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**TOPIC: Algae as a food product and characterization of its
nutritive values**

Project Report

Submitted in partial fulfillment of the requirements for the degree of
Master of Science
(Biotechnology) Hons.

Submitted By
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Under the guidance of
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DECLARATION

I hereby declare that the project entitled “**Algae as a food product and characterization of its nutritive value**” is an authentic record of my own work carried out at School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, for the partial fulfillment of the award of Master of Science in Biotechnology under the guidance of **Dr. Mohammad Amin-ul Mannan**.

This work is my original work and has not been submitted for any degree/diploma in this or any other University. The information furnished in this report is genuine to the best of my knowledge and belief.

Place: Lovely Professional University
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(Rupinder Kaur)
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Date:

CERTIFICATE

This is to certify that **Rupinder Kaur (Registration no. 11616577)** have partially completed the project, entitled “**Algae as a food product and characterization of its nutritive value**” under my guidance and supervision. To the best of my knowledge, the present work is the result of their original investigation and study.

No part of the report has ever been submitted for any other degree at any University. The report is fit for submission for session 17181 and the partial fulfillment of the conditions for the award of **M.Sc. Biotechnology (Hons.)**

Date:

(Supervisor Signature)

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Rupinder Kaur

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INTRODUCTION

Microalgae are a group of photosynthetic organisms which utilize light, carbon dioxide (CO₂) and nutrients for the generation of biomass. Microalgae belong to benthic groups and can thrive in marine and fresh water. It can colonize rocks, soil, sand, polar and mountain ice. The term microalgae include the tiny algae *Sensu stricto* and the photosynthetic microbes like cyanobacteria (Tomaselli, L. *Blackwell Science*, 2007). Algae are a chlorophyll-bearing and autotrophic life form. Most of them appear in a relationship with fungi i.e. lichen. The shape and size of algae are exceedingly factor. The size extends from the unicellular structures like *Chlamydomonas* to provincial structures and filamentous structures like *Volvox* and *Spirogyra*. Reproduction in algae occurs via vegetative, asexual and sexual methods. In the vegetative method, vegetative parts give rise to new plants. Each section forms into a thallus. Asexual reproduction is by the creation of various sorts of spores (eg. Zoospores). Sexual reproduction is the combination of two gametes which further form zygote. It has specific environmental conditions such as water conditions, light source, pH, temperature and salinity (Edward et al., 2015).

Algae produce essentially by-products like biomass, oils, food, and other products like bio-diesels. Algae are a rich source for bio-medicine that they contain organic matter, for example, polyphenols, unsaturated fat, polysaccharides, and peptides. Algae comprise of lipids, proteins, polysaccharides, vitamins, and minerals. They produce hydrogen gas, bio-oils, bio-ethanol, antifungal, antiviral, anti-infection agents, cancer prevention agents. Algae give some water-solvent cancer prevention agents like polyphenols, phycobilin-proteins and vitamin (Jyotirmayee et. al., 2014).

On the basis of sizes, algae can be classified as (Hardy et al., 2010).

1. Microalgae: unicellular and microscopic forms involves blue-green algae, green algae, brown algae, etc.
2. Macroalgae: Multicellular forms involves marine algae like seaweeds.

Growth of algae occurs in three main phases (P. Perumal et al., 2012)

- **Exponential phase:** It is the stage in which development rate of the population is constantly positive in time and reliant on supplements, temperature furthermore, lighting accessible in the earth.
- **Stationary phase:** It is the stage in which of population development rate has backed off or wiped out and algal focus achieves a high importance.
- **Descending phase:** It is the stage in which the cells tend to die, being suspended both the progression of separating both the metabolic stage.

NUTRITION

Algae play an important role in human food for a large number of years due to their high nutrient content. Especially in East-Asian areas, protein-rich algae for e.g. the microalgae *Spirulina*, *Nostoc*, or macroalga *Porphyra* are part of regular food (Tseng, C.K. **2004**).

In the mid of the 20th century, the subsequent deficiency in protein supply for humanity expanded activities for investigating novel and optional protein sources, for example, single-cell proteins. And this gave rise to create improved development procedures for huge scale generation of algal biomass. The first examples for a business scale generation were acknowledged in Taiwan, the USA, China and India (utilizing *Chlorella*, *Arthrospira*, and *Spirulina*). Additional genera that are dominatingly developed for their high protein content are *Scenedesmus*, *Anabaena*, or *Synechococcus* (Olaizola, M. **2007**).

