situ bioremediation refers to a procedure in which the treatment of pollutants is carried out inside the contaminant itself with the help of processes such as bio stimulation, bioventing, bio augmentation etc. It does not need excavation. These methods should, ideally, result in the lowest possible costs. Important environmental parameters for this process include the availability of

nutrients, the temperature, the acidity of the solution, the amount of moisture present, and the electron acceptor status.[29]

ALGAL BIOREMEDIATION OF WASTEWATER:

Algal bioremediation, which refers to the process of using living algae to remove excess dissolved nutrients from aquaculture effluents, is generally recognised as an effective and cost-efficient technique of treating wastewater[30]. Algae have a great capacity for adaptation. Depending on the type of substrate and the amount of light that is available, they are capable of growing either heterotrophically, autotrophically, or mixotrophically. Because of this, it would have a better chance of surviving in hostile environments. Microalgae have the ability to take in a wide variety of contaminants during the process of photosynthesis in water. The creation of oxygen by microalgae minimises the requirement for external aeration, which is essential for aerobic biodegradation. This is because the increased oxygen content in the water caused by microalgae encourages the development of numerous decomposers[31].

The proper species must be chosen for algal bioremediation in order to achieve a substantial decrease in the nutrient loads present in the effluents produced by aquaculture operations. While selecting species for bioremediation, a wide variety of factors has to be properly considered. It is essential for species to have large growth rates since this is generally a good indicator of strong

bioremediation potential. Selecting target species is the first step in algal bioremediation. In algal bioremediation, it's important to evaluate strains from multiple locations under different environmental conditions[30].

Algae's are eco-friendly and produce no pollution problems if their biomass is reused. Bioremediation biomass is a promising biofuel feedstock. Microalgal species like Chlorella, Scenedesmus, Botryococcus, Chlamydomonas, Spirulina, Oscillatoria, were used for bioremediation. Ulva lactuca, Kappaphycus alvarezii, etc. are some macroalgae used for bioremediation. During growth and development, macroalgae assimilate large amounts of nutrients. Cultivating macroalgae is the most promising way to reduce the

impacts eutrophication and hazardous algal blooms as a result of animal mariculture and human activities[32].

Microalgae can use waste products as a source of nutrients while simultaneously degrading pollutants through the action of their enzymes. It is known that the xenobiotics and heavy metals can be detoxified, transformed, or volatilized through the metabolic processes of microalgae. They have the capability of absorbing a wide variety of nutrients, including nitrogen and phosphorus, among others[33]. Microalgae remove pollutants through bio adsorption, degradation, and accumulation. Microalgae-mediated pollutant removal combined with biodiesel, biochemical, and bioelectricity production can be cost-effective and efficient[34].

2.8 EUTROPHICATION

The over-enrichment of aquatic environments with nutrients, which can lead to algal blooms and anoxic episodes, is referred to as eutrophication. Effects of eutrophication are excessive plant production, blooms of hazardous algae, increased frequency of anoxic conditions, and fish deaths. Eutrophication is blamed for a variety of economic reasons, like high cost of purifying water, decrease in fish and animal population, loss of recreational activities. Global population growth, food consumption, land conversion, fertiliser use, and nitrogen deposition have all combined to make eutrophication a major issue that will only become worse in the future decades[35].

Drinking water sources are prone to experiencing periodic surface blooms of cyanobacteria, sometimes known as blue-green algae, which can present a significant risk to both animal and human health. The overabundance of nutrients enters aquatic bodies from sewage, industrial discharges, agricultural run-off, urban areas, and building sites. Eutrophication can be reduced to a manageable level by the regulation of nutrient supplies, the reduction of fertiliser usage, the use of mathematical models, effective soil management techniques, phytoremediation, and other methods[36].

Plants and predators are affected by algal blooms, which reduce light penetration, resulting in decreased growth. Photosynthesis linked with

eutrophication may also reduce dissolved inorganic carbon and elevate pH to harmful level during the daytime. When the thick algal blooms die, microbial breakdown reduces the amount of dissolved oxygen, resulting in an anoxic "dead zone" where most species are unable to survive[37].

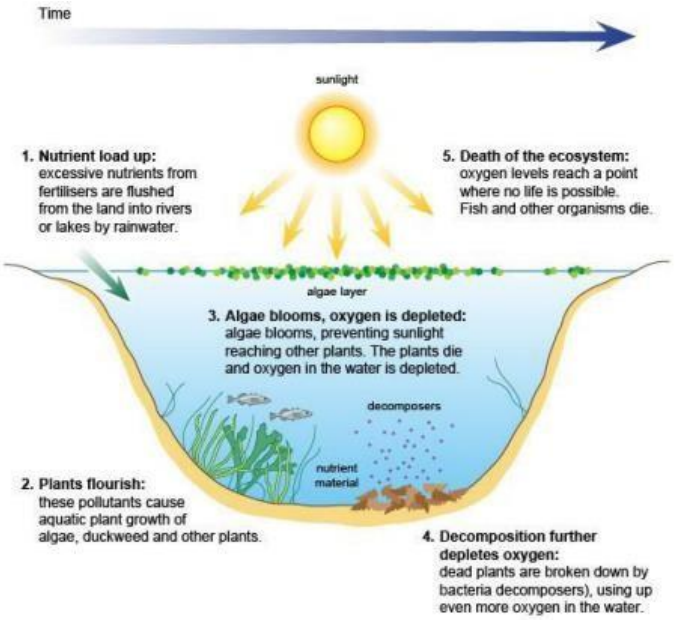


Figure 13: Eutrophication process[38]

Controls :

Even though many cities have passed laws to control the loading of nutrients from point sources, eutrophication and cyanobacterial blooms are widespread everywhere. The effects of cultural eutrophication are often reduced by water resource managers through the implementation of a number of different strategies. These include: the diversion of excess nutrients, the alteration of nutrient ratios, the physical mixing of water, the shading of water bodies with opaque liners or water-based stains, and the application of potent algaecides. The use of algaecides such as copper sulphate is effective at reducing high rate algal blooms temporarily. For big and complex ecosystems these solutions have shown to be ineffective. It is often possible to improve water quality by lowering the amount of nitrogen or phosphorus that is introduced into aquatic systems and bottom up nutrient management has increased the water quality in some cases. Bio-manipulation, which is the process of modifying a food web in order

to restore ecosystem health, is yet another approach that has been considered for enhancing water quality in nutrient-dense lakes[38].

2.9 ALGAL USE IN WASTE WATER MANAGEMENT

Algae contribute to wastewater treatment by providing oxygen that enables aerobic bacteria to degrade organic pollutants in the water and by absorbing excess nitrogen and phosphate. Additionally, it is a long term solution that is also economical to conventional wastewater treatment methods. Up to 60 percent of current wastewater treatment procedures (WWTP) operating expenses can be related to power expenditures. Ultimately, the usage of algal systems might minimise these costs while also helping the sector by collecting fertiliser materials[39].

A single hectare of algae can remove tonnes of CO2 and produce dry biomass each year. Algae also has the potential to be used in the manufacture of sustainable fuels like biodiesel and hydrogen, which is another advantage of using algae. Because the algae are able to consume the CO2 that is released when these biofuels are burned, the consequence is that there is no overall increase in the amount of CO2 that is released into the atmosphere. Apart from being a source of nutrients such as vitamins, minerals and proteins, algae may also balance the expense of CO2 bioremediation[40].

2.10 DNA BARCODING

DNA barcoding is the method used to identify the target taxon using a specific DNA sequence that works as a species-specific nucleotide. Meta- and mini-barcoding are the two types of barcoding that is used to detect and identify particular taxa from mixed samples and degraded DNA from multiple sources. Mitochondrial cytochrome oxidase (COI) is the most commonly used barcode. It helps taxonomists to distinguish between creatures that have cryptic morphological characteristics or similar species by using this method[41]. Instead of depending on a single marker, DNA barcoding uses two or more markers. Plastid Rubisco large subunit(rbcl), elongation factor tufA, universal plastid amplicon (UPA),and 5S rDNA spacer region are the markers that used in green

macroalgae[42]. This effective approach has improved taxonomy, ecology, biosecurity, and food product control. Currently 4,449 rbcL sequences from chlorophyte species in Barcode of Life Data Systems (BOLD) collection, is taxonomically maintained for genetic information[43].

The use of DNA barcoding methods is becoming more significant in the process of identifying algae. This method often makes use of a segment of a gene with a high degree of variability, which has been demonstrated to vary across species[44]. The core barcode matK is 1,550 bp long and encodes a type II intron splicing enzyme in chloroplast lysine tRNA (trnK) gene12. matK is one of the genes with the quickest rate of mutation in the chloroplast genome's sections that code for proteins. It only exists in a single copy. This gene has an evolution rate that is about two to three times faster than rbcL. Despite the fact that matK has a rather low success rate when it comes to amplification, it has been widely employed in investigations of both evolutionary botany and systematic botany[45].

DNA barcoding uses an amplified, sequenced, and compared mitochondrial gene area that uses molecular taxonomy to establish an organism-identifying barcode and a standardised reference library for DNA-based species identification. It relies on standardised PCR techniques and procedures and expanding data as research advances. DNA barcoding has three steps:

- Extraction of DNA
- Amplification by PCR
- DNA sequencing and analysis

Primers were used for the purpose of amplifying a specific area (COI gene).If a single gene is amplified using multiple species. It is feasible to establish a library of gene sequences that has been evaluated. It is essential to have a good understanding of the taxonomic group that the organism of interest belongs because PCR primers are unique to taxonomic groups. PCR products are tested on an agarose to confirm amplification. If there's a band, DNA sequencing can be done. If there's no amplification, examine the problem. This may involve

redoing DNA extraction, attempting a new primer combination and adjusting the master mix. A sequencing organisation uses a PCR product to identify the organism.

Without bioinformatics, the sequencing company's 700-base DNA sequence is meaningless. The Barcode of Life Data Systems (BOLD) and NCBI Basic Local Alignment Search Tool (BLAST) can examine DNA sequence. DNA BOLD is a free application that matches the DNA sequence to taxonomist- identified samples that include extra information about the sample. This websiteis a centre for information and analysis on DNA barcoding. NCBI BLAST is afree, online software where researchers may submit non-verified DNA sequences to a database. This software compares a sequence against both vouchered and non-vouchered samples. Both of these algorithms use alignment techniques to identify the unknown sequence[46].

