LARGE SCALE OUTDOOR ALGAL CULTIVATION IN OPEN POND SYSTEM

A internship Report submitted

For the partial fulfilment of the Degree of Master of Science

By

Ranipa Brindaben Ravjibhai

210621038

[M.Sc. Biotechnology]

Under the supervision of

Mr. Prafulla A. Sabbanwar(M.Sc.)

DEPARTMENT OF BIOTECHNOLOGY

ATMIYA UNIVERSITY

'YOGIDHAM GURUKUL' KALAWAD ROAD

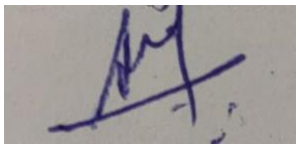
RAJKOT (GUJARAT) – 360005

2022-23

ATMIYA UNIVERSITY
(Established under the Gujarat Private University Act 11, 2018)
Yogidham Gurukul, Kalawad Road, Rajkot - 360005, Gujarat (INDIA)

CERTIFICATE

This is to certify that this training report entitled "Large scale outdoor algal cultivation in open pond system" " was successfully carried out by Miss Ranipa Brindaben Ravjibhai towards the partial fulfilment of requirements for the degree of Master of Science in Biotechnology of Atmiya University, Rajkot. It is an authentic record of her own work, carried out by her under the guidance of Prafulla A. Sabbanwar for a period of three months during the academic year 2022–23. The content of this manuscript, in full or in parts, has not been submitted for the award of any other degree or certificate in this or any other university.



Dr. Nutan Prakash Vishwakarma
Head of the Department

Prafulla A. Sabbanwar
Supervisor

2011 ASTD BEST AWARD WINNER

Reliance
Industries Limited

Golden Peacock National Training Award

C.No:106227201

Date : 03 Apr 2023

To Whomsoever It May Concern

CERTIFICATE

This is to certify that ***Miss. Brinda Ranipa*** , a student of M.Sc. Biotechnology from Atmiya Institute of Technology & Science has undergone training from 04 Jan 2023 to 31 Mar 2023 . The Trainee has developed a project on "*Large Scale Outdoor Algal Cultivation In Open Pond System* ".

The Trainee has shown keen interest in the training and was punctual and sincere during training.

We wish the trainee all the best for future endeavors.

For Reliance Industries Limited

Reliance Industries Limited Jamnagar

Head of Department

Learning & Development, Jamnagar

This is system generated certificate.

CIN: L17110MH1973PLC019786

P.O. Reliance Greens Sub-Post Office, Motikhavdi, Dist.: Jamnagar, - 361 142. Phone: +91-288-661 0000.

Refinery Division - Village Meghpar / Padana, Taluka Lalpur, Dist. Jamnagar, Gujarat.

Registered Office: 3rd Floor, Maker Chambers IV, 222 Nariman Point, Mumbai – 400 021, India.

D E C L A R A T I O N

I hereby declare that the work incorporated in the present dissertation report entitled "**Large scale outdoor algal cultivation in open pond system**" is my own work and is original. This work (in part or in full) has not been submitted to any University for the award of any Degree or a Diploma.

Date: Ranipa Brindaben Ravjibhai

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to everyone who has contributed to the completion of my report.

First and foremost, I would like to thank my mentor Mr. Prafulla A. Sabbanwar for their invaluable guidance and support throughout my research journey and thankful to my supervisor Mr. Madhur Khajuria, Mr. Jayesh Sakariya, Mr. Rahul Patel. Their expertise, insight, and encouragement have been instrumental in shaping my ideas and helping me to develop as a researcher.

I would also like to extend my thanks to the employees who have provided me with the necessary resources and intellectual support to carry out my research. Their feedback and comments have helped me to refine my ideas and improve the quality of my work.

I deem it a privilege to express my sincere thanks to Mrs. Ragini Raghav and head of department Dr. Nutan Prakash Vishwakarma and other staff members of atmiya University for their incompatible patience in guiding me through my work, their support in solving those problems that I encountered and opening up a different approach to any situation at hand. This accomplishment would not have been possible without you. Thank you.

I am grateful to my family and friends for their unwavering support and encouragement. Their love and understanding have been a constant source of motivation for me throughout this process.

