

EXTRACTION AND ESTIMATION OF PROTEIN FROM MICROALGAE

Submitted in partial fulfillment of the requirements for the award of Bachelor of
Science degree in Biochemistry

By

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SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Harsha Varthini G (40070002)**, **Sabeetha M (40070004)**, who carried out the project entitled **"EXTRACTION AND ESTIMATION OF PROTEIN FROM MICROALGAE"** under our supervision from December 2022 to May 2023.

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ABSTRACT

The Xanthophyceae is a clade of stramenopilan photoautotrophs containing about 118 genera and 600 species. Morphology ranges from free-living or attached unicells to colonies and unbranched or branched filaments and siphons. A large majority are found in freshwater and soil, while some occur in brackish and marine habitats. Although abundant growth of a few species can occur in nature, none are known to be of practical importance. They are characterized by possession of chlorophylls *a*, *c*₁, and *c*₂ and a range of xanthophylls, but not fucoxanthin, in generally yellowish-green, discoidal, parietal chloroplasts. Thylakoids are in groups of three, and most species investigated have a single thylakoid forming a girdle band around the periphery of the chloroplast. Chloroplasts are surrounded by chloroplast endoplasmic reticulum. Pyrenoids, when present, are typically semi-immersed and are not associated with granules of storage products. A cell wall consisting of two overlapping parts occurs in some coccoid and filamentous species. Reproduction is generally asexual but some, e.g., *Vaucheria*, exhibit sexual reproduction. The taxonomic status of a significant number of species is uncertain, especially those that are rarely observed, e.g., species of Chloramoebales, Heterogloales, and Rhizochloridales. Transfer of species to the Eustigmatophyceae and other groups is likely. There is molecular phylogenetic data for fewer than 20 % of species. Four major clades are recognized. Two of these contain both coccoid and filamentous species. Many traditional orders, families, and genera are paraphyletic or polyphyletic. It is presently convenient to retain the traditional classification of seven orders based on morphology until these difficulties are resolved following the inclusion of more species in phylogenetic analyses.

Keywords: Algae, Protein, Estimation, Extraction.

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LIST OF ABBREVIATION

BBM - Bold Basal Medium
BCA - Bicinchoninic Acid
CG - Gas chromatography
TLC - Thin layer chromatography
ACS - American chemical society

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CHAPTER 1

INTRODUCTION

Yellow-green algae or the Xanthophyceae (xanthophytes) are an important group of heterokont algae. Most live in fresh water, but some are found in marine and soil habitats. They vary from single-celled flagellates to simple colonial and filamentous forms. Xanthophyte chloroplasts contain the photosynthetic pigments chlorophyll *a*, chlorophyll *c*, β -carotene, and the carotenoid diadinoxanthin. Unlike other Stramenopiles (heterokonts), their chloroplasts do not contain fucoxanthin, which accounts for their lighter colour. Their storage polysaccharide is chrysolaminarin. Xanthophyte cell walls are produced of cellulose and hemicellulose. They appear to be the closest relatives of the brown algae. The species now placed in the Xanthophyceae were formerly included in the Chlorophyceae. In 1899, Lüther created the group Heterokontae for green algae with unequal flagella. Pascher (1914) included the Heterokontae in the Chrysophyta. In 1930, Allorge renamed the group as Xanthophyceae. The monadoid (unicellular flagellates) and also sometimes the amoeboid species have been included by some authors in the Protozoa or Protista, as order Heterochloridina (e.g., Doflein and Reichenow, 1927-1929), as class Xanthomonadina, with orders Heterochloridea and Rhizochloridea (e.g., Deflandre, 1956), as order Heterochlorida (e.g., Hall, 1953 Honigberg et al., 1964), as order Heteromonadida (e.g., Leedale, 1983), or as subclass Heterochloridia (e.g., Puytorac et al., 1987). These groups are called ambiregnal protists, as names for these have been published under either or both of the ICZN and the ICN. Microalgae are largely a diverse group of microorganisms comprising eukaryotic photoautotrophic protists and prokaryotic cyanobacteria. These microbes contribute to half of global photosynthetic activity and are virtually found in euphotic niches.