CHAPTER 3
MATERIALS AND METHODS

3.1 MORPHOLOGY AND MEASUREMENT OF ALGAE

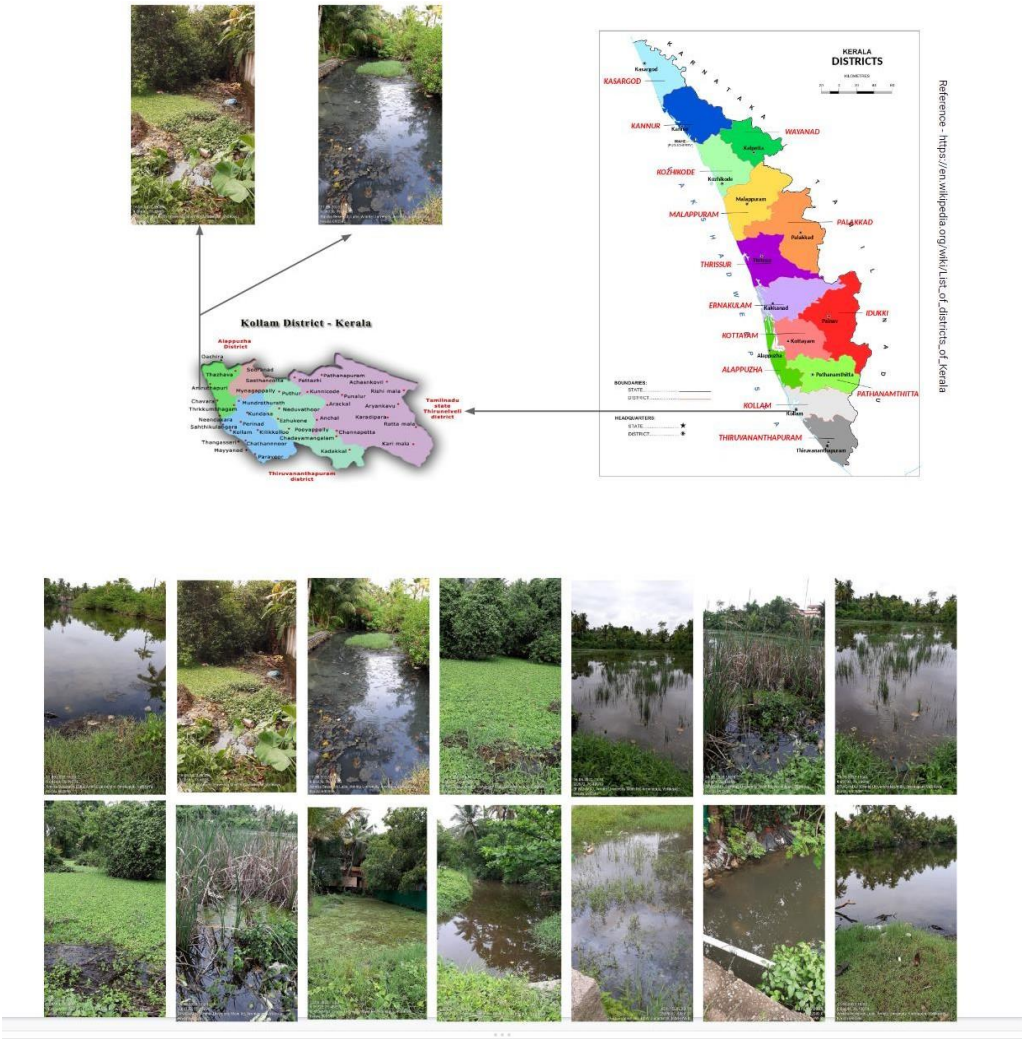
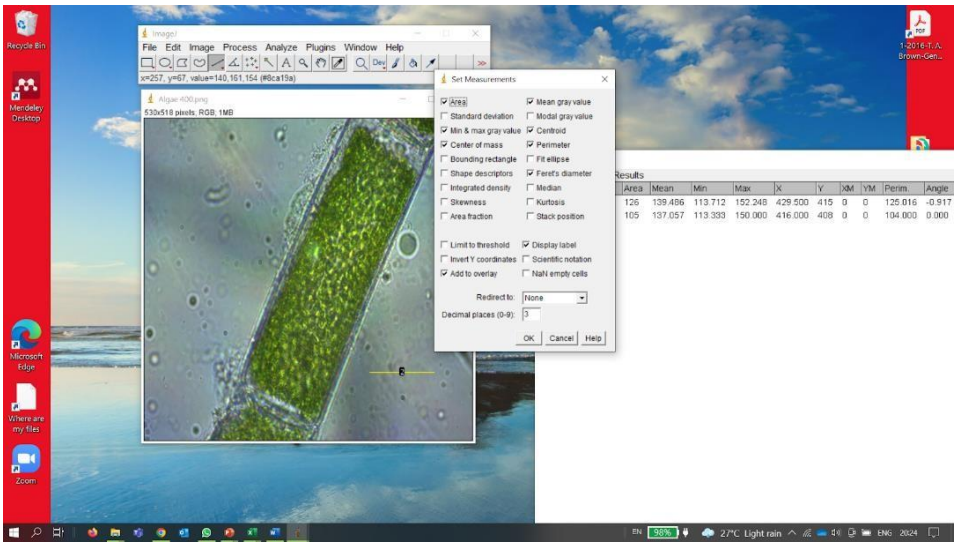


Figure 14 : Locations

Morphometry of Algal strains: To identify the taxonomic position of different algae by using a microscopic picture of the morphology of the algal strain captured by a microscope camera with CatCam software. Measurement of the cells/filaments was done by calibrating pixels with with micrometer image of defined scale with the help of ImageJ software. A screenshot of the ImageJ measurement tool is shown below:



3.2 DNA ISOLATION

The basic principle of DNA isolation is disruption of cell wall, cell membrane and nuclear membrane. This is followed by precipitation of DNA and removal of other contaminating biomolecules such as proteins, polysaccharides, lipids and phenols. The algal sample was grind with 1ml of CTAB and glass beads in a mortar and pestle. The sample was transferred to a micro centrifuge tube and then centrifuged at 13000 rpm for 10 mins. Collect the supernatant and transfer to another centrifuge and then add 250ul of chloroform isoamyl alcohol. Then the sample is inverted and mixed. Then again spin at 12000 rpm for 5 mins and collect supernatant, and transfer to a new tube. Add 50ul of 7.5M ammonium acetate and 500ul of ethanol to the sample. Centrifuge the sample again and add 70% ethanol. Then invert the tube and centrifuge for 5 mins at 12000rpm. Remove the supernatant and allow the DNA to dry and add 50ul of milli-q water. Add 2ul of RNase solution and incubate at 65 degree Celsius.

3.3 DNA QUANTIFICATION USING BIOPHOTOMETER

A Bio photometer is a micro-spectrophotometer and is used to evaluate the quality of nucleic acid samples like DNA, RNA and proteins. Mostly photometers detect the light with photoresistors, photodiodes, or photomultipliers. To analyse the light, a photometer either measures the light after it has been passed through a filter or through a monochromator for the analysis of the spectral distribution of the light. 1ul of the sample and 59ul of

milli-q water was added to a cuvette. And then the cuvette was placed in a bio photometer and the absorbance was noted.

3.4 GEL ELECTROPHORESIS

Gel electrophoresis is a technique that is used for the separation of charged molecules like DNA, RNA and proteins based on their size. Agarose gel is used as the separation medium. The charged molecules move through the gel when an electric current is applied. Electric current is applied through the gel so that one end of the gel has a positive charge and the other end has a negative charge. Negatively charged molecules will be pulled towards the positive end and positively charged molecules will move to the negative end. The gel has a permeable matrix, through which molecules can migrates when electric current is applied. Smaller molecules migrate more quickly and travel longer distance than larger fragments that migrate slowly and travel a shorter distance. Hence DNA fragments of different lengths are separated using electrophoresis. As DNA is negatively charged when electric current is applied to the gel, DNA will migrate towards the positively charged electrode. 0.24 g of agarose was mixed with 30ml TAE in a microwavable flask. Then the agarose was microwaved for 1-3 mins until it was completely dissolved. And to this agarose, 2.5ul of EtBr was added. The coating tray was cleaned, the sides were taped and comb was placed. Then the dissolved agarose was poured onto the coating tray. The gel was kept to dry for 20 mins and after the gel was dried the comb was removed. After which the trays were placed in a 1X TAE buffer. 1ul of the sample and the dye was mixed using a micropipette and placed carefully in the wells. The voltage was set at 100V and ran for 1 hour, and the gel was observed under UV in the digital gel documentation.

3.5 PROTEIN ESTIMATION USING BRADFORD METHOD

The Braford protein assay is used to measure the concentration of total protein in the sample. The principle is that binding of protein molecules to coomassie dye under acidic conditions results in a colour change. From each of the dilution 5ml was added into a 96 well plate triplicate. Then 125 CBB G250 wasadded and incubated for 5 mins. After which, the plate was placed under plate

reader and the absorbance was recorded at 595 nm. 5ml sample and 125ml CBB G250 was placed as a triplicates in 3 wells and incubated for 15 mins, and then the absorbance was observed.

3.6 FOLCH METHOD

The Folch method is based on the principle of partitioning lipids in a biphasic mixture of chloroform and methanol. Methanol disrupts hydrogen bonds between lipids and protein following addition of chloroform. A chloroform methanol stock was prepared by mixing 14ml chloroform and 1ml methanol. 1g dried sample was weighed and added into a falcon tube. The sample was vortexed for 1 min after adding chloroform-methanol stock. Then vortex again for 1 min after adding 5ml of distilled water. Then after centrifuging the for 10 mins for 2500 rpm, the chloroform and methanol layers were separated. The chloroform layer was transferred to a petri plate and left overnight in the LAF.

3.7 MEASUREMENT OF GROWTH RATE

Increasing amounts of sunlight, temperature, and available nutrients increases the algal growth. Growth rate is the change in biomass per change in time. The samples collected was isolated and grown in wastewater (a mixture of both grey and black water). The growth rate of 3 samples were observed over a time span of 15 days and weighed at an interval of 4 days. The collected sample was poured into a preweighed petri plate and the weight was noted by placing it on a weighing machine.

3.8 POLYMERASE CHAIN REACTION

Polymerase chain reaction is a commonly used laboratory technique to amplify a particular region of DNA. In PCR short synthetic DNA fragments called primers are used to select a segment of the genome that has to be amplified, and then multiple rounds of DNA synthesis is done to amplify that particular segment. The template was added to sample 1 in a PCR tube and non-template was added to sample 2 in a PCR tube. Then both the tubes were placed in the

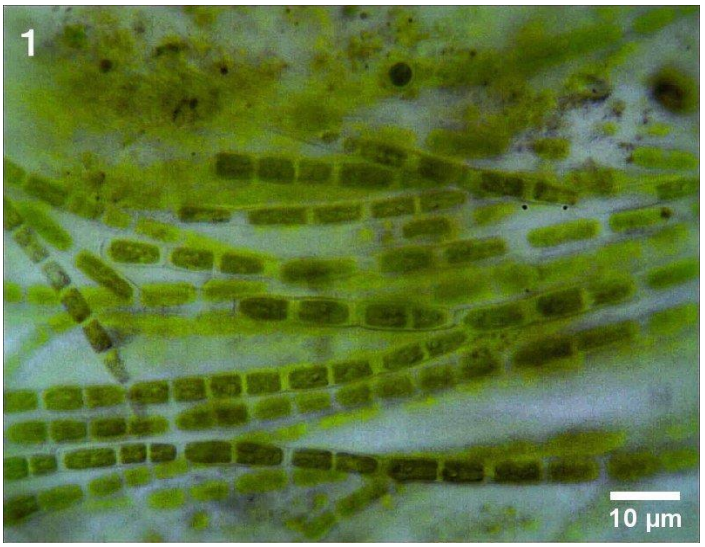
PCR machine for 1.5 hours. The DNA was amplified and the amplified DNA was evaluated by agarose gel electrophoresis

CHAPTER 4
RESULT

4.1 Taxonomic identification:

SAMPLE 1 : *Rhizoclonium*

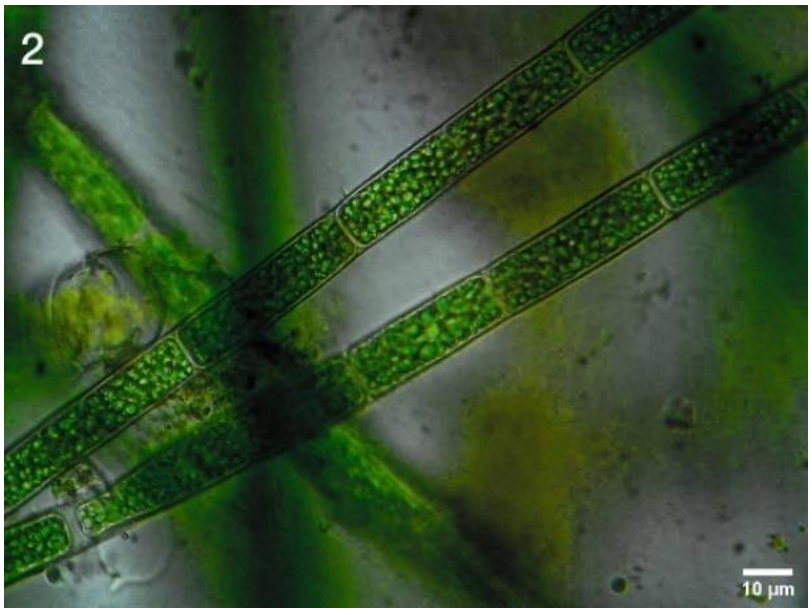
Rhizoclonium constitute unbranched filaments, short, unicellular, rhizoidal branches that arise at right angles to the axis.