Finally, I would like to acknowledge the participants who took part in my study. Without their willingness to participate and provide their valuable insights, my research would not have been possible.

In conclusion, I am deeply grateful to everyone who has contributed to the completion of my thesis. Their support and encouragement have been invaluable, and I am truly thankful for their help in making this achievement possible.

Contents

ABSTRACT

Large-scale algal cultivation refers to the process of growing algae in large quantities for various purposes, such as biofuel production, food, and cosmetics. Algae are attractive as a biofuel source due to their high growth rates, ability to grow in a variety of environments, and ability to capture carbon dioxide.

In large-scale algal cultivation, several factors must be taken into consideration, such as the choice of species, the type of cultivation system, and the cultivation conditions. Algae can be grown in open ponds, photo bioreactors, or hybrid systems, and each system has its advantages and disadvantages.

Crop protection is also a crucial aspect of large-scale algal cultivation, as algae are susceptible to various diseases and pests that can reduce yield and quality. Common crop protection measures for algal cultivation include the use of biocontrol agents, such as predatory microorganisms or enzymes, and physical barriers, such as netting or screens.

In addition to crop protection, other challenges in large-scale algal cultivation include the high cost of production, the need for large amounts of water and nutrients, and the development of efficient harvesting and processing techniques. Overall, large-scale algal cultivation has the potential to be a sustainable and renewable source of energy and other products.

COMPANY INFORMATION

Reliance Industries Limited is an Indian multinational conglomerate company, Jamnagar. It has diverse business including energy, petrochemicals, natural gas, retail, telecommunications, mass media and textiles. Currently I am trainee at Reliance Gagva site also known as RIL biofuel R & D Site. It covers area of 40 acres of land. This Pilot plant work to develop algae on sea water and covert into biofuel and biorefinery projects. Comprised of 150+ scientists, engineers are working. This technology that takes carbon dioxide waste from refinery, then combines it with algae and sunlight to produce bio crude oil that could one day fuel carbon- neutral air travel.

OBJECTIVE

1. Inoculum scale-up and study culture growth pattern.
2. Crop protection study to help the good cultivation process.
3. Compare the different parameters for data analysis.

INTRODUCTION

The booming world population, climate change, Depletion of fossil fuels and ever-increasing demand for food and energy are some of the concerns of the century. The ever-increasing dependence on non-renewable fuel sources has sparked an interest in securing alternative sustainable options when the fossil fuels run dry. The Main external source of energy to earth is from the sun. The significant piece of this energy is saddled by developing oil yields to photo synthetically convert sunlight-based energy into fuel. Analysts have investigated yields, for example, sugar stick for bioethanol, soybean, palm oil and assault seeds for biodiesel to get what's to come requests through sustainable sources. A second era of biofuels were tested by using remaining waste from farming biomass. Albeit, these biofuels possess different downsides, one of them was the insufficient or unpredictable inventory of the biomass required for fuel creation. What's more, these harvests contend with the assets expected for food security like rich land and freshwater. In the ongoing situation, just unambiguous parts or compounds of these oil crops/plants are used for biofuel age. Microalgae species are reported to have high efficiency for photosynthetic conversion of sunlight compared to the first and second-generation biofuel sources. Third generation biofuel is basically advanced algae-based biodiesel while fourth-generation biofuels are created using petroleum-like hydro processing or advanced biochemistry.

Why algae is preferred as for biodiesel production?

- Relatively high oil content and rapid biomass production.
- Photosynthetic organism
- Use CO_2 as energy source
- Uses environmental energy so energy consumption is less
- Reduce Greenhouse effect and global warming

Etc....

In this report we are discussing about large scale outdoor algal cultivation process in open pond system.