Figure 1.1 Algae in pond water

Algae are defined as a group of predominantly aquatic, photosynthetic, and nucleus-bearing organisms that lack the true roots, stems, leaves, and specialized multicellular reproductive structures of plants. Algae are photosynthetic organisms. Algae can be either unicellular or multicellular organisms. Algae lack a well-defined body, so, structures like roots, stems or leaves are absent. Algae are found where there is adequate moisture (Allorge, 1930). Reproduction in algae occurs in both asexual and sexual forms. Asexual reproduction occurs by spore formation. Algae are free-living, although some can form a symbiotic relationship with other organisms. Algae are neither bacteria nor plants. The term algae is used to describe a large collection of photosynthetic, eukaryotic organisms. Most of these organisms grow in freshwater or seawater, they also grow on soil, trees, and animals such as turtles and polar bears and even under or inside porous rocks, such as sandstone and limestone (Emend Fritsch, 1935). They can tolerate a wide range of temperatures and they can grow in hot springs, on snow banks, or deep within the polar ice. Algae do not have true roots, stems and leaves. Hence, they need to be near a moist or watery environment to survive. In other words, they do not possess vascular tissue necessary for the conduction of water and minerals. Algae are generally harmless to humans. However, certain species of algae can form algal blooms, which can have detrimental effects on human health and the environment (Hibberd, 1981). Algae

are known to fix 50% carbon dioxide. Being photosynthetic, they increase the oxygen content of their environment. They are primary producers and many organisms derive food from them. Many commercial products are obtained from algae such as align, etc. Algae form the primary source of food for many organisms in the food chain (Leedale,1983). From an economical perspective, algae are very useful for creating biomass or fertilizers as they grow very fast. Algae are the most important photosynthesizing organisms on Earth. They capture more of the sun's energy and produce more oxygen than all plants combined. They form the foundation of most aquatic food webs, which support an abundance of animals. Algae have been used for centuries, especially in Asian countries, for their purported powers to cure or prevent illness as varied as a cough, gout, gallstones, goitre, hypertension, and diarrhoea. Recently, algae have been surveyed for anticancer compounds, with several cyan bacteria appearing to contain promising candidates (Pascher,1912). Diatoms also have been used in forensic medicine, as their presence in the lungs can indicate a person died due to drowning

1.1 USES

- A range of microalgae species are produced in hatcheries and are used in a variety of ways for commercial purposes, including for human nutrition, as biofuel, in the aquaculture of othe organisms, in the manufacture of pharmaceuticals and cosmetics, and as biofertilizer (Luther, 1899).
- Food and health seaweed cultivation forms a major industry, particularly in japan, china and korea, where many algae species are grown by aquaculture.
- Algae are packed full of essential micronutrients such as magnesium, calcium, potassium, iodine, iron, and zinc and are therefore used in many food supplements (Luther, 1899).
- Algae has long been used to fertilise crops as they provide a rich source of nutrients and organic matter. Algae can be directly deposited on the land where it decomposes or algae extract can be sprayed on the crops (Allorge, 1932).
- The large diversity of pigments found in different algal species offer a source of natural pigments to be used as alternatives to artificial chemical dyes and colouring agents, as well as for food colouring.

- Agar, the medium used in the labs to culture bacteria as well as other lab techniques such as gel electrophoresis is derived from red algae. A white powdered substance, known as diatomaceous earth is made of fossilised diatoms, a single-celled alga, that contains silica (Emend Fritsch, 19350).
- Algae also hold great potential to be used as carbon-neutral fuels. Many species of microalgae have high lipid contents that can be readily extracted and converted to biodiesel. They also process high concentrations of fermentable sugars making them suitable for bioethanol production.
- Algae naturally produce polymers that have a great potential for bioplastic production such as starch or polyhydroxyalkanoates (PHAs). PHAs possess thermoplastic properties that don't need to be modified to create a plastic film.
- Algae can also be used in wastewater treatment as they metabolise and breakdown waste substances, thereby reducing the need for toxic chemicals to be used (Kolb N,1995).
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Algae are primarily classified into the following types:

- Blue-green Algae
- Red Algae
- Green Algae
- Yellow-green algae

1.2 CHARACTERISTICS OF MICROALGAE

- Microalgae are microscopic, single-celled, and mostly photosynthetic. Although they are single-celled, some of them can form colonies, such as filaments or spheres with the same species. Their ability to photosynthesize is due to the presence of photosynthetic pigments (Winget G.D, 1966).
- Algal cells are eukaryotic. For instance, algae can photosynthesize like plants, and they possess specialized structures and cell-organelles, like centrioles and flagella, found only in animals. The algal cell walls consist of mannans, cellulose and Galatians. Listed below are some of the general characteristics of algae.
- Algae are photosynthetic organisms. Algae can be either unicellular or multicellular organisms. Algae lack a well-defined body, so, structures like roots, stems or leaves are absent. Algae are found where there is adequate moisture. Reproduction in algae occurs in both asexual and sexual forms (Heasman, M. 2001).
- Asexual reproduction occurs by spore formation. Algae are free-living, although some can form a symbiotic relationship with other organisms. Algae provide much of Earth's oxygen, serve as the food base for almost all

aquatic life, and provide foods and industrial products, including petroleum products. Their photosynthetic pigments are more varied than those of the classification of algae is changing rapidly because new taxonomic information is being discovered (Hoffman J.R., 2004).