Order	Cladophorales
Family	Cladophoraceae
Genus	Rhizoclonium
Species	hizoclonium
Measured Length	9 µm
Measured breadth	5.2 µm

SAMPLE 2*: Chaetomorpha minima*

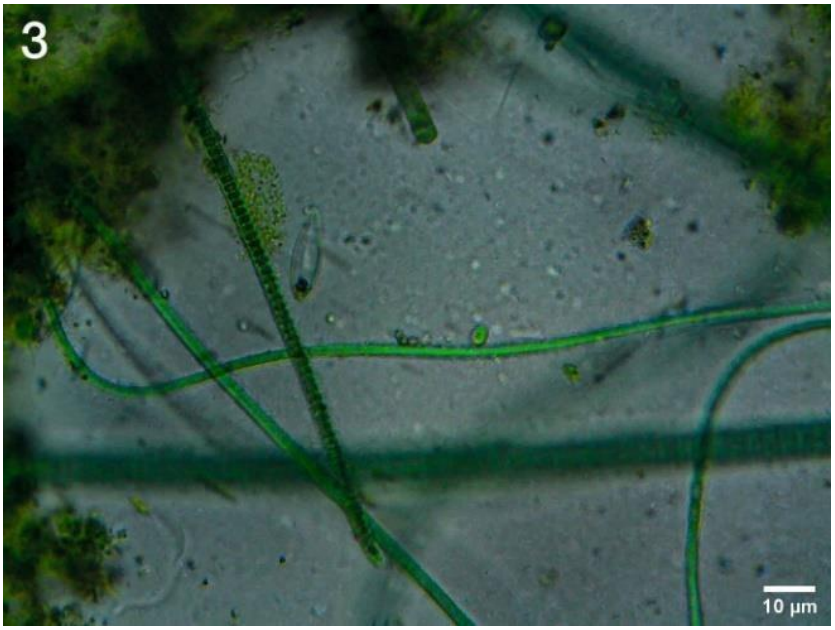
Chaetomorpha is a macroscopic algae, consisting of un-branched filaments, wth intercalary growth, free living or attached basally by rhizoids.



Kingdom	Plantae
Division	Chlorophytao – green algae, algues vertes
Subdivision	Chlorophytina
Class	Ulvophyceae
Order	Cladophoralesc
Family	Cladophoraceae
Genus	Chaetomorpha Kuetzing
Measured Length	15 µm
Measured Breadth	10.99 µm

SAMPLE 3*: Geitlerinema splendidum*

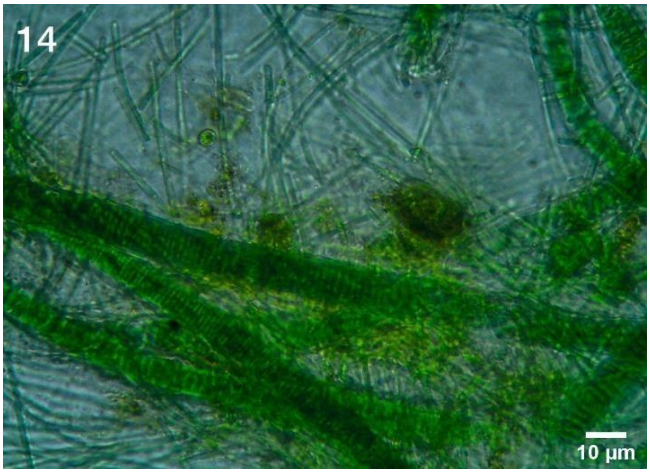
Geitlerinema splendidum belongs to the family Coleofasciculaceae. It is a common species in the periphyton of north temperate region.

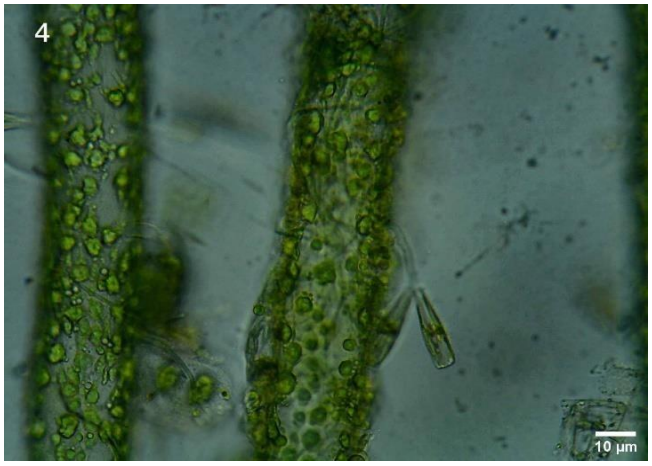


Domain	Bacteria
Phylum	Cyanobacteria
Class	Cyanophyceaee
Order	Oscillatoriales
Family	Coleofasciculaceae
Genus	Geitlerinema
Measured Length	114µm
Measured Breadth	4.2µm

SAMPLE 4,14: *Pithophora*

This is a filamentous algae and it has a coarse texture so it is called horse hair. They are irregularly branched algae.

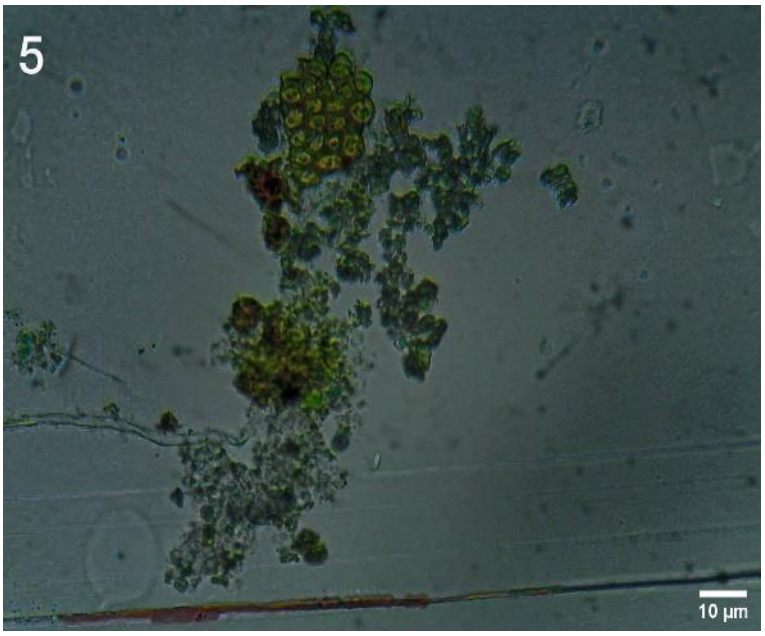




Genus	Pithophora
Division	Chlorophyta
Class	Ulvophyceae
Order	Cladophoraceae
Family	Pithophoraceae
Measured length	159.27um
Measured breath	12,093um

SAMPLE 5: *Oocystis*

Oocystis is a planktonic genus mainly of freshwater green algae of the family Oocystaceae.



Division	Chlorophyta
Class	Trebouxiophyceae
Order	Chlorellales
Family	Oocystaceae
Genus	Oocystis
Measure Length	6.7 µm

SAMPLE 6: *Lyngbya aesturaii*

Lyngbya, is a genus of cyanobacteria, unicellular autotrophs and it forms the basis of the oceanic food chain.

Kingdom	Bacteria
Subkingdom	Negibacteria
Phylum	cyanophytes
Class	Cyanophyceae
Order	Nostocales
Family	Oscillatoriaceae
Genus	Lyngbya Agardh
Measured Length	42.644 µm
Measured Breadth	23.44 µm

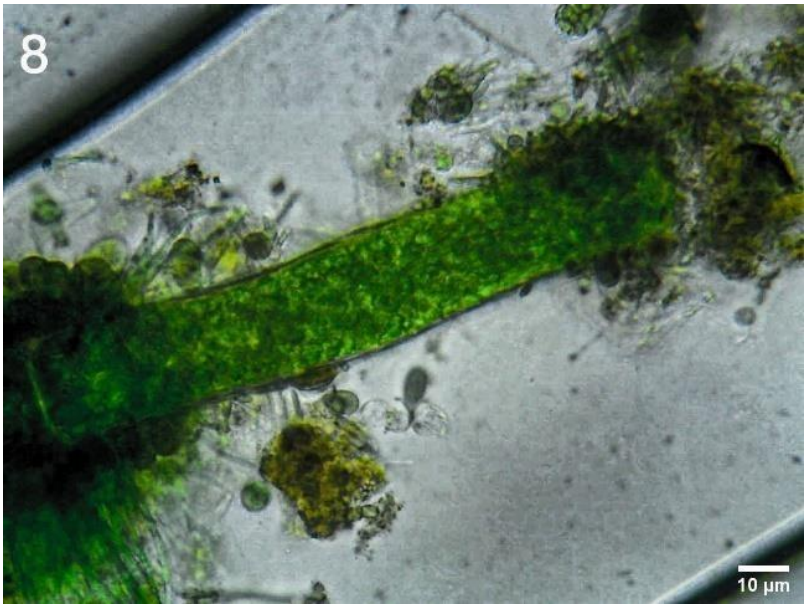
SAMPLE 7: *Lyngbya*

Lyngbya is a genus of cyanobacteria, unicellular autotrophs that form the basis of the oceanic food chain.