OVERVIEW OF ALGAE

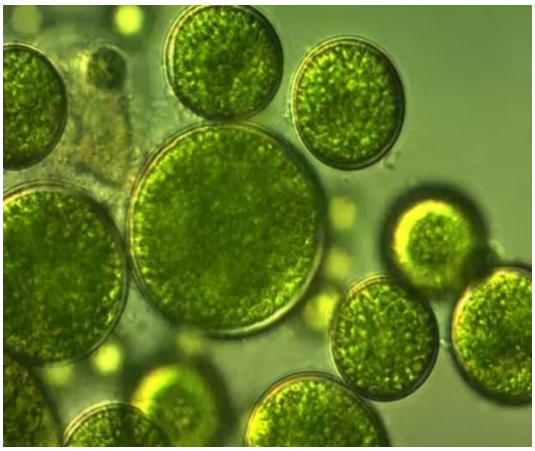


Figure 1 .Microalgae

Algae are a diverse group of aquatic organisms that range from unicellular microalgae to multicellular seaweeds. They are found in a wide variety of environments, including freshwater, saltwater, soil, and even snow and ice.

Algae are photosynthetic, which means they use sunlight to produce their own food through the process of photosynthesis. They are responsible for producing a significant portion of the world's oxygen and are the foundation of many aquatic food chains.

Algae can be classified into several groups based on their cell structure, pigments, and other characteristics. Some of the most well-known groups include:

1. Cyanobacteria:
    - Cyanobacteria and Blue green algae – gram negative bacteria
    - Survive on harshes habitats on earth
    - Nitrogen fixation, carbon fixation or both – essential for life
    - E.g. Prochlorococcus, Spirulina, Nostoc, Anabena etc.
2. Glaucocystophytes
    - Uncommon unicellular eukaryotic algae contain plastid
    - Structurally like cyanobacteria
    - One of the descendants of product of early endosymbiosis
    - E.g. Cymphora, Glaucocystics, Peliaina, Glacochacte etc.
3. Rhodophytes
    - Rhodophytes or red algae composed of multiple photosynthetic eukaryotes
    - Characterized by distinctive red colour compound phycoblisomes in chloroplast

- E.g. Chroodactylon, Bangia, Cyanidium, Compsopogan etc.

4. Chlorophytes
    - Chlorophytes or green algae are photosynthetic eukaryotes which contain chlorophylls as their main photosynthetic pigments
    - E.g. Haemotococcus, Chlorella, Dunaliella, Graesiella, Scenedesmus etc.
5. Charophytes
    - Territorial and freshwater algae
    - Features as territorial plants and ancestors of charophytes gave rise to land plants.
    - E.g. Coleochaete, Micrasterias, Chara, Prenium, Klebsormidium etc…
6. Chlorarachinophytes
    - Marine photosynthetic protists possess secondary plastids originated from secondary endosymbiosis
    - E.g. Chlorarachunion, Lotharella, Bigelowella etc…
7. Euglenoids
    - Unicellular flagellated eukaryotes
    - Exhibit both animal and plant like characteristics
    - Most are freshwater, some are marine
    - E.g. Euglena, Discoplastics, Phacus, Coacium, Strombomonas etc.
8. Apicomplexans
    - Group of obligate intercellular parasites
    - Responsible of various disease in plants and animals.
    - E.g. Plasmodium, Toxoplasma, Cryptosporidium etc.
9. Dinoflagellates
    - Class of unicellular parasites
    - Characteristized by relatively large nucleus, green golden plastid and unique method of swimming
    - E.g. Gymnodium, Karenia, Dinophysis, Alexandrium etc.
10. Heterokontophytes

- Flagellated photosynthetic eukaryotes
- Characterized by biflagellate, which is different in length
- E.g. Chrysophyacae, Parmophyacceae, Xanthophyaceace etc.

11. Haptophytes
    - Unicellular photosynthetic microalgae with chloroplast originated from the endosymbiosis of red algae
    - Usually 2 equal or unequal flagella
    - E.g. Chysochromulnia, Prymmesium, Pavlova, Diacronema etc…
12. Crptophytes
    - Motile, photosynthetic unicellular organisms
    - Characterized by presence of 2 flagella
    - Freshwater and marine water algae
    - E.g. Guillardia, Camphlomonas, Geminigera, Rhodomonas etc…

Algae have a wide range of applications in areas such as food, medicine, biofuels, and wastewater treatment. They are also being studied for their potential to remove carbon dioxide from the atmosphere and reduce greenhouse gas emissions

The microalgae Biomass can be directly converted to bio-fuel via four techniques.