PROBLEM BACKGROUND

Vitality is essential for monetary development and personal satisfaction. The worldwide interest for vitality is always on the expansion. The subsequently builds oil costs and furthermore adds to the accumulation of greenhouse gases in the environment. (Demirbas, A. *Energy Conversion, and Management*, **2010**). Biofuels are characterized as strong, fluid or vaporous powers got from natural material. The organic solvents such as hexane, ether, chloroform, methanol, benzene, etc are used for the extraction of secondary metabolites from algae (Maschek et al., 2008). Soxhlet extraction is the best and widely used method for the extraction of secondary metabolites (Ramananath et al., 2015). Algae cultures were isolated and cultured

under favorable conditions in shaker incubator and extracted by centrifugation so that it can express antimicrobial properties (Gianluca et al., 2015).

Algae comprise antimicrobial activity so it can produce medicines like antibiotics (Kolanjinathan et al., 2014). The significance of water as a worldwide asset for human life is verifiable. A conspicuous risk to worldwide water quality, all in all, is its tainting with human-determined squanders of private, modern and business inceptions. This is especially the case for freshwater assets, where human-determined wastewaters are one of the real wellsprings of sullyng and contamination. The decrease in water quality has offered to ascend to huge natural issues and open wellbeing concerns (Craggs, Oswald et al., 1996).

BENEFITS OF MICROALGAE:

- Fast growth - Algae can twofold their numbers at regular intervals, can be collected day by day, and volume of biomass increases.
- Yield - Algae store vitality as oils and sugars, which, joined with their high efficiency.
- Co2 consumption
- Production of useful products - Algae can be developed to create a variety of products for substantial to little markets: plastics, concoction feedstocks, greases, composts, and even beauty care products.
- Algae can be grown to produce hydrogen (Hans Gaffron, 1939)
- Play a major role in medicinal fields such as antibiotics and algae supplements.
- Increases immunity level and decrease cholesterol level.
- Fights against cancer.
- Widely used in therapeutics as it contains a large amount of protein and chlorophyll in it.

REVIEW OF LITERATURE:

On the basis of class, algae can be classified into three parts:

1. Chlorophyceae (green algae)
2. Phaeophyceae (brown algae)
3. Rhodophyceae (red algae)

Table 1: Division of algae and their characteristics

Classes	Common name	Major pigments	Stored food	Cell wall	Flagellar No. & Position of insertions	Habitat
Chlorophyceae	Green algae	Chlorophyll a, b	Starch	Cellulose	2-8, equal, apical	Freshwater, brackish water, and saltwater
Phaeophyceae	Brown algae	Chlorophyll a, c, fucoxanthin	Mannitol, laminarin	Cellulose and algin	2, unequal, lateral	Freshwater (rare), brackish water and saltwater
Rhodophyceae	Red algae	Chlorophyll a, d, phycoerythrin	Floridean starch	Cellulose	Absent	Freshwater (some), brackish water and salt water (most)

HISTORICAL BACKGROUND

Algae is the main part of the food chain in the aquatic environment. They performed photosynthesis and produces the complex natural compound. These natural compounds help to discharged essential and optional metabolites .by the existences of a number of chemical compounds like terpenes, phenol, unsaturated fat, acetogenic and unpredictable halogenated

hydrocarbon (Helena *et al.*, 2011). *Dunaliella salina* produces a few unsaturated fats and compound like alpha and beta-ionine, beta-cyclocitrial, neophytodiene and phenol, those reactions in the antimicrobial exercises in human due to their antimicrobial properties. Different natural dynamic mixes demonstrating against microbial properties has been disconnected from algae to be specific *C. vulgaris*, *D. Salina*, *Euglena viridis* (B.Digamber Rao, 2015).