- Algae were formerly classified into three major groups the red, brown, and green seaweeds based on the pigment molecules in their chloroplasts. Many more than three groups are now recognized, each sharing a common set of pigment types.
- Algae are not closely related to each other in an evolutionary sense. Specific groups can be distinguished from protozoans and fungi only by the presence of chloroplasts and by their ability to carry out photosynthesis; these specific groups thus have a closer evolutionary relationship with the protozoa or fungi than with other algae. Algae are common on “slimy” rocks in streams and as green sheens on pools and ponds. Use of algae is perhaps as old as humankind; many species are eaten by coastal societies (Ingelfinger J.R. 2008).
- Yellow-green algae which contain chlorophyll but lack chlorophyll b. Instead of chlorophyll b possess chlorophyll e or c.
- Besides chlorophyll they possess xanthophylls and beta carotene. Mostly fresh water unicellular to siphonous body. Cells are eukaryotic with silica and pectic in the cell wall. Chief pigments include chlorophyll a and e, beta carotene, neoxanthin and violaxanthin (Volkman H., 2008).
- Reserve food includes chrysolaminarin and oils; Starch and pyrenoids absent. Sexual reproduction is isogamous, anisogamous or oogamous. Zoospore formation is common (Wang T., 2009).
- Male gametes are flagellate.
- Flagella are heterokont (Unequal).
- Life cycle is mostly haplontic.



Figure 1.2: sources of algae

Some algae can also be used to capture fertiliser runoffs, therefore preventing harmful effects of eutrophication as less enters aquatic systems. Algae are also used in some power plants to reduce CO₂ emissions as it is captured and utilised but the algae for photosynthesis. Algae are utilised as a feed for a diverse set of farm animals, especially seaweed. For example, cows and chickens, *Rhodomenia palmate* or alleged “Sheep’s weed” is utilized (Mariotti F., 2004)

Xanthophyceae are in general less frequent in marine than in freshwater environment, although several benthic species are common in brackish waters. They occur often under dystrophic or mesotrophic conditions, showing their highest diversity in acidic waters enriched with dissolved organic matter.

Xanthophytes include a variety of cell organizations. Single flagellated cells are relatively rare, except in brackish waters, where they are usually metaphytic or epiphytic among submersed vascular plants (Karsten U., 2012). A trichal xanthophyte common in freshwater plankton, which forms straight filaments where cells are held together by overlapping H-shaped cell walls. Several epiphytic species prefer cold, nutrient rich waters, where they can be found on reeds and other vegetal

substrate especially in spring and autumn. Other trichal xanthophytes (i.e., *Heterodendron*) form branched filaments (Eilertsen K.E., 2016).

The most common and best known xanthophytes have siphonal organization, characterized by globose (*Botrydium*) or tubular thalli, where numerous nuclei and chloroplasts are dispersed in the protoplast and not separated by cell walls (Batan, L. 2010).