I. Bio-chemical conversion
II. Thermochemical conversion
III. Transesterification
IV. Microbial fuel cell

The choice of selecting a suitable process depends on various factors such as specification of the project, type and availability of crude biomass feedstock and budget of the project.

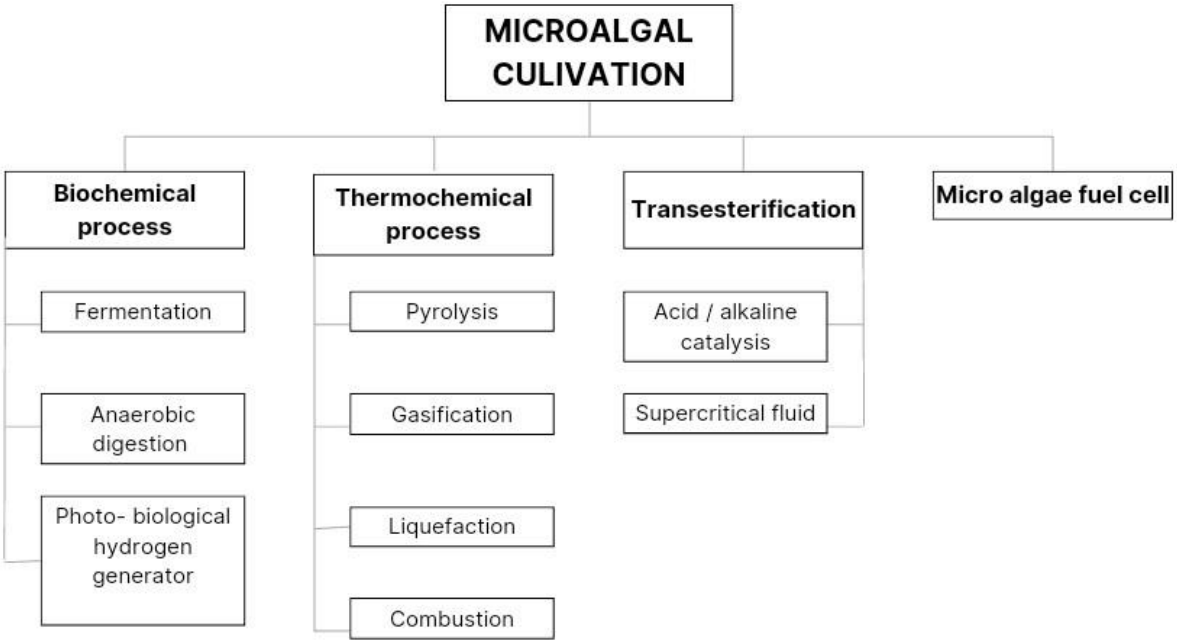


Figure 2. Techniques for microalgae biomass converted into biofuel

OVERVIEW OF BIIOFUEL

The concept of biofuels dates to Rudolf Diesel who envisioned vegetable oil as a fuel source for his newly invented engine. The process the Initiative uses to produce biodiesel was discovered in 1937.

There are divided into following;

1. Bioethanol & Biodiesel: Fermentation of sugar and starch produces bio alcohols like ethanol, propanol and butanol.
2. Biogas: produce from any kind of biomass
3. Bio ethers: primary source is wheat & beat sugar. Also prepared from waste glycerol produced during production of biodiesel.
4. Bio hydrogen: produced with mixture of other gases include carbon monoxide, carbon dioxide.
5. Wood: type of solid biofuels.
6. Energy crops: produce for burning purposes
7. Algae based: Algae are the highest source of energy in the class of biofuels. Because of the food problems. Algae gain most attention to use as a fuel.