Algae are the good source of the bioactive compound indicating microbial properties. Blue-green algae are a rich source of basic and naturally dynamic metabolic products like against parasitic, bacterial, viral and allergic. Micro-algae are critical crude materials for generation of amino corrosive, vitamins and other natural metabolites. The development of smaller scale algae helps in term of many fields like useful application and biotechnology field and they help in biotherapeutic exercises (Vivek k.bajpai, 2016)

Nowadays, there are a number of diseases that are caused by microbial agents. These can be treated by the algae-based products. Microalgae are used because they are unicellular and can be found either individually or in a group. They consist of biologically active compounds such as lipid, fatty acid or phenol etc that acts against microbial activities by using many organic solvents in different temperature (Jyotirmayee et al., 2014)

Table 2: Composition % of selected algae

Strain	Protein	Carbohydrates	Lipids	Nucleic acids
<i>Scenedesmus obliquus</i>	50-56	10-17	12-14	3-6
<i>Scenedesmus quadricauda</i>	47	-	1.9	-
<i>Scenedesmus dimorphus</i>	8-18	21-52	16-40	-

GROWTH CONDITIONS:

- Media used: we have used BG 11 media for Scenedesmus where the growth is slow. Instead of BG11, TAP media can also be used but the problem with TAP medium is that the growth is fast that cells may die.
- Light source: Converts inorganic matter into organic matter through photosynthesis. It can also be provided through fluorescent tubes.
- pH: 8.2-8.7
- Temperature: 16-24 degree C

BIOLOGICAL CHARACTERIZATION OF ITS NUTRITIVE VALUES:

Algae which are chlorophyll-containing (Bold and Wynne, 1985) play a very important role in the efficiency of ocean and constitute the basis of the food chain (Hillison, 1977). Microalgae are notable for their high nourishing values including protein, sugar, lipid, basic amino acids, polyunsaturated unsaturated fats (PUFAs), vitamins, minerals and non-caloric dietary strands. The advantageous estimation of microalgae could be exchanged to animals through the basic food chain (Kumar and Singh, 1976). They are used in aquaculture and have wide applications in biodiesel (Schenk et al., 2008), food nourishment (Natrah et al., 2007), animals feed and manures (Spolaore et al., 2006), nutraceuticals and pharmaceuticals (Shahidi and Wanasundara, 1998) and bioplastics (Murphy, 2006). Efficiency and lipid arrangement of microalgae rely upon their development stage and arrangement, irradiance (Thompson et al. 1993), saltiness and temperature (Renaud et al. 2002) of the medium.

OBJECTIVES:

- To develop algae-based products.
- To study anti-microbial properties of algae on human health.
- Characterization of nutrient components of microalgae.
- To optimize growth conditions of algae.

MATERIALS AND METHODOLOGY

Materials

Labware used:

Beaker, Conical flask, Microfuge tubes, Refrigerator, Autoclave, Microwave oven, Micropipette, Gloves, Cryovials, Inoculating loop, Incubator Shaker, Petri plates

Chemicals used:

Nutrient Agar, BG 11 broth, Glycerol, *Scenedesmus* strain, Ethanol

Methods

Media used: BG11

Table 3: Composition of BG11 media

Chemicals	Quantity
NaNO ₃	1.5g
K ₂ HPO ₄	0.04g
MgSO ₄ .7H ₂ O	0.075g
CaCl ₂ .2H ₂ O	0.036g
Na ₂ CO ₃	0.02g
EDTA (chelating agent)	0.001g
Citric acid	0.006g
Ferric ammonium citrate	0.006g
Trace metal mix A5	1.0ml
Agar	10.0g
Distilled water	1.0L

GROWTH CONDITIONS

Light: It is a source of photosynthesis and it converts inorganic matter into organic matter. This is supplied through two ways, either by natural or through fluorescent tubes.

pH: Optimum pH is 8.2-8.7.

Temperature: Optimum temperature is 16- 22 degree C.

Photoperiod (light: dark): 16:8

STERILIZATION: It is of two types:-

1. Wet sterilization: Autoclaving is done at 121 degree C at 15 psi for 15 mins.
2. Dry sterilization: the oven is used at 180 degrees C, for 2 hrs.

CULTURING: *Scenedesmus sp* .strains was selected for the screening of their antimicrobial activity against some pathogenic bacteria and fungi and cultured by using BG11 (Composition: 0.135gm in 500ml) broth media in 28°C with the light condition.

Preparation of media

For agar media,

0.3254 gm in 200 ml of double distilled water + 5 gm of agar.

For broth media,

0.1627 gm in 100 ml of double distilled water.

Culture Vessel

1. It should be transparent to light.
2. Must be non-toxic.
3. Must be cleaned and sterilized properly.
4. Must be grown in conical flasks.
5. Bioreactor should be used for the performance and its maintenance.