Class	Cyanophyceaex
Order	Nostocales
Family	Oscillatoriaceae
Genus	Lyngbya Agardh Ex Gomont, 1892
Species	Lyngbya aestuarii (Mert.) Lieb
Measured Length	110.8 µm
Measured Breadth	7 µm

SAMPLE 8: *Cladophora*

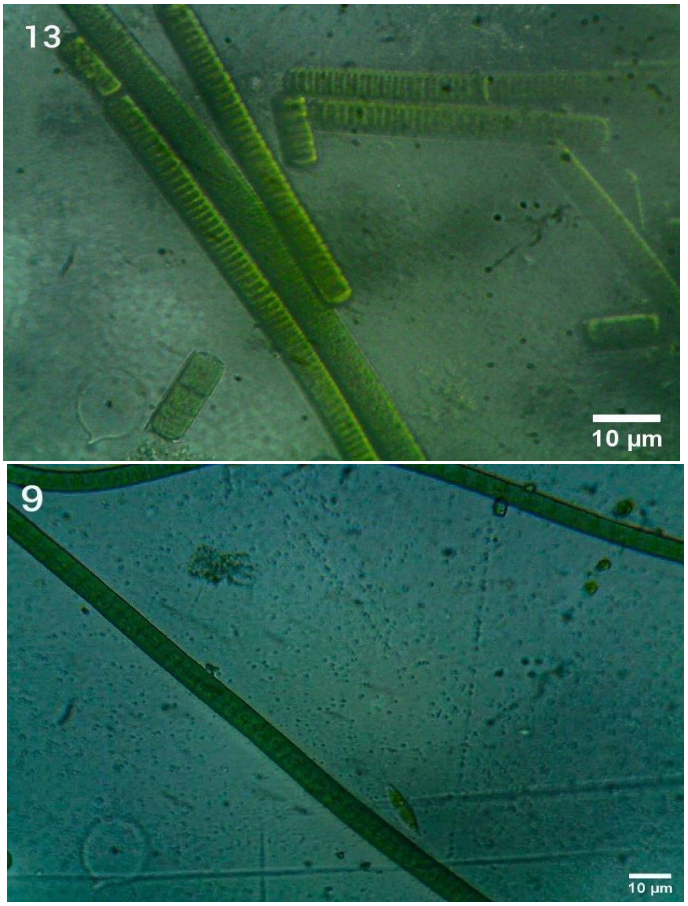
Cladophora is a genus of reticulated filamentous Ulvophyceae. The genus Cladophora contains many species that are very hard to tell apart and classify, mainly because of the great variation in their appearances, which is affected by habitat, age and environmental conditions.



Order	Cladophoralesc
Family	Cladophoraceae
Genus	Cladophora Kützing,
Species	Cladophora glomerata (Linnaeus) Kuetzing
Measured Length	162.7 µm
Measured Breadth	23.496 µm

SAMPLE 9, 13: *Cyanobacterium oscillatoria*

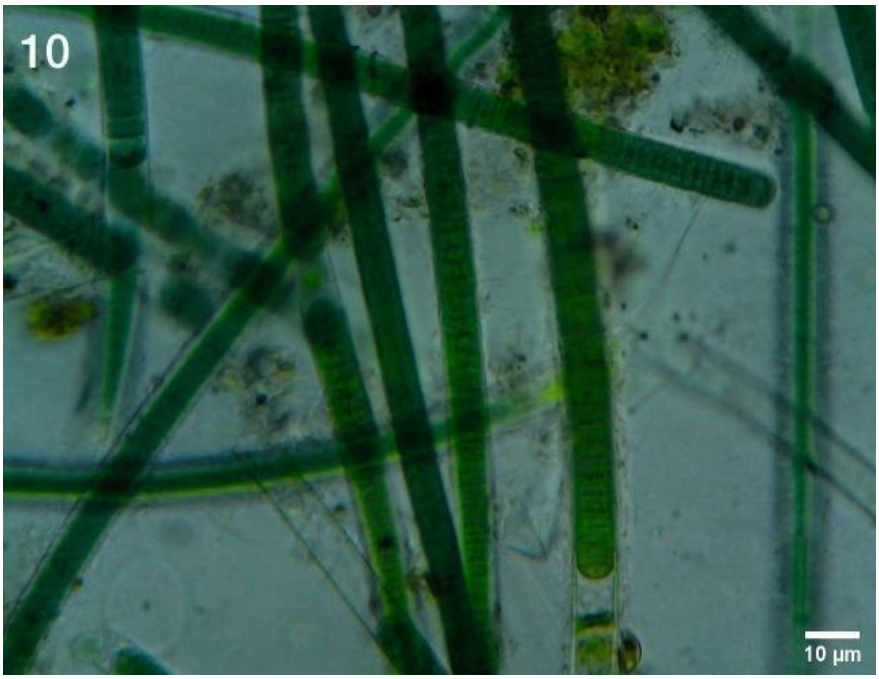
Oscillatoria is a filamentous cyanobacterium.It is mostly found in freshwater environments, such as hot springs. It has a blue-green appearance.



Phylum	Cyanobacteria
Domain	Bacteria
Class	Cyanophyceae
Order	Oscillatoriaceae
Family	Oscillatoriaceae
Measured length	43.86um
Measured breadth	5.359um

SAMPLE 10: *Phormidium* is

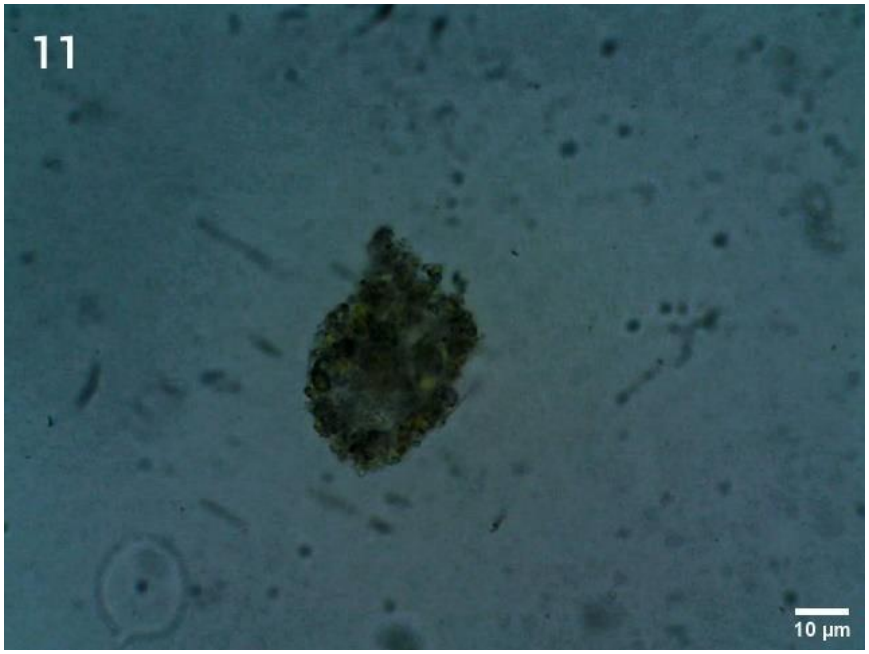
Phormidium is a cyanobacteria.They are unbranched. And they are commonly found in ponds and lakes.



Family	Oscillatoriaceae
Phylum	Cyanobacteria Cavalier-Smith
Class	Cyanophyceaex
Order	Nostocales
Genus	Phormidium Kuetzing
Measured Length	143 µm
Measured Breadth	9.2 µm

SAMPLE 11: *Zoochlorellae*

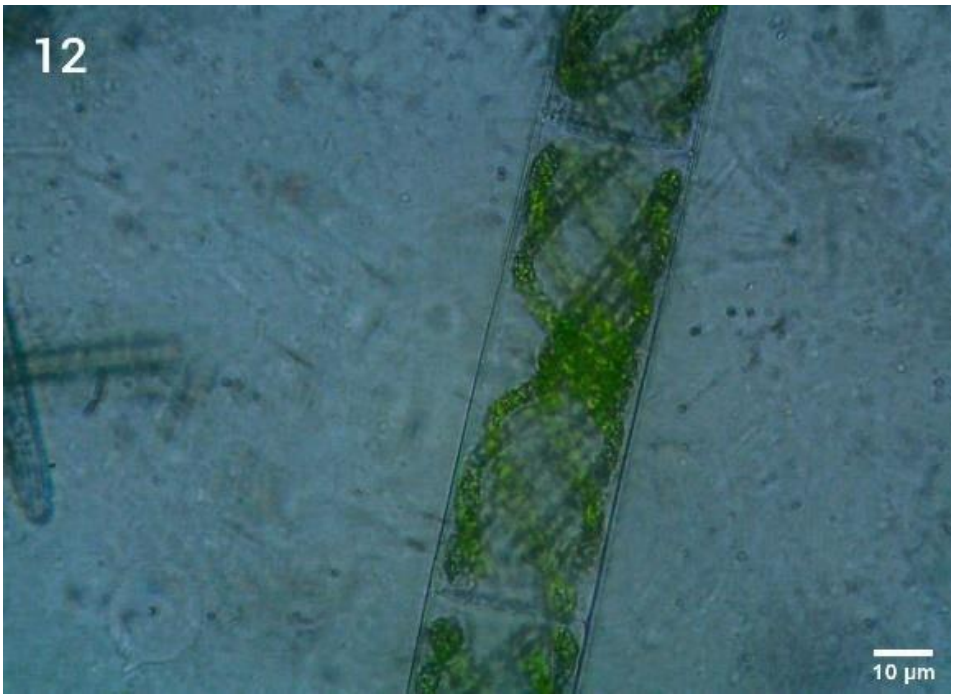
Zoochlorella is a nomen rejiciendum for a genus of green algae assigned to Chlorella. The term zoochlorella is sometimes used to refer to any green algae that lives symbiotically within the body of a freshwater or marine invertebrate or protozoan.



Division	Chlorophyta
Class	Trebouxiophyceae
Order	Chlorellalesz
Family	Chlorellaceae
Genus	Zoochlorella
Measured Length	39.48 µm
Measured breadth	27.807µm

SAMPLE 12: *Spirogyra*

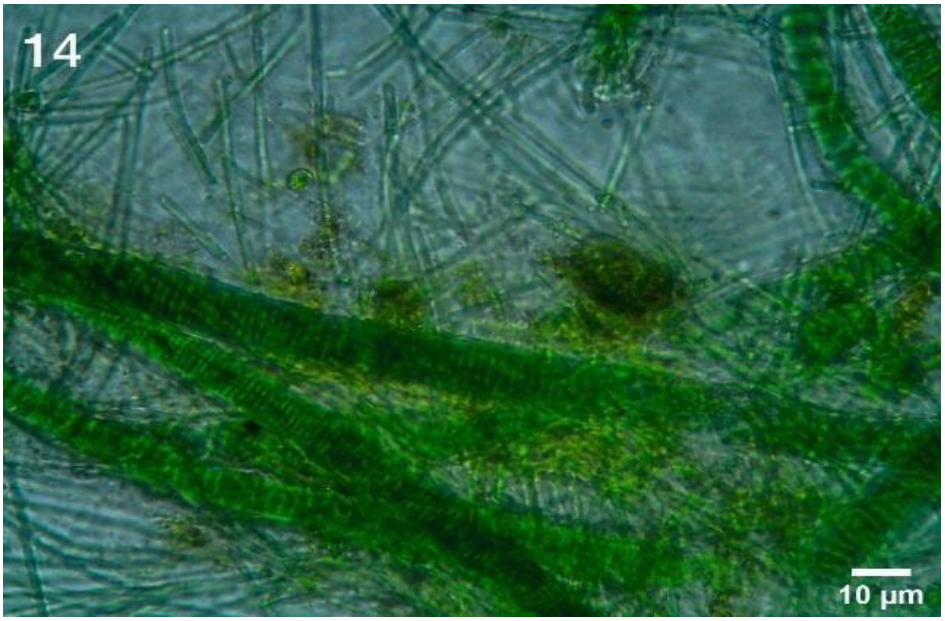
Spirogyra are free-floating green algae seen in freshwaters such as ponds, lakes, etc. And it is commonly known as water silk or pond silk.