OPRATING PROCEDURE

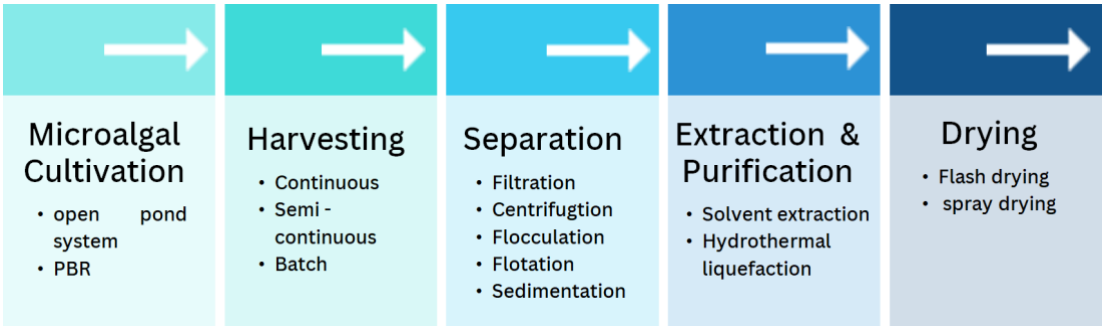


Figure 3. Operating procedure for biorefinery project

MICROALGAL CULTIVATION

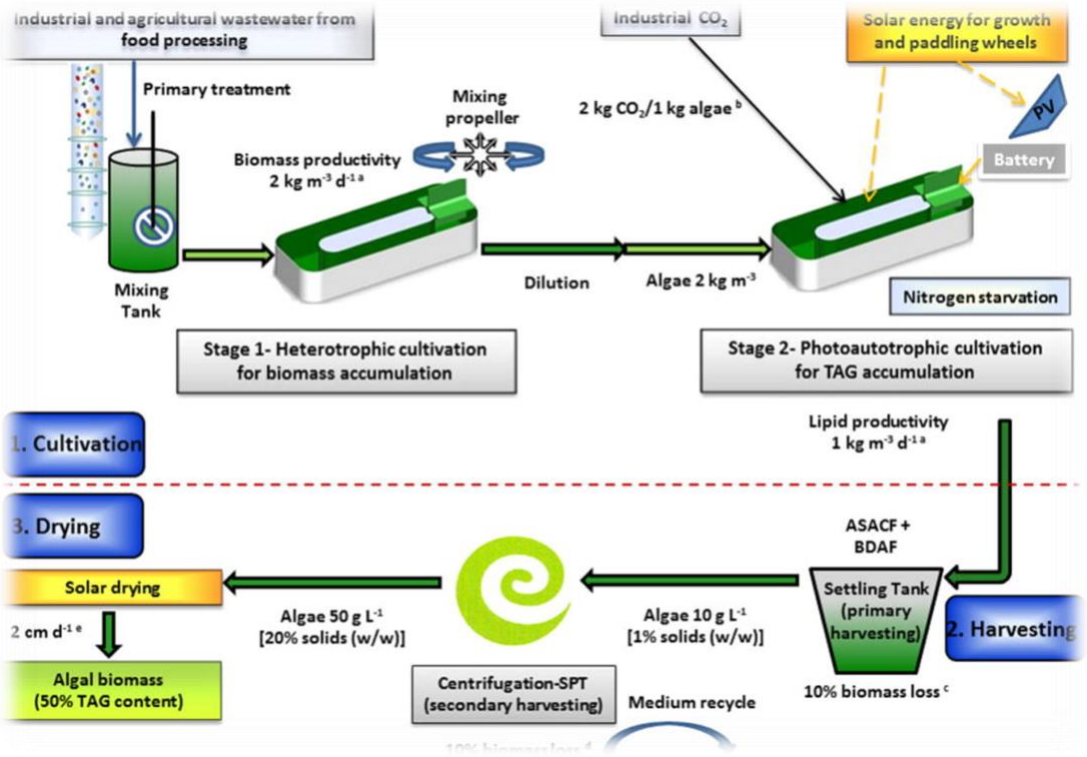


Figure 4. Microalgal cultivation process

Microalgae are capable to grow rapidly due to it's short life cycle. It convert solar radiation energy into chemical energy via photosynthesis [1]. Their cells can accumulate a broad array of value-added bioactive substances, such as proteins, lipids, carbohydrates, fatty acids, pigments and vitamins [2]. Their high photosynthesis efficiency coupled with the ability to accumulate a large amount of bio products within their cells make them a suitable candidate to serve as industrial raw material [3]. Besides, cultivation of microalgae does not require fertile land, a large quantity of freshwater, and herbicides and pesticide when compared to the other crops and thus will not be competing for resources [4]. Furthermore, cultivation of microalgae can even be performed using wastewater such as domestic sewage water and palm oil milling effluents which can assist in bioremediation of wastewater [5,6]. Apart from wastewater treatment, cultivation of microalgae can also help with reduction of atmospheric carbon dioxide through photosynthesis, effectively contributing to the efforts of tackling greenhouse effect and global warming and also reduce carbon foot print.one of the major drawback of using waste water is low biomass production and the small

size of cells, Low cell density, reduced growth rate and difficulty in harvesting when they are cultured in liquid medium render the harvesting process of microalgae very costly.