HARVESTING

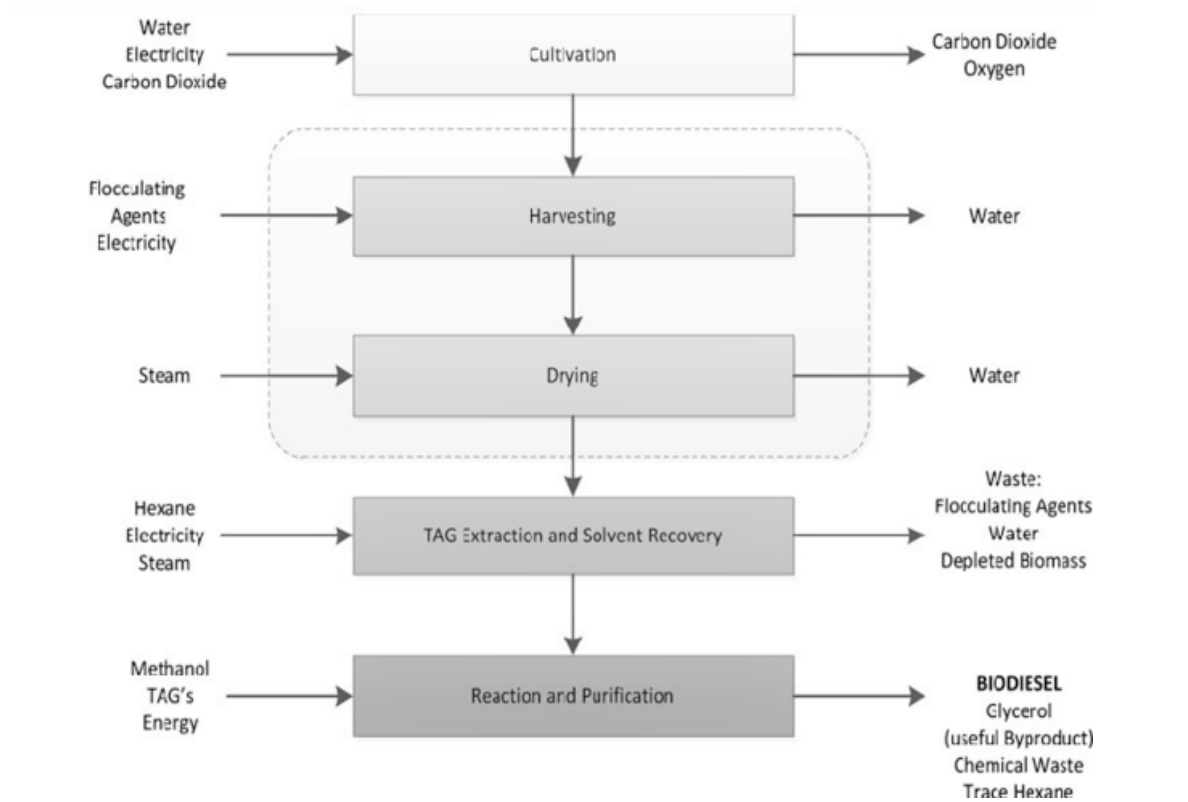


Fig 1: Flowchart of Harvesting algae culture(Barros et al., 2015)

Harvesting and Cultivation:

- The sample must be checked regularly for the cell development rates.
- Culture got circulated air through utilizing air pumps.
- The constant blending of the algal culture in the tank.
- The temperature must be kept up at 21degree C-32 degree C.
- There are two techniques for harvesting algae culture: thickening and drying

EXTRACTION METHODS:

1. Disc diffusion method
2. Soxhlet method (Cardozo et al., 2007)
3. Sonication (Efsthathic et al., 2007)
4. Esterification

5. Lipid- lipid extraction (Rosenberg et al., 2008)

DISC DIFFUSION METHOD

The agar diffusion test (Kirby–Bauer antibiotic testing, KB testing, or disc diffusion antibiotic sensitivity testing) is a trial of the anti-infection affectability of microscopic organisms. It utilizes anti-toxin circles to test the degree to which microbes are influenced by those anti-infection agents. This is known as a zone of a hindrance.