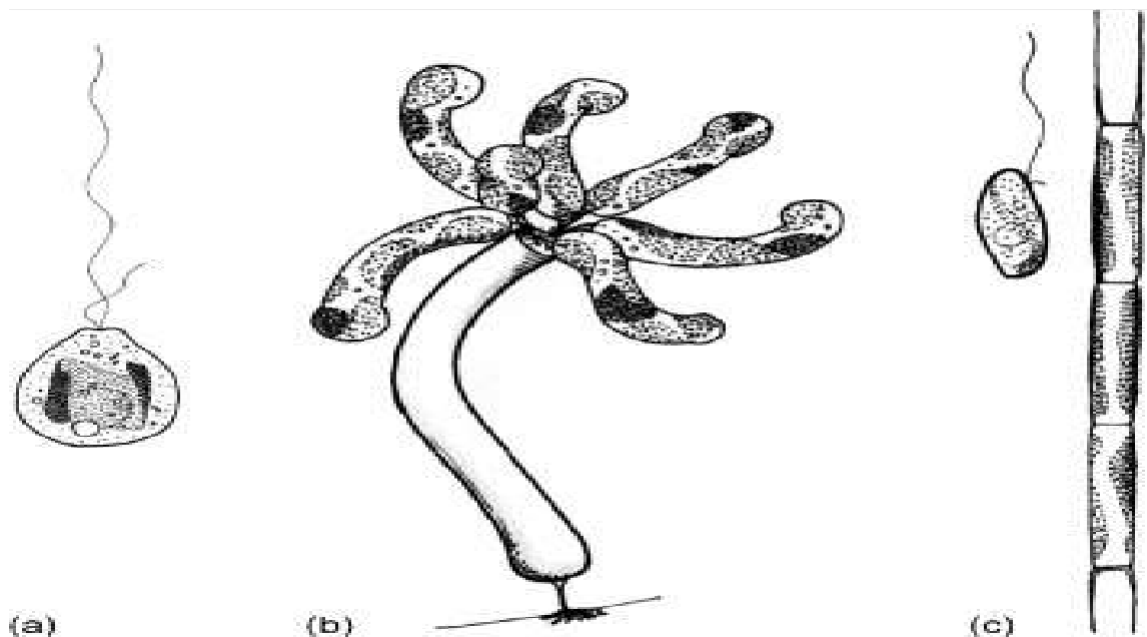


Figure 1.3 Example of xanthophyceae organization

The thallus of *Vaucheria* shows a zonation of nuclei, chloroplasts, and vesicles near the filament apex and a peculiar light adaptation, which consists in changing chloroplast distribution and orientation according to changing light intensity and spectral composition (Biancarosa L., 2017). Chloroplast displacement occurs by protoplast currents and is aimed at optimizing light harvesting and reducing light induced pigment damages. Several *Vaucheria* species live in brackish and sea waters, while others are very common on damped ground and in well-aerated ditches, pools, and slow-flowing small streams, where they can form mats and cushions (Jones D.B. 1941).

For the majority of Xanthophyceae only vegetative reproduction is known, which can occur by vegetative cell division, filament fragmentation, nonmotile spores (aplanospores) or flagellated spores (zoospores). Although free-swimming xanthophytes are very few, reproduction by flagellated spores is very common in the whole class. Several taxa (e.g., *Tribonema* and *Botrydium*) can also produce akinetes, modified vegetative cells with thickened cell wall that can survive adverse environmental conditions (Grobelaar, J.U., 1985).

Sexual reproduction is less common and has been reported only in *Tribonema*, where isogamous union of gametes produces a dormant zygote, *Botrydium*, which isogamous or anisogamous gametes, and *Vaucheria*, which presents a very characteristic oogamy, with male and female reproductive structures arising near the apex of vegetative filaments on monoecious or dioecious thalli (Hoek C., 1995). Although each species has its own temperature optimum and range, several studies have shown that Bacillariophyceae (diatoms) tend to dominate at approximately 5–20°C, Chlorophyceae (green algae) and Xanthophyceae (yellow-green algae) at 15–30°C, and Cyanophyceae at >30°C (Kjeldahl J. 1983)

CHAPTER 2

REVIEW OF LITERATURE

1. Due to their rich composition (wide variety of biomolecules of various chemical nature), greater areal productivities in comparison to traditional crops, and independence from fresh water and arable land, microalgae have been seen as a viable feedstock for the feed and food sectors.(Draaisma *et al.*, 2013)
2. The main sources of energy are frequently lipids and carbohydrates; alterations in these stores might reveal how an organism sees its surroundings and how it intends to adapt. Total protein variations may be an indicator of how quickly cells are metabolising nutrients as they expand. In reaction to environmental stress, these components' cellular distribution changes. (A. Singh *et al.*Appl. Energy (2011))
3. Transporting flue-gas CO₂ to the pond and putting the gas into solution requires a significant amount of energy. This input is influenced by a variety of variables, such as the distance between the power plant and the algal ponds and the amount of CO₂ present in the flue gas, among others. Assuming there are algal ponds all around the power plant (A. Singh *et al.*Appl. Energy 2011)
4. For the microalgae production platform to remain viable, multiple high-value product extraction is advised. This paper suggests a method for selectively extracting carotenoids and chlorophylls from wet, frozen-thawed *Chlorella vulgaris* biomass before extracting proteins at an alkaline pH. (Benemann and Oswald 1996)
5. In which a phytoplankton bloom was induced to occur in a free-floating 20-ft diameter thin transparent plastic sphere has been repeated. Daily measurements were made of nutrients, particulate matter, and photosynthetic rates with less frequent assays for vitamins and dissolved organic matter (S. Kulkarni *et al.*2018, Antia, N. J., Mc Allister)
6. The heated biuret-Folin method for determining protein consistently measures 90% of the total nitrogen of filtered algae samples as protein-N without the need of mechanical disruption as long as the heating period in biuret is 100

min at 100 C. (T Iwamura, H Nagai, SE Ichimura - Internationale Revue der ..., 1970)