An ideal microalgae culturing system should possess the characteristics, including:

I. Adequate light source
II. Effective transfer of material across liquid-gas barrier
III. Simple operation procedure
IV. Minimal contamination rate
V. Cheap overall building and production cost
VI. High land efficiency [7]

There are 2 methods for microalgal cultivation

1. Open pond
2. Photobioreactor

Open pond system requires a large area compare to photobioreactor. Photo bioreactor requires less area but once contamination occurs in photobioreactor it is highly problematic to reduce or remove those contaminations. Both methods have their own advantages and disadvantages. Our topic for report is large Scale outdoor algal cultivation in an open pond systems. So we could not discuss photobioreator. So let's discuss about large scale algal cultivation in an open pond system.

OPEN POND SYSTEM

Open pond is used in microalgal cultivation because it is relatively cheaper in construction, and less operating and maintenance cost. There are several advantages and disadvantages to open pond system.

ADVANTAGES:

- Simplistic operation and maintenance
- Low energy demand and easily scale-up
- Cost effective cultivation system

DISADVANTAGES:

- It requires large cultivation area
- Growth condition such as salinity and pH erosion of banks that resulting in leakage turbidity

There are 2 types of ponds present in open pond system such as

1. Circular ponds
2. Raceway ponds

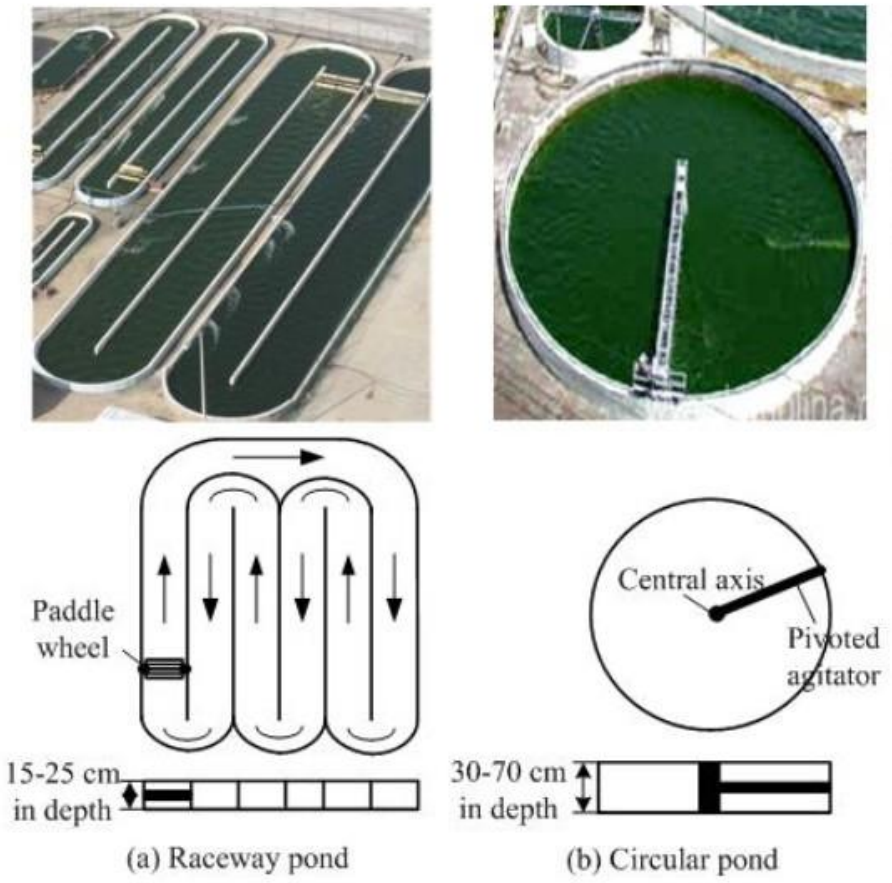


Figure 5.Types of open pond system

Circular ponds

- First artificial pond used in large scale algal cultivation.
- Depth- 30 to 70cm and width – 45 meters

- Due to rotary agitator present in centre of pond effective mixing and prevent sedimentation of algal biomass occurs.