Phylum	Chlorophyta
Domain	Eukaryota
Class	Zygnematophyceae
Order	Zygnematales
Family	Zygnemataceae
Measured length (fragment)	83.32 µm
Measured Breadth	27.708 µm

SAMPLE 14: *Pithopora*

This is a filamentous algae and it has coarse texture,so it is referred to as "horse hair". They are irregularly branched algae.

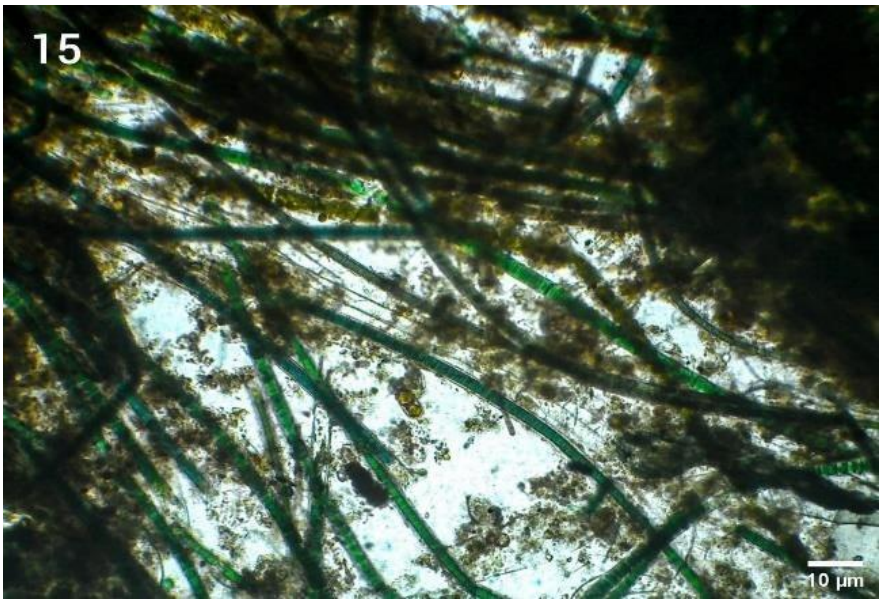


Genus	Pithophora
Division	Chlorophyta
Class	Ulvophyceae
Order	Cladophorales
Family	Pithophoraceae
Measured Length	159.27µm
Measured Breadth	12.093µm

SAMPLE 15: *Lyngbya*

Lyngbya is a unicellular autotrophs that form the basis of the oceanic food chain.They are usually unbranched. They are found in fresh water

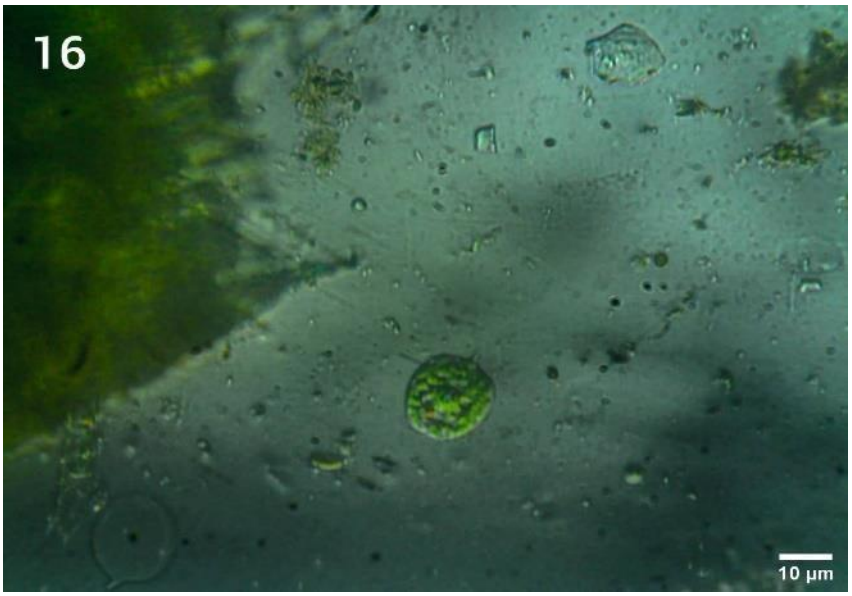
.



Domain	**Bacteria**
Phylum	**Cyanobacteria**
Class	**Cyanophyceae**
Order	**Oscillatoriales**
Family	**Oscillatoriaceae**
Genus	**Lyngbya**
Measured Length	70.88µm
Measured Breadth	2.62µm

SAMPLE 16: *Dictyochloropsis*

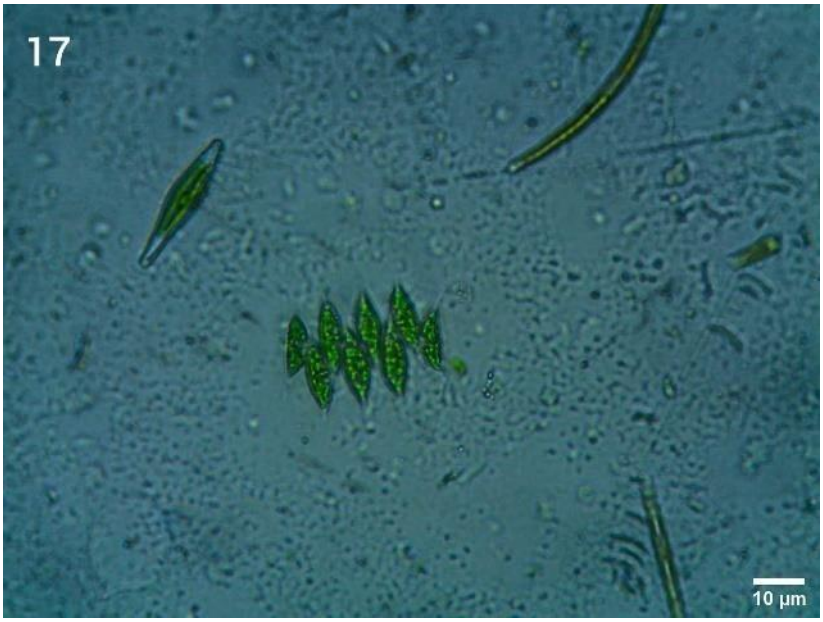
Dictyochloropsis is a unicellular green alga of the phylum Chlorophyta. They consist of free-living algae. The reticulate chloroplast varies in morphology between species.Dictyochloropsis is asexual and reproduces using autospores.



Division	**Chlorophyta**
Class	**Trebouxiophyceae**
Order	**Trebouxiaceae**
Genus	**Dictyochloropsis**
Family	**Trebouxiaceae**
Measured Length	16.249µm
Measured Breadth	18.579µm

SAMPLE 17: ***Nitzschia palea***

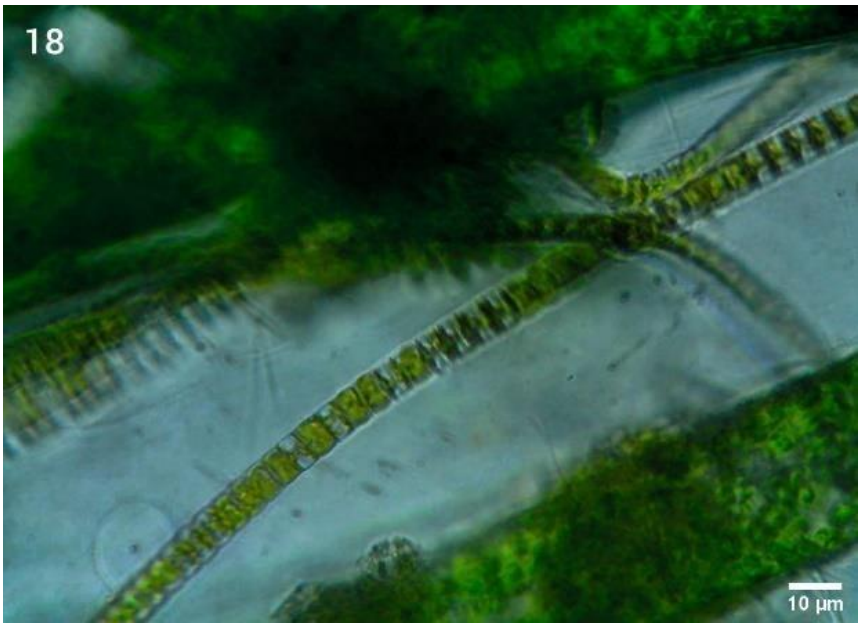
Nitzschia is a pennate marine diatom. Nitzschia is commonly found in cold waters, and they are associated with both Arctic and Antarctic polar sea ice.



Phylum	Ochrophyta
Class	Bacillariophyceae
Order	Bacillariales
Family	Bacillariaceae
Genus	Nitzschia
Measured Length	28.324µm
Measured Breadth	12.44µm

SAMPLE 18: *Ulothrix*

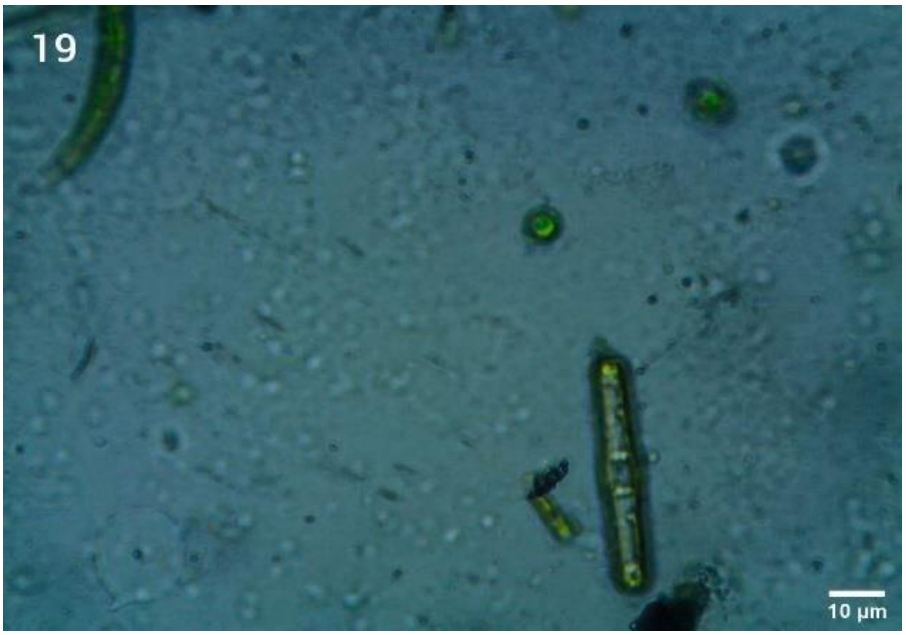
Ulothrix is a filamentous green algae. They are found in marine and fresh waters.



Division	Chlorophyta
Class	Ulvophyceae
Order	Ulothrichales
Family	Ulotrichaceae
Genus	Ulothrix
Measured length	1353.3µm
Measured Breadth	58.13µm

SAMPLE 19: *Nitzschia*

Nitzschia is found mostly in cold waters.It is a common pennate marine diatom.