7. An increase of the nutritive value can be achieved by heating the cells in solution or during drying. The heat treatment increases the permeability of the cell envelope to digestive enzymes and an increase of the nutritive value up to the same level as after complete mechanical cell disruption is obtained. (TE Dorsey, P McDonald, OA Roels - Journal of Phycology, 1978)
8. Because changes in the chemical composition of cells may occur as a result of the washing and storage conditions utilised, the preparation of samples for analysis is covered. Wet, since analytical results must make reference to one of these values, dry-weight determinations of bacterial samples and assays of total cell counts are presented. (Hedenskog, G., 1978)
9. Growing algae is a viable method for reducing the effects of population expansion and rising carbon dioxide emissions. Microalgae are a diverse group of photoautotrophic microorganisms that can grow using anthropogenic emissions as sources of nutrition and transform tiny molecules like ammonium or carbon dioxide into macromolecules with added value like proteins. Microalgae exhibit great photosynthetic productivity and growth rates, which lead to high productivities of carbohydrates. Growing algae is a viable method for reducing the effects of population expansion and rising carbon dioxide emissions. (D Herbert, PJ Phipps, RE Strange)
10. Microalgae are a diverse group of photoautotrophic microorganisms that can grow using anthropogenic emissions as sources of nutrition and transform tiny molecules like ammonium or carbon dioxide into macromolecules with added value like. (Abomohra et al. Citation2016; Chen et al. Citation2018; Su et al. Citation2017)
11. The chemical properties of algae's biomass can be learned a lot from the determination of their protein content. (Lowry et al., 1951)
12. Because not all amino acids can equally oxidise the Folin phenol reactive or bind to the Coomassie dye, the reactivity of both techniques in comparison to a particular protein is highly impacted by its amino acid composition. (Stoscheck, 1990)

13. Protein analysis involves so many variables at once, writers may overlook certain parameters and compromise the accuracy of protein analysis. However, research on protein analysis in algae is comparatively understudied, and to fill this gap, experimental data are required. In order to make data on algal protein accessible to academics, algae growers, people interested in the nutritional value of algae, and soon, it is also crucial to design a straightforward and affordable protocol employing affordable tools and consumables. In this work, various methods for extracting and measuring the amount of protein in marine algae were assessed. The study's specific objectives were to (i) develop a methodology for the extraction and measurement of marine algae's protein. (Lowry and Bradford)
14. Microalgae are tiny photosynthetic microbes that can adapt to nearly any environment. Currently, microalgae are being grown commercially all over the world in a number of small- to medium-scale systems to produce goods for human nourishment. Proteins, carbohydrates, fatty acids, and other nutrients are abundant in microalgae. (Shaila Hiremath and Pratima Mathad, 2010).
15. Microalgae are a key primary producer for a variety of consumers, including Rotifers, Copepods, Shrimps, and others, which in turn feed juvenile and late larval fish and crustaceans. (Richmond, 1996)
16. Microalgae can improve the nutritional value of typical meal preparations, which benefits both human and animal health. One of the key factors for considering microalgal species as an unconventional source of protein is their high protein content. (Cornet, 1998; Soletto, 2005)
17. Algal cells in a volume of 40 ml were centrifuged; the pellets were then twice-washed in tris HCl buffer (pH-7.0) and re-suspended in a volume of 5 ml of the same buffer. The suspension was centrifuged at 2000 rpm for 15 minutes after being sonicated in an Ultra-sonicator for 15 minutes. After adding 10% Trichloro Acetic Acid (TCA) to the supernatant, the precipitate was produced by centrifuging the mixture for 15 minutes at 5000 rpm in a cooled microfuge. The precipitate is then neutralised with an unknown amount of 2N NaOH and tested for protein. (Lowry *et al.*, 1951).
18. On the fifth, tenth, fifteenth, and twentieth days, samples of algae were taken from an outdoor algal pond in order to analyse the total protein level. This was done using Lowry's method from 1951. According to the findings, the total

protein level was 117 mg/gram on day 5, 137 mg/gram on day 10, 182 mg/gram on day 15, and 83 mg/gram on day 20. According to the aforementioned findings, total protein levels peaked on the 15th day and then slightly decreased after that. (Lowry's 1951)