Raceway ponds

- Most frequently uses in microalgal cultivation
- Series of closed loop channels around 30cm deep
- Due to the presence of paddlewheel recirculation of microalgal biomass, equal distribution of nutrients, prevent sedimentation of microalgal biomass, etc. occurs
- One of the successful raceway pond cultivation by Sapphire Energy's Columbus, Algal biomass Columbus, US(2 years- 520metric tonnes dried microalgal biomass)

INOCULUM SCALE UP AND STUDY CULTURE GROWTH PATTERN

Inoculum scale-up and studying culture growth patterns are critical steps in large-scale algal cultivation. Here are some key considerations:

Inoculum preparation and scale up:

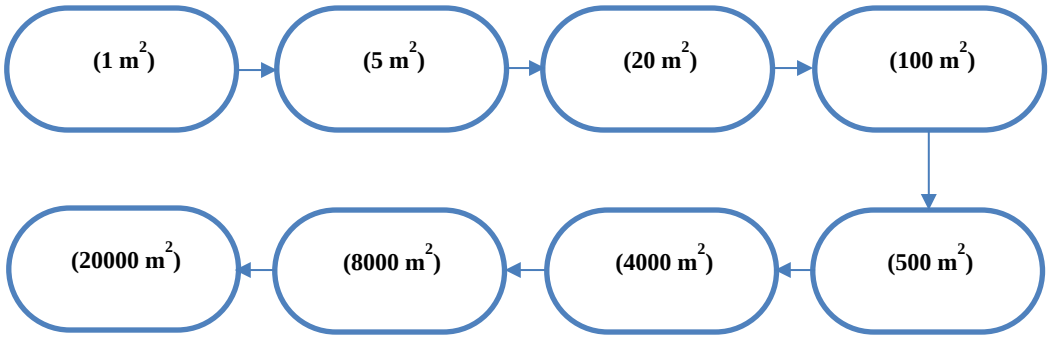


Figure 6. Scale up of microalgal culture

The inoculum should be prepared from a healthy, well-maintained culture that has been checked for contaminants. The inoculum should be grown in a small volume of medium to reach a high density before it is added to the larger culture vessel.

There are different stages at site for inoculum development. This stages is called inoculum ponds First of all culture inoculate in stage 1 and leave this for 6 days to adopt the environmental conditions and increase culture cell density by double. Then transfer into stage 2 and follow the process as mention up to stage 5. It's takes 25-30 days to reach at stage 5 now culture is ready to grow on production pond transfer the culture into 1 acre pond and follow the process mention above. After some day cell density increase by double no. of cell transfer into other production ponds.

The many steps and the extended time required not only increase costs, but also increase the risk of contamination. The process of producing the inoculum for large scale commercial cultures is not trivial and is a critical factor to the successful operation of a production facility. It is therefore not surprising that most commercial producers generally guard the exact details of

their process. One factor that may help in the process of scaling up and shortening the period from laboratory scale to the production units is modification of the culture depth so that the areal productivity of the culture is high even when the biomass concentration is relatively low due to the dilution. Another way to do in greenhouse so, cultures to get better control the daily fluctuation in light and temperature. One option to reduce the time and cost of inoculum scale-up is to operate the large-scale cultures as semi continuous cultures rather than as batch cultures.

Culture growth pattern: The growth pattern of the culture should be monitored closely throughout the cultivation process. This can be done by measuring the biomass concentration, pH, dissolved oxygen, and other relevant parameters. By analysing the growth pattern, it is possible to determine the optimal time for harvesting the culture.

Yield optimization: To optimize yield, it is important to understand the factors that affect algal growth, such as light intensity, temperature, nutrient availability, and carbon dioxide concentration. By optimizing these factors, it is possible to achieve higher yields and more consistent growth patterns.