Phylum	Ochrophyta
Class	Bacillariophyceae
Order	Bacillariales
Family	Bacillariaceae
Genus	Nitzschia
Measured Length	47.09 µm
Measured Breadth	7.435 µm

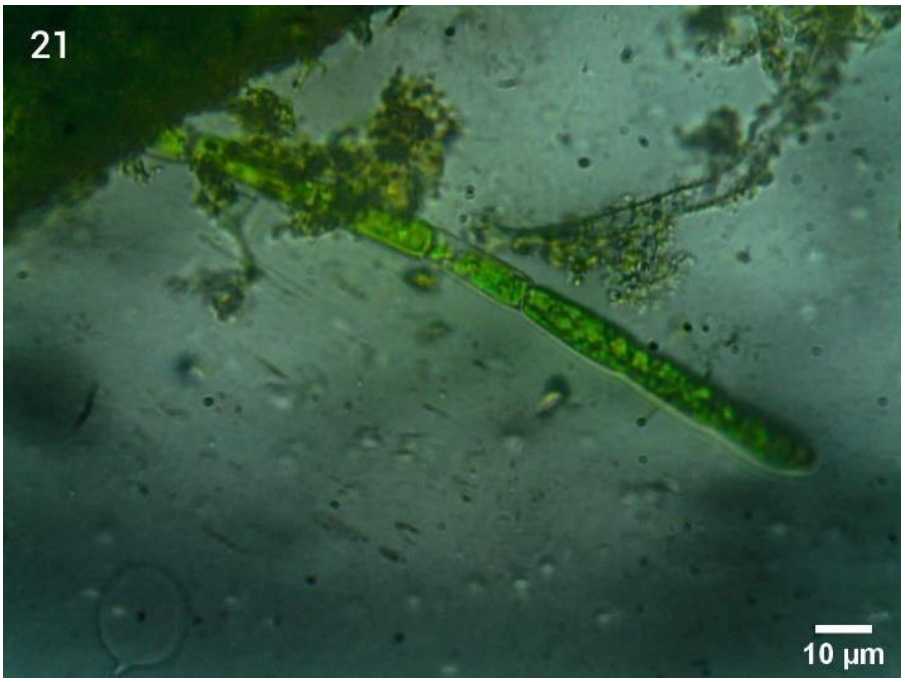
SAMPLE 20: *Closterium*

Closterium is a unicellular charophyte green algae. Closterium has a key phylogenetic position as an ancestor of land plants

Class	Zygnematophyceae
Order	Desmidiales
Family	Closteriaceae
Genus	Closterium
Measured Length	50.748 µm
Measured Breadth	5.007 µm

SAMPLE 21: *Tribenoma*

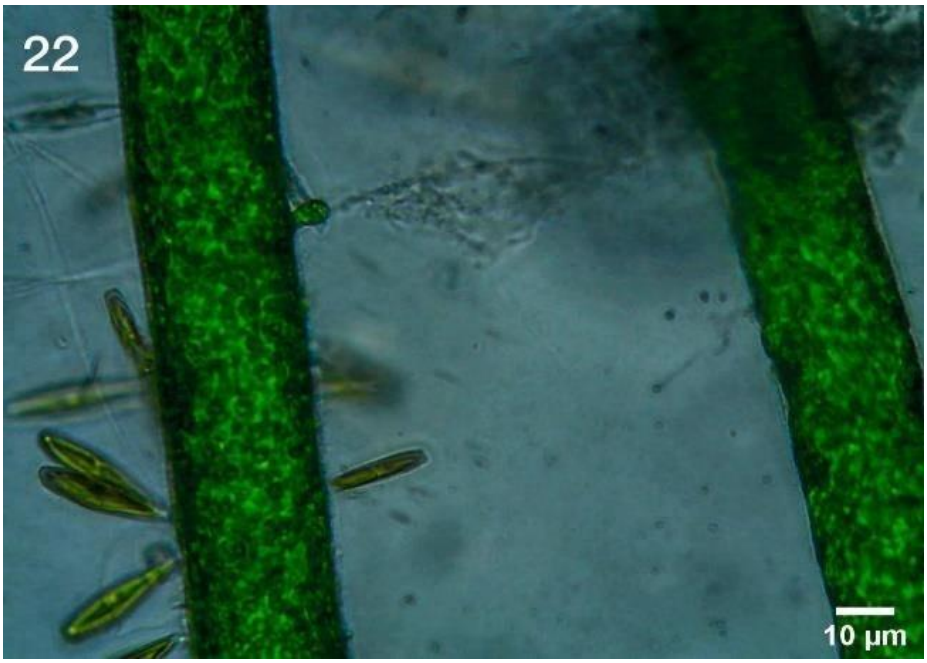
Tribonema is a filamentous, freshwater yellow-green algae.They may be branched or unbranched.



Phylum	**Ochrophyta**
Class	**Xanthophyceae**
Order	**Trbonematales**
Family	**Tribonemataceae**
Genus	**Tribonema**
Measured Length	**1050.9 µm**
Measured Breadth	**52.15 µm**

SAMPLE 22: *Vaucheria*

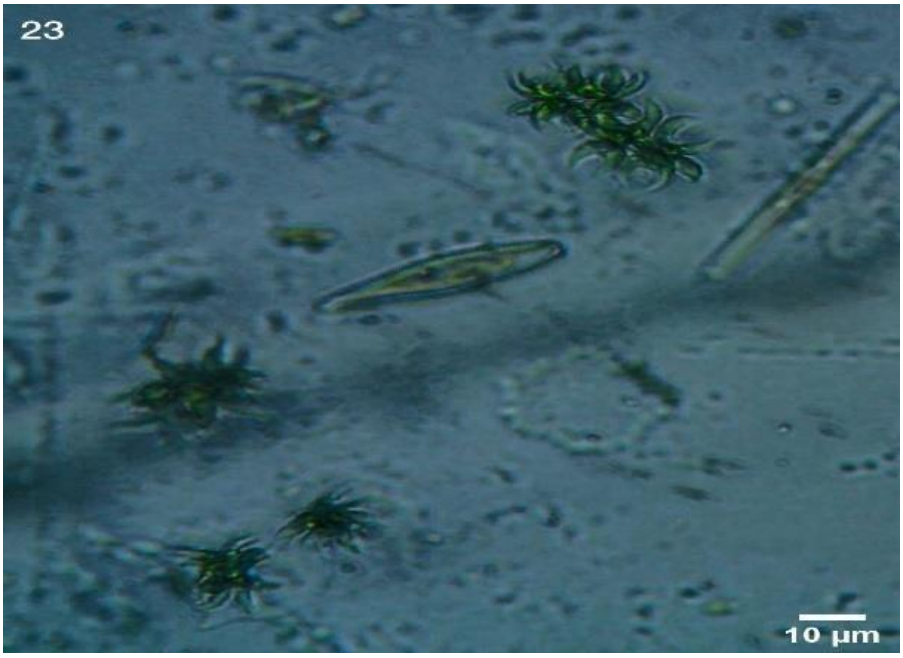
Vaucheria is a yellow-green algae known as water felt. They have apical growth from the tip of filaments and this forms mats in terrestrial environments or freshwater environments.



Phylum	Ochrophyta
Class	Xanthophycease
Order	Vaucheriales
Family	Vaucheriaceae
Genus	Vaucheria
Measured Length	958.5 µm
Measured Breadth	220.9 µm

SAMPLE 23: *Selenastrum*

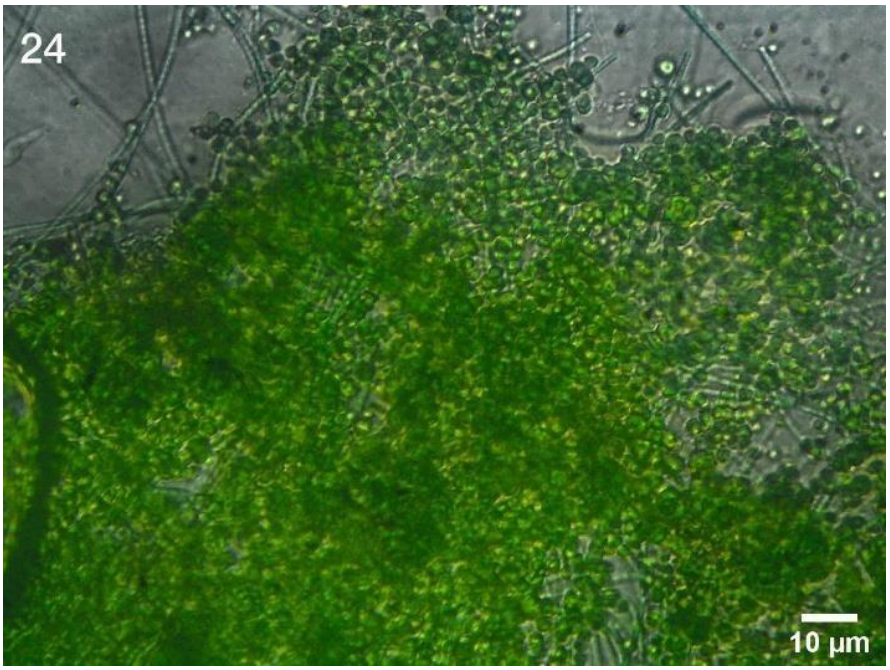
Selenastrum is a genus of green algae.Selenastrum has crescent-shaped or sickle-shaped cells.



Division	Chlorophyta
Class	Chlorophyceae
Order	Sphaeropleale
Family	Selenastracea
Genus	Selenastrum
Measured Length	290.5 µm

SAMPLE 24: *Chlorella*

Chlorella is a species of single- celled green alga. It's a good source of several vitamins, minerals and antioxidants.



Division	Chlorophyta
Class	Trebouxiophyceae
Order	Chlorellales
Family	Chlorellaceae
Genus	Chlorella

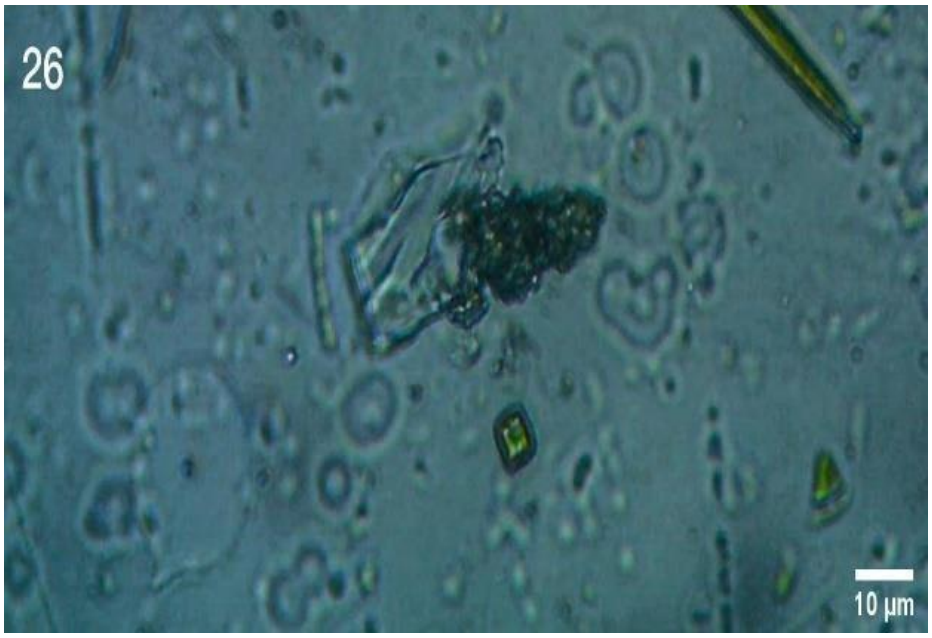
SAMPLE 25: *Ankistrodesmus*

Ankistrodesmus freshwater chlorophyte that is found worldwide . It is used to study biofuel production, and study the effects of toxins on aquatic communities.