19. An enriched source of CO₂, such as flue gas, must be available for microalgae production in open ponds or enclosed photobioreactors. The use of closed photobioreactors to produce fuels, feeds, or reduce greenhouse gas emissions is neither technically nor commercially practical. (Weissman *et al.* 1988)
20. During the austral summer, a microalga was isolated from the pack-ice in Wood Bay (Antarctica). Its pigmentation and ultrastructural characteristics were compared to those of a legitimate strain obtained from the Sammlung von Algenkulturen at the University of Göttingen. Comparable in composition to *Pleurochloris meiringensis*. However, comprehensive 18S rRNA gene sequence analysis indicated significant genetic divergence. (C Andreoli, I Moro, N La Rocca, F Rigoni... - European Journal of ..., 1999)
21. Under axenic circumstances, the alga *Vaucheria dichotoma* was isolated and grown. Temperature, pH, and salinity maxima of 22–23° C and 8.0–8.5, respectively, were found. Glycerophosphate was as effective as inorganic phosphate as a source of P, and good growth could be attained in a variety of concentrations of all three P-compounds. The organic N-source had no effect on growth at all, and NO₃ produced the highest yield out of the six N-sources that were examined. (H Åberg, L Fries - *Phycologia*, 1976 - Taylor & Francis)
22. Although precise measurements of carbon intake were acquired by the ¹⁴C approach, it was discovered that chlorophyll a content was a good criterion for prospective carbon generation. The best markers are estimations of ATP, active proteins, and amino acids of cellular metabolism. Dry weight is used to evaluate the standing crop well. (BR Berlan *et al.* 1970)
23. In order to isolate proteins from microalgae, yeasts and bacteria, cell disintegration in a special ball-mill was performed. The degree of disintegration of the different microorganisms was compared. The dependence of disintegration on bead size and on the ratio between the volume of suspension and the volume of glass beads was also investigated. Nondisintegrated and disintegrated cells were extracted with sodium

hydroxide and then amount of extractable nitrogen and the amount of nitrogen precipitable at pH 4.0 (G Hedenskog *et al.* 1970)

24. Biofuels like biomass, methane, and ethanol are examples of renewable energy sources, and microalgae is one potential biological fuel source. Microalgae are a renewable resource since they obtain their energy and carbon from light and carbon dioxide. Microalgae appear to be more effective in fixing carbon dioxide than terrestrial plants at photosynthetic processes. Algae's capacity to fix carbon dioxide has been suggested as a way to remove carbon dioxide from power plant exhaust gases and so minimise greenhouse gas emissions. (A.H. Scragg *et al.* 2002)
25. The metabolic activity of microalgae, which is constrained by photosynthesis, regulates the rate of carbon intake. Numerous writers have researched the ability of microalgae to repair CO₂. Theoretically, microalgae may sequester or absorb up to 513 tonnes of CO₂ while producing 280 tonnes of dry biomass per hectare each year. 8 days of xanthophyce cultivation in 3% CO₂ resulted in a carbon dioxide fixation of 865 mg. This translates to about 1.7 times more carbon dioxide fixed than Bilanovic's estimate. (Bilanovic *et al.*, 2009)
26. Hormones, carbohydrates, and others. This suggests that the fate of carbon may be fairly complex and that the creation of microalgal biomass is only one aspect of the carbon destination. Additionally, limited data is known about the concurrent study of the worldwide rates of carbon dioxide sequestration and the rates of carbon incorporation into the biomass of microalgae. (Chiu *et al.*, 2008)
27. Due to intrinsic benefits such as high biomass and lipid productivities, the ability to grow microalgae on non-arable land, the use of low-quality and saline water, and integration with point source waste streams, microalgae production has received a great deal of attention in recent years. (Khan *et al.*, 2009; Mata *et al.*, 2010)
28. Cells are exposed to circumstances during microalgae formation that guarantee lipid accumulation and biomass expansion. Environmental factors like nutrient load, carbon dioxide, light, and temperature are properly controlled and optimised for microalgae development and stability by the growing system. However, these conditions abruptly end during harvesting and preprocessing, and new conditions that were previously avoided in a

regulated development environment emerge. Conditions and exposure time vary depending on the harvesting technique and processing capacity. The cells are often not exposed to sunlight during the collection and transportation of microalgae from the cultivation system to a centralised separation facility, and the culture temperature depends on the weather. Microalgae are still performing physiological functions throughout this time, albeit under less-than-ideal circumstances. (Mishra and Gamage, 2007)

29. The literature on microalgae that addresses these issues is few, and there are few studies that give data on biomass and lipid breakdown. Prior research concentrated on the fractionation of carbon isotopes, the respiration rate of microalgae, and the decomposition of microalgae in their natural habitats. Results have limited applications to microalgae biorefinery systems. The effect of storage duration or preprocessing temperatures on yield is also not taken into account by a number of techno-economic and life cycle studies of the microalgae biofuel process since they all presume a smoothly integrated co-located growth and processing facility. (Batan *et al.*, 2010)
30. Cultivation systems, which include photobioreactors (PBR) and open raceway ponds, have been used at pilot plant scale to produce biomass, which is typically harvested, preserved, and processed off site. Large-scale systems will integrate on site processing based on environmental and economic benefits. (Lundquist *et al.*, 2010)

CHAPTER 3

AIM & SCOPE

3.1 AIM:

To extraction and estimation of protein from microalgae.

3.2 SCOPE:

- The process of protein extraction starts with very crude samples that are cleaned up through filtration, centrifugation, solubilization and precipitation.
- To determine the amount of protein present in the algae by Lowry's method.