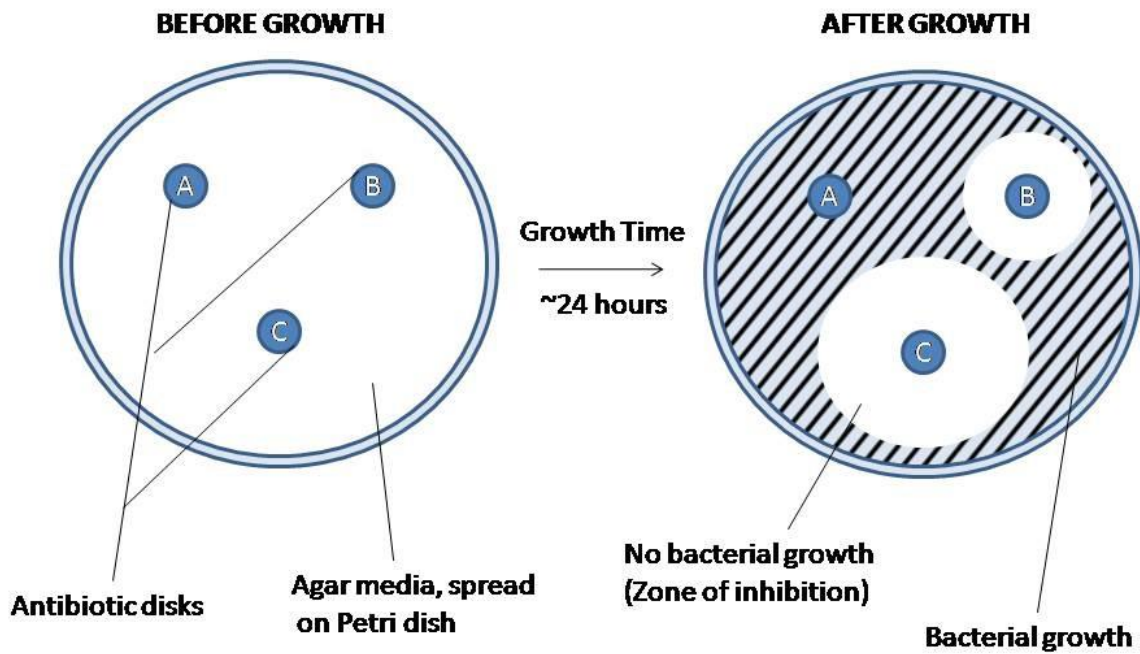


Fig 2: Disc diffusion method (mohanty et al., 2010)

SOXHLET METHOD

A Soxhlet extractor is a bit of research facility device developed in 1879 by Franz von Soxhlet. It was initially intended for the extraction of a lipid from a strong material. Consists of following components: Thimble

- Soxhlet extractor
- Distillation flask
- Condenser
- Distillation arm
- Siphon arm

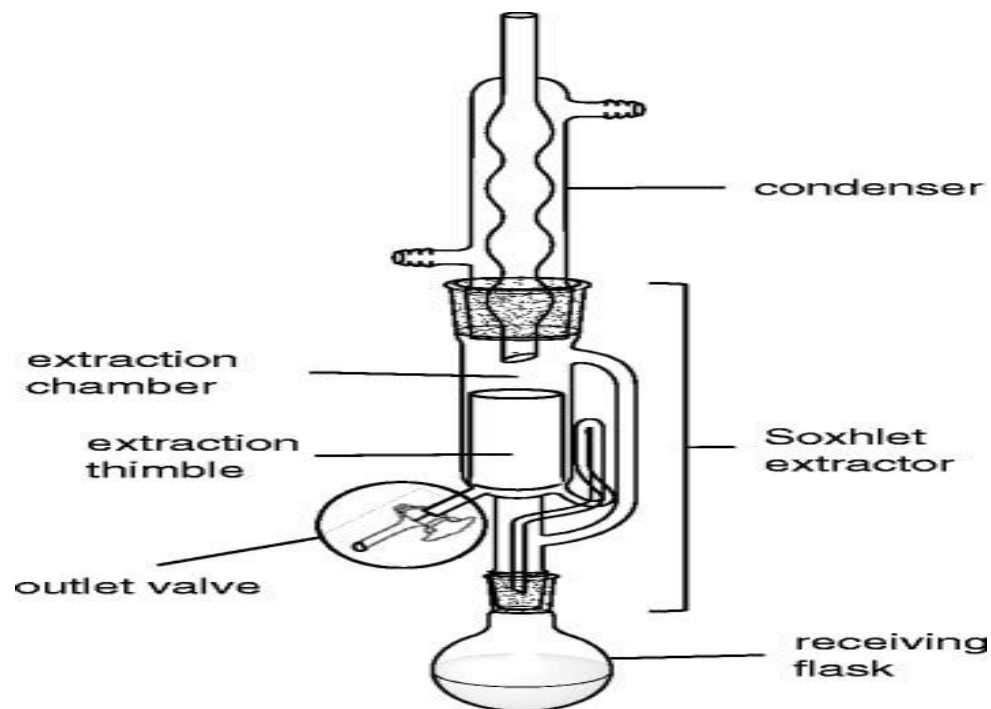


Fig 3: Soxhlet extractor(Franz von Soxhlet, 1879)