Division	Chlorophyta
Class	Chlorophycea
Order	Sphaeropleales
Family	Selenastraceae
Genus	Ankistrodesmus
Measured Length	393.1 µm
Measured Breadth	69.9 µm

SAMPLE 26: *Cephaleuros virescens*

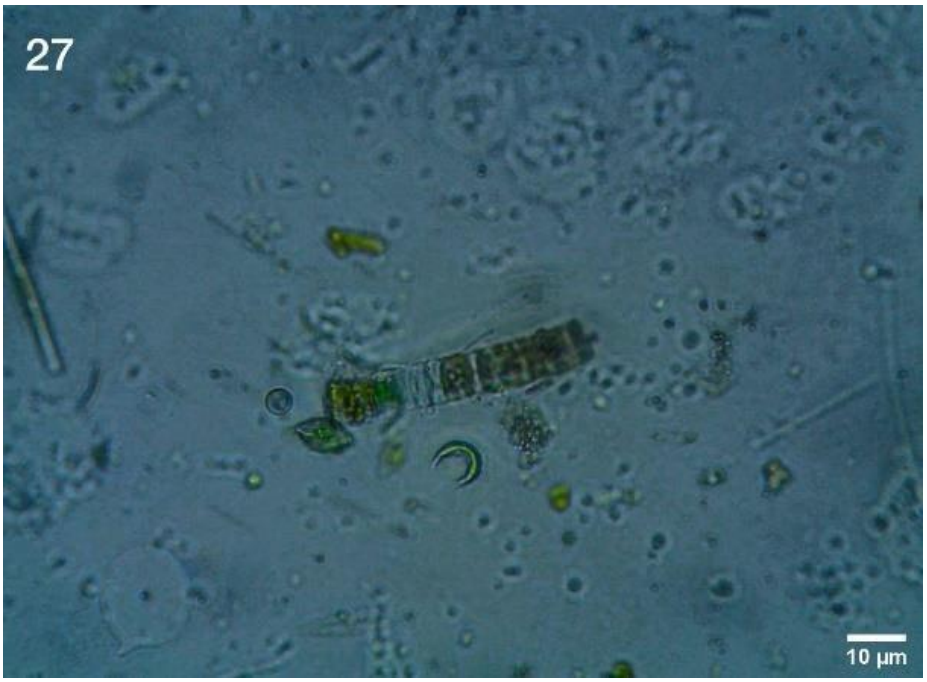
Cephaleuros virescens is a pathogen of algal plant.It infects tea, coffee and coconut plants, and this causes algal leaf spot or algal rust.



Class	Ulvophyceaex
Order	Trentepohlialesc
Family	Trentepohliaceae
Genus	Cephaleuros Kunze
Species	Cephaleuros virescens
Measured Length	51 µm
Measured Breadth	55.47 µm

SAMPLE 27: *Monoraphidium*

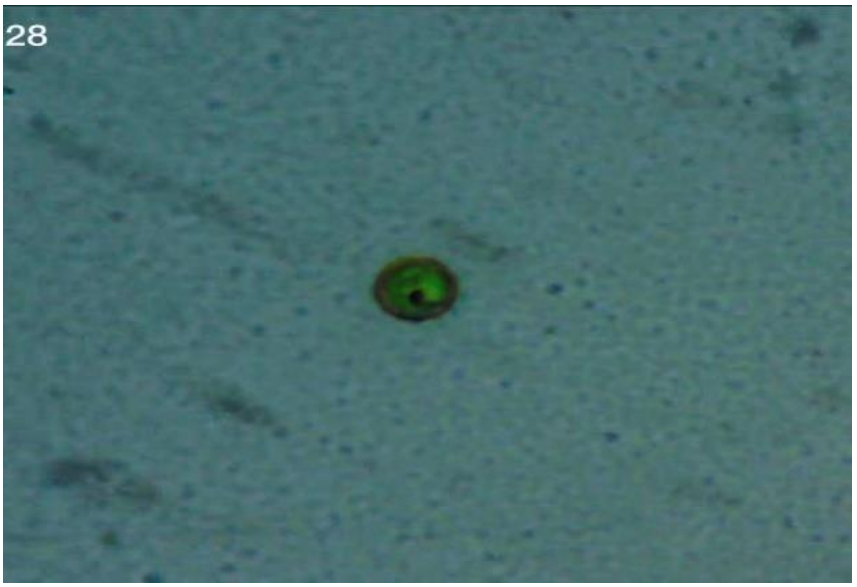
Monoraphidium is a genus of green algae in the family Selenastraceae.



Division	Chlorophytaw
Class	Chlorophyceae
Order	Sphaeropleales
Family	Selenastraceaex
Genus	Monoraphidium
Measured Length	58.41 **µm**

SAMPLE 28: *Chlorella vulgaris*

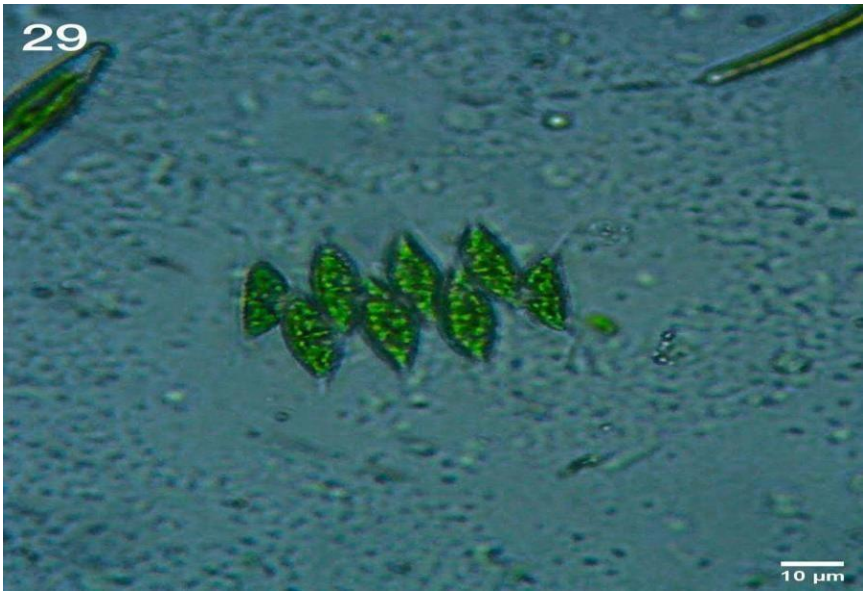
Chlorella vulgaris is a green microalga. It is used as a dietary supplement or protein-rich food additive in Japan.



Order	Chlorellales
Family	Chlorellaceae
Class	Trebouxiophyceae
Species	C. vulgaris
Genus	Chlorella
Division	Chlorophyta
Measured Length	11.271 µm

SAMPLE 29: *Chlamydomonadales*

Chlamydomanales belongs to green algae and is unicellular flagellates,that is found in stagnant water and on damp soil, in freshwater, seawater etc



Class	Chlorophyceae
Order	Chlamydomonadales
Family	Chlamydomonadaceae
Genus	Chlamydomonas
Species	Chlamydomonas
Measured length	244.2µm
Measured breadth	98.08µm

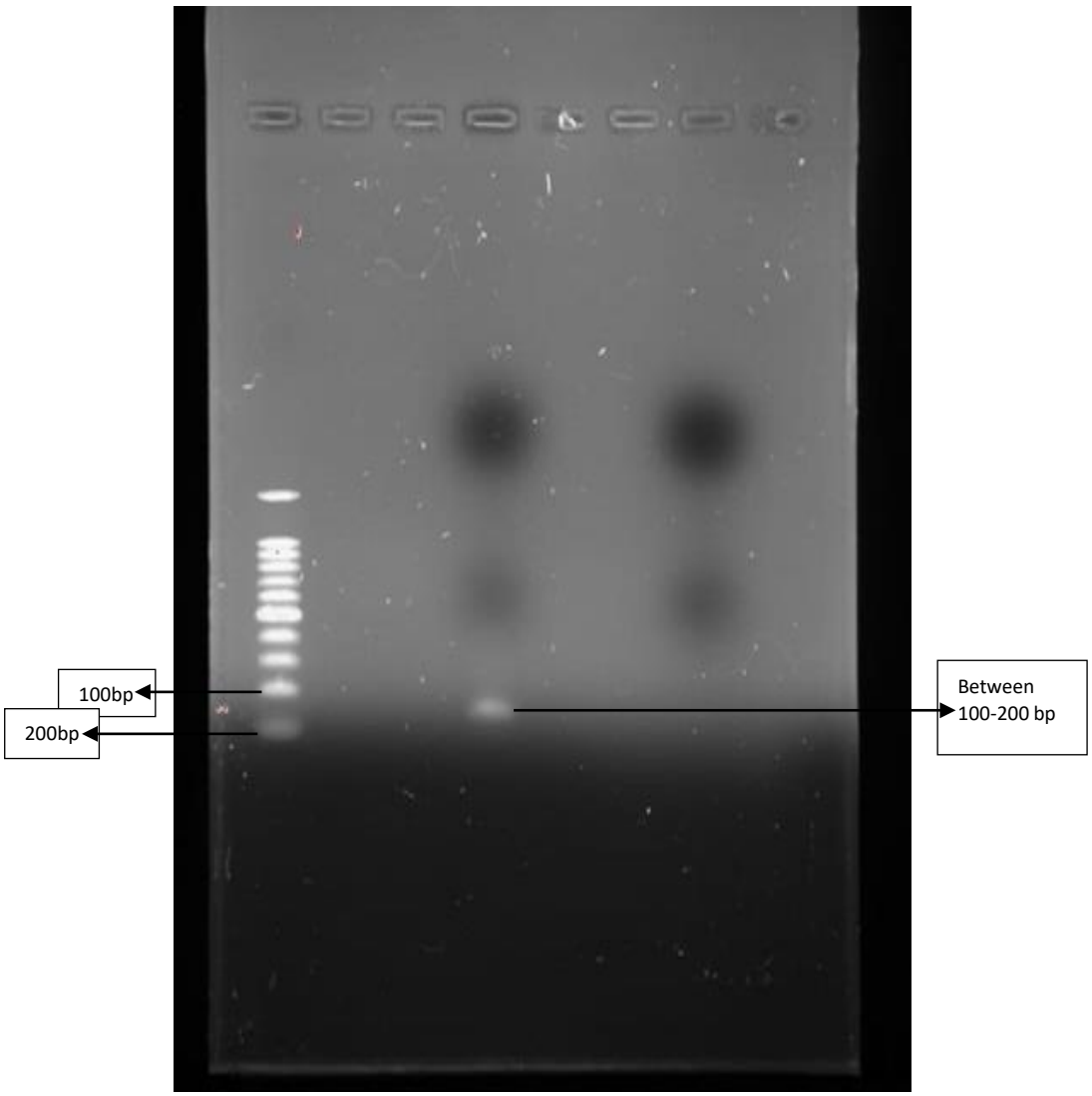
S.NO	SAMPLE NO:	***ALGAE***	***FAMILY***	***CLASS***
1	Sample 17	*Nitzschia palea*	*Bacillariaceae*	*Bacillariophyceae*
2	Sample 19	*Nitzschia cf. communis*	*Bacillariaceae*	*Bacillariophyceae*
3	Sample 11	*Zoochlorellae*	*Chlorellaceae*	*Trebouxiophyceae*
4	Sample 24	*chlorella*	*Chlorellaceae*	*Trebouxiophyceae*
5	sample 28	*Chlorella vulgaris*	*Chlorellaceae*	*Trebouxiophyceae*
6	Sample 1	*Rhizoclonium*	*Cladophoraceae*	*Ulvophyceae*
7	Sample 2	*Chaetomorpha minima*	*Cladophoraceae*	*Ulvophyceae*
8	Sample 8	*Cladophora species*	*Cladophoraceae*	*Ulvophyceae*
9	Sample 20	*Clostrium moniliferum*	*Closteriaceae*	*Zygnematophyceae*
10	Sample 3	*Geitlerinema splendidum*	*Coleofasciculaceae*	*Cyanophyceae*
11	Sample 5	*Oocystis*	*Oocystaceae*	*Trebouxiophyceae*
12	Sample 6	*Lyngbya aestuarii*	*Oscillatoriaceae*	*Cyanophyceae*
13	Sample 7	*Lyngbya*	*Oscillatoriaceae*	*Cyanophyceae*
14	Sample 9	*Cyanobacterium oscillatoria*	*Oscillatoriaceae*	*Cyanophyceae*
15	Sample 10	*Phormidium*	*Oscillatoriaceae*	*Cyanophyceae*
16	Sample 13	*Cyanobacterium oscillatoria*	*Oscillatoriaceae*	*Cyanophyceae*
17	Sample 15	*Cyanobacteria lyngbya*	*Oscillatoriaceae*	*Cyanophyceae*
18	Sample 4	*pithophora*	*Pithophoraceae*	*Ulvophyceae*
19	Sample 14	*Pithophora*	*Pithophoraceae*	*Ulvophyceae*
20	Sample 23	*Selenastrum*	*Selenastracea*	*Chlorophyceae*
21	Sample 25	*Ankistrodesmus*	*Selenastraceae*	*Chlorophycea*
22	Sample 29	*Chlamydomonadales*	*Chlamydomonadaceae*	*Chlorophyceae*
23	Sample 27	*Monoraphidium*	*Selenastraceaex*	*Chlorophyceae*
24	Sample 16	*Dictyochloropsis*	*Trebouxiaceae*	*Trebouxiophyceae*
25	Sample 26	*Cephaleuros virescens*	*Trentepohliaceae*	*Ulvophyceaex*
26	Sample 21	*Tribenoma species*	*Tribonemataceae*	*Xanthophyceae*
27	Sample 18	*Ulothrix*	*Ulotrichaceae*	*Ulvophyceae*
28	Sample 22	*Vaucheri*	*Vaucheriaceae*	*Xanthophycease*