CHAPTER 4

MATERIALS AND METHODOLGY

4.1 MATERIALS AND METHODS FOR EXTRACTION:

Mortor and pestle, Weighing machine, Micro centrifuge tube.

4.1.1 REAGENTS:

1. Sodium Chloride (NaCl)
2. Tris HCl
3. Sodium Dodecyl Sulphate (SDS)

4.1.2 PROCEDURE:

- Weigh 1-1.5g of algae sample add 1ml of protein extraction buffer per gram of algae in a cold mortor and pestle.
- Grind the tissue, collect the paste(1.5ml) and place them in refrigerator for 20 mins at 12000 rpm at 4°C.
- Transfer the supernatant to another 1.5ml micro centrifuge tube.
- Place about 100muL of the supernatant protein by Lowry method.



Figure: 4.1 Extraction of algae

4.2 MATERIALS AND METHODS FOR ESTIMATION:

Test tubes, standard measuring flask, test tube stand, conical flask, beaker, pipette, cylindrical measure, micro pipette

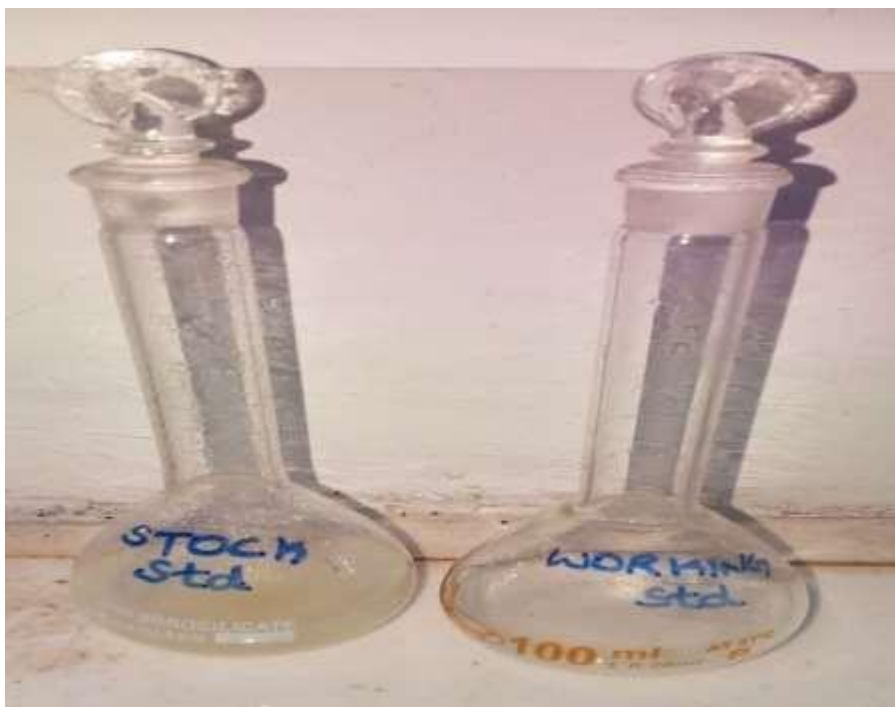


Figure: 4.2 sample preparation



Figure 4.3 Reagent preparation

4.2.1 REAGENT REQUIRED

- A. Sodium carbonate (Na_2CO_3)
- B. Sodium hydroxide (NaOH)
- C. Sodium potassium Tartarate
- D. Copper sulphate (CuSO_4)
- E. Folin's reagent

SOLUTION A: 4g sodium carbonate in 0.2N of sodium hydroxide.

SOLUTION B: 1g copper sulphate in 2g of sodium potassium tartarate.

SOLUTION C: 100ml of solution A and 2ml of solution B.

STOCK SOLUTION:

- 100mg of BSA(Bovine serum albumin) dissolved in water and make up into 100ml.

WORKING STANDARD SOLUTION:

- 10ml of BSA stock solution make up into 100ml.

4.2.2 PROCEDURE:

- Pipette out into clean test tubes 0.5, 1, 1.5, 2, and 2.5 of BSA working standard and make up into 2.5 ml using distilled water.
- Add 0.5 and 1ml of test solution.
- To each test tube add 4.5ml of alkaline copper sulphate. Mix well allow to stand at room temperature for 10 mins.
- Pipette out 0.5ml of folin's reagent in each test tubes. Mix well after each addition.
- Allow the tubes to stand for 30 minutes.
- Measure the absorbance of optical density at 660nm.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 RESULT

TABLE: 5.1 Estimation of protein (before heat shock)