DETERMINATION OF BIOCHEMICAL CHARACTERISTICS:

Carbohydrate extraction and determination:

- It was done by the method of Mian and Percival.
- Firstly it was done by homogenizing the samples with acetone (10ml/g) to remove pigments and it was done in Brinkman Polytron homogenizer.
- Centrifugation was done where the supernatant was discarded and resulting sediments were washed 3 times with 200ml of acetone for half an hour during stirring.
- Nitrogen evaporation is done to remove acetone and then place in an oven at 50 degrees C for 24 hrs.
- At last, the algal samples were extracted with 80% ethanol with stirring.

Fat Extraction and determination:

- Take a clean flat bottom flask and keep it in the oven at 95-100 degree C for approximately for half an hour.
- Cool it in desiccators and note down the weight of empty flask.

- Prepare the thimble and weigh approx. 10g of the sample in it.
- Note down the sample weight.
- Put the thimble in siphon tube and pour the solvent.
- Arrange the assembly and let it run for 16 hrs.
- Evaporate the solvent containing fat in a water bath.
- Put the flask containing fat in the oven at 95-100 degree C for approx. half an hour.
- Cool it in desiccators and weigh it.
- Repeat this process of heating, cooling and weighing at half-hour intervals until the loss in weight between successive readings is less than 1mg.

Formula:

$$\text{Fat \% (g/100g)} = \frac{W3 - W1 * 100}{W2}$$

W1 = weight of empty flask in gm

W2 = weight of sample

W3 = weight of flask containing fat

Protein extraction and determination:

- Take 0.8g sample, 0.5g CuSO₄, 10g Na₂SO₄, 25ml H₂SO₄ in the flask.
- Boil it up to 390 degrees C.
- Gases like CO₂, SO₂, and water are liberated at the time of digestion.
- After complete digestion, the sample looks like sea green or blue in color.
- Cool it and then add 50% NaOH to the dropping funnel.
- Add 350ml H₂O in a Kjeldahl flask and then transfer it to the round bottom flask.
- Take 50ml of 0.1N H₂SO₄ in the beaker in which we need to collect nitrogen.
- Methylene red is added to the beaker too and collects it till 150ml.
- After this, titrate it with methylene red.

Formula:

$$\text{Protein\%} = \frac{(B - S) * N * 14.01 * 6.25 * 100}{W * 1000}$$

B = Blank titre value

S = Sample titre value

N = Normality of NaOH

W = Weight of the sample

RESULT:

Scenedesmus sp. strains were cultured and were grown in sterile test tubes and Petri-plates.

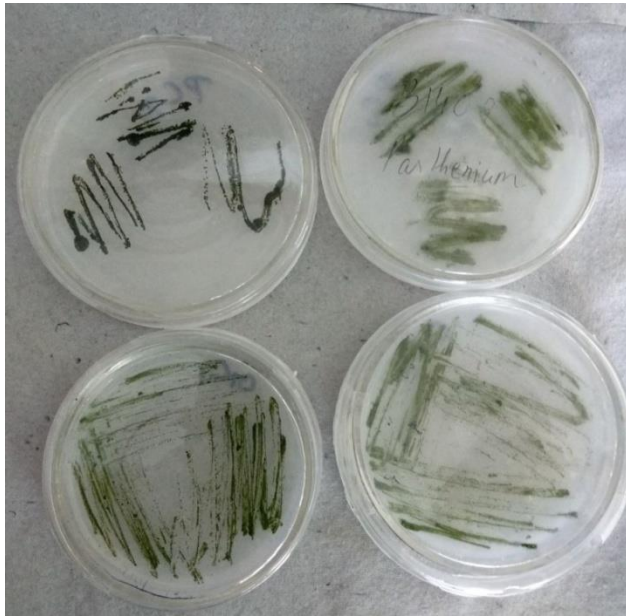


Fig 4: Algae culture in Petri plates

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