4.2 Qualitative Analysis of DNA Sample

DNA sample was quantified using biophotometer. Quantity of DNA obtained was 9.2ug/µl with a purity of 1.14 (A260/280).

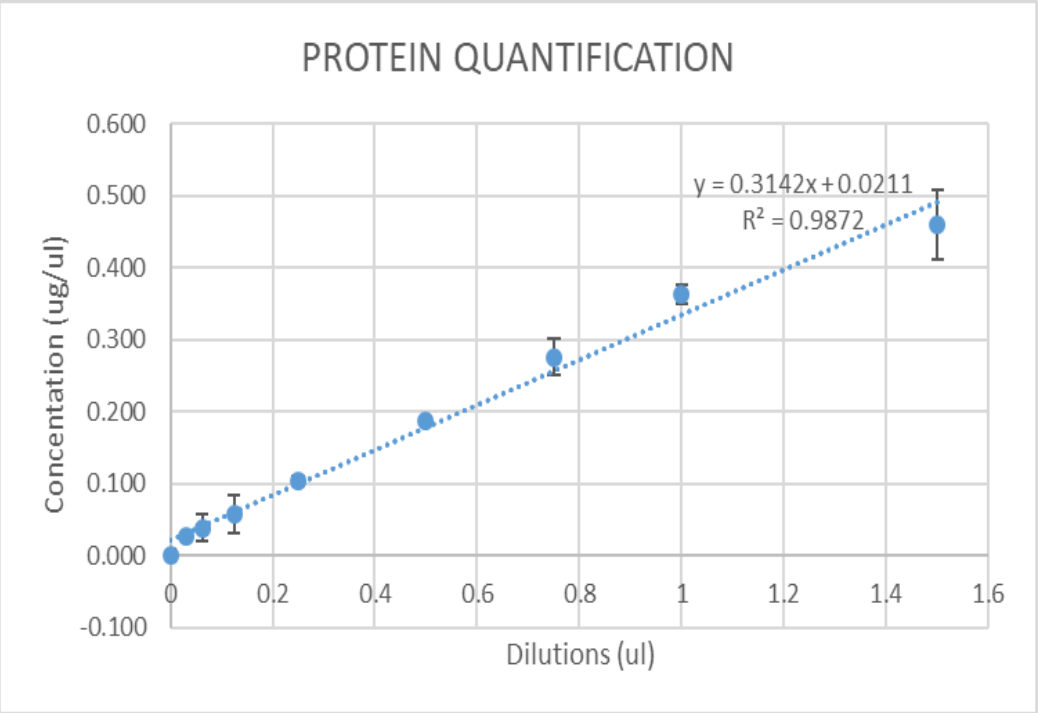
4.3 Agarose Gel Electrophoresis

DNA was separated using Gel electrophoresis. The voltage was set to 100W and the gel ran for 1 hour. The band was observed between 100-200bp.



4.4 Protein Quantification by Bradford's Method

Protein extraction of different algal strains was done using the Bradford method. Protein samples were mixed with Bradford reagent CBB G-250 and absorbance was measured at 595 nm using an ELISA reader. The readings were observed and calibration curve of protein concentration were done by using BSA as standard. Concentration of protein obtained as 1.123ug/ml.

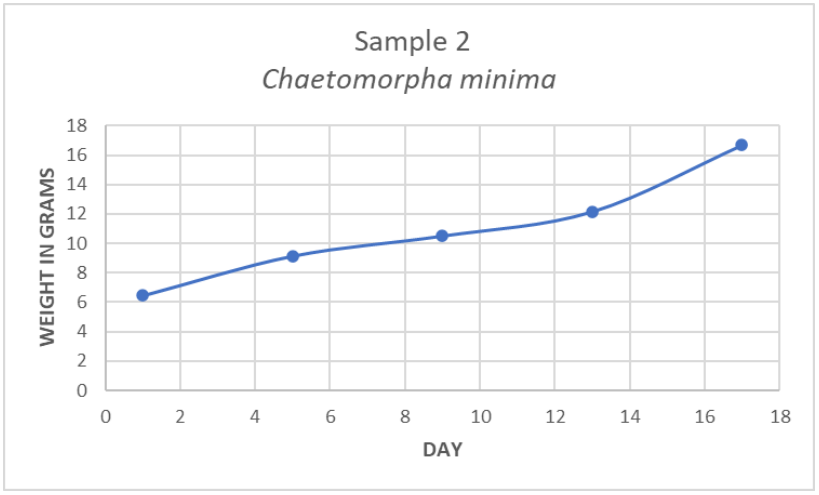


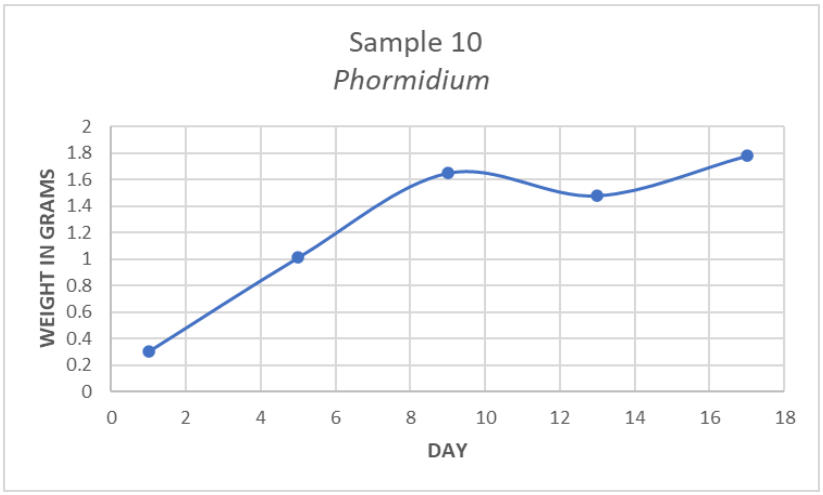
4.5 Folch Method

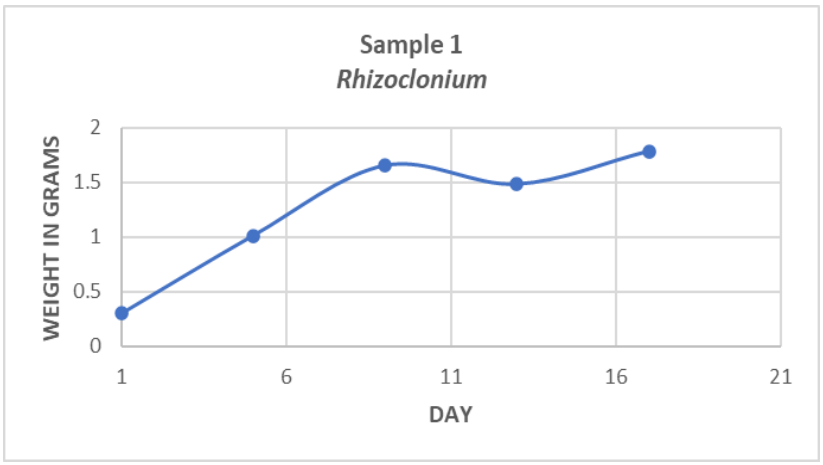
After drying the chloroform layer for 24 hours on a pre-weighed aluminium sample pan, the lipid content was weighed in grams. The lipid weight obtained was 0.16 grams

4.6 Algal Growth Rate

The 3 algal growths were plotted into graph and the maximum growth was observed for sample 2- *Chaetomorpha minima*







CHAPTER 5

CONCLUSION AND FUTURE PERSPECTIVE

CONCLUSION & FUTURE PERSPECTIVE

Twenty-nine species of algae/cyanobacteria were collected from Amritapuri and different parts of Kerala, and they were identified by comparing the morphometric measurement of the microscopic pictures and habitat with taxonomic keys available in the literature. Out of 29 species, the growth rate of three species was measured. *Chaetomorpha minima* showed the fastest growth in the local sewage, doubling-time of approximately six days.

Chaetomorpha minima, previously identified as having high antioxidant activity, was studied in detail and collected from Amrita School of Biotechnology's vertical garden. The sample DNA was extracted using the CTAB method, followed by amplification of DNA by PCR using rbcL primers. The size of algal DNA was found to be between 100 - 200bp using gel electrophoresis. The DNA has been sent for sequencing to confirm the identity of the algae. The protein content of the algae is 1.12 mg/mL using Bradford Assay. The identified algae had a lipid content of 0.16g/g dry weight.

In future, the potential of the fast-growing algae can be explored for their nutrient removal capacities and be used for the bioremediation of wastewater. *Chaetomorpha minima* have already been shown to have high antioxidant activity and have the potential to be used as a sun protection factor (SPF) in pharmaceutical/food/cosmetic industries. DNA barcoding protocol needs to be followed for remaining species to confirm their identities. Economic valorisation potential can be explored with respect to their biological activities: sun protection factor (antioxidant), animal feed, biofertilizer.

CHAPTER 5

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