	B	S1	S2	S3	S4	S5	T1	T2
WORKING STANDARD	-	0.5	1.0	1.5	2.0	2.5	-	-
Conc. Of PROTEIN	-	50	100	150	200	250	-	-
TEST SOLUTION	-	-	-	-	-	-	0.5	1.0
DISTILLED WATER	2.5	2.0	1.5	1.0	0.5	-	2.0	1.5
ALKALINE COPPER SULPHATE	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
FOLIN'S REAGENT	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
MIX WELL AND INCUBATE FOR 30 MINUTES AT ROOM TEMPERATURE								
OD AT 660nm	0	0.09	0.18	0.27	0.36	0.47	0.08	0.14

Figure: 5.1 Graphical representation of estimation of protein before heat shock

CALCULATION:

T1=0.5ml of unknown protein solution compounds to 0.08 OD

T1 0.5ml of unknown protein solution contains 45 µg of protein

Therefore, 100ml of unknown solution contain

$$= 100 \times 45 \mu\text{g} \div 0.5$$

$$= 13,500 \mu\text{g}$$

$$= 13.5\text{mg}$$

T2=1ml of unknown protein solution compounds to 0.14 OD

T2 1ml of unknown protein solution contains 82.5 µg of protein

Therefore, 100ml of unknown solution contain

$$= 100 \times 82.5 \mu\text{g} \div 1$$

$$= 8250 \mu\text{g}$$

$$= 8.2\text{mg}$$

Table 5.2 Estimation of protein (after heat shock)

	B	S1	S2	S3	S4	S5	T1	T2
WORKING STANDARD	-	0.5	1.0	1.5	2.0	2.5	-	-
Conc.Of PROTEIN	-	50	100	150	200	250	-	-
TEST SOLUTION	-	-	-	-	-	-	0.5	1.0
DISTILLED WATER	2.5	2.0	1.5	1.0	0.5	-	2.0	1.5
ALKALINE COPPER SULPHATE	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
FOLIN'S REAGENT	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
MIX WELL AND INCUBATE FOR 30 MINUTES AT ROOM TEMPERATURE								
OD AT 660nm	0	0.14	0.28	0.44	0.56	0.66	0.12	0.36

Figure: 5.2 Graphical representation of estimation of protein after heat shock

CALCULATION:

T1=0.5ml of unknown protein solution compounds to 0.12 OD

T1 0.5ml of unknown protein solution contains 40 µg of protein

Therefore, 100ml of unknown solution contain

$$= 100 \times 40\mu g \div 0.5$$

$$= 4000 \div 0.5$$

$$= 8,000 \mu g$$

$$= 8mg$$

T2=1ml of unknown protein solution compounds to 0.36OD

T2 1ml of unknown protein solution contains 130µg of protein

Therefore, 100ml of unknown solution contain

$$= 100 \times 130\mu g \div 1$$

$$= 1,300 \mu g$$

$$= 1.3mg$$



Figure 5.3 Estimation of protein (Before heat shock)



Figure 5.4 Estimation of protein (After heat shock)

5.2 DISCUSSION:

With the objective of enhancing the accumulation of proteins in xanthophyceae sp., four cultures were growing under different stress strategies. The extraction and estimation of proteins from those cultures of xanthophyceae sp. consist in four principle phases: development of a method for extraction of proteins quantitative (by BCA protein quantification) and qualitative (by 2D-PAGE) estimation of the protein extraction, fractionation of salt stress protein extracts by anion exchange chromatography and its gelation capacity study.

CHAPTER 6

SUMMARY AND CONCLUSION

6.1 Summary

Algae are defined as a group of predominantly aquatic, photosynthetic, and nucleus-bearing organisms that lack the true roots, stems, leaves, and specialized multicellular reproductive structures of plants. Pond water is collected. Prepare Media (Bold -Basal Medium) to grow algae. Algae Kept for 45 days. Heat Pressure Given in one fish tank for 7 days (1000ml). Heat pressure is given for calculate the difference between the protein present in algae before heat shock and after heat shock. Algae Separated & Kept in two fishtank. Identification of Microalgae xanthophyceae (yellow-green algae). Protein is extracted from the microalgae. Sample collected and dried protein buffer is prepared Grind the tissue, collect the paste and then place in refrigerator transfer the supernatant to micro centrifuge tube Place the supernatant in a tube for quantification of extracted protein by Lowry method. Protein is estimated by Lowry's method, Pipette out clean tubes, add the protein solution and make up the total volume to 2ml with addition of distilled water. With the help of heat shock method to determine the protein, attained a value before heat shock of 13.5mg and 8.2mg, after the heat shock 8mg and 1.3mg.

6.2 Conclusion:

In this study it was showed that the protein estimate from the microalgae it has different values before and after heat shock. The protein value decreases after heat shock.

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