PARAMETERS AFFECT GROWTH OF ALGAE

There are several parameters that can affect the growth of algal cultivation which is harder to control such as

LIGHT (µmol/m²/s)	NUTRIENTS (PHSOPHATE, AMMONIA, UREA)
TEMPERATURE(° C)	pH
SALINITY	CARBON DIOXIDE

Figure 7. Several parameter affect growth of algae

Light: Light is a critical parameter for algal growth as it is the primary source of energy for photosynthesis. Algae are autotrophic organisms, meaning they produce their own food through photosynthesis, which requires light energy. The amount and quality of light available to algae can affect their growth rate, pigment composition, and nutrient uptake. Algae require a specific range

of wavelengths for photosynthesis, typically between 400 and 700 nm, which corresponds to the visible spectrum of light. The intensity and duration of light exposure also play a crucial role in algal growth. The optimal light conditions for algal growth vary depending on the species, but typically fall within a range of 100 to 2000 µmol photons/m^2/s, with some species requiring higher or lower intensities. Additionally, the duration of light exposure should be long enough to allow for sufficient photosynthesis but not too long to cause photoinhibition, which can damage the algal cells. Therefore, controlling light intensity, quality, and duration is essential for optimizing algal growth in various applications, including wastewater treatment, biofuel production, and food production.

Temperature: Temperature is a critical parameter for algal growth because it influences many important physiological and biochemical processes in algae. Algae are photosynthetic organisms, and their growth rate is closely related to the rate of photosynthesis, which is directly affected by temperature. Algae have a temperature range within which they can grow optimally. Below this range, their metabolic processes slow down, and growth is reduced, while above this range, their metabolic processes can become impaired or even stop altogether, leading to cell damage and death. The optimal temperature range for algal growth varies depending on the species of algae. Some algae can grow at temperatures as low as 4°C, while others require temperatures above 30°C. Most algae grow best in temperatures ranging from 20°C to 30°C.Temperature affects the rate of cell division, respiration, and photosynthesis in algae. As temperature increases, the rate of these processes also increases, up to a certain point, beyond which they start to decline. Thus, temperature influences the biomass yield and lipid content of algal biomass. In addition to the direct effects on growth and metabolism, temperature also affects the availability of nutrients and dissolved gases, which can further impact algal growth. Therefore, understanding the optimal temperature range for specific algal species is critical for optimizing the growth conditions for commercial-scale algal cultivation.

Nutrients: Algal cultivation requires a number of nutrients to support the growth and development of the algae. Some of the key nutrients required include:

I. Carbon dioxide (CO_2): Algae use CO_2 as a carbon source for photosynthesis, and it is typically supplied via aeration or sparging.
II. Nitrogen (N): Nitrogen is essential for algal growth and is often the limiting nutrient in algal cultivation systems. Common nitrogen sources include nitrate, ammonium, and urea.
III. Phosphorus (P): Phosphorus is important for cellular growth and energy transfer in algae. It is typically supplied in the form of phosphate.
IV. Potassium (K): Potassium is essential for maintaining cell turgor and regulating enzyme activity in algae.
V. Iron (Fe): Iron is a key component of photosynthetic pigments and is necessary for photosynthesis and respiration in algae.
VI. Trace elements: Algae also require a range of trace elements, such as zinc, copper, manganese, and molybdenum, to support various metabolic processes.

The exact nutrient requirements of algae can vary depending on the species being cultivated and the specific conditions of the cultivation system. Additionally, excess nutrient levels can lead to algal overgrowth and potentially harmful algal blooms, so careful management of nutrient inputs is important for successful algal cultivation

pH: pH is a crucial factor in algal cultivation, as it affects many aspects of algal growth, metabolism, and productivity. Algae are sensitive to changes in pH levels and can thrive only within a certain pH range. The optimal pH range for algal cultivation varies depending on the type of algae being grown. Most commonly cultivated microalgae prefer a neutral to slightly alkaline pH range of 7.0-9.0, although some species may have more specific requirements. On the other hand, some macroalgae species can grow in a wider range of pH levels, from 6.0 to 10.0. The pH of the cultivation medium affects the availability of nutrients and the toxicity of certain ions. For example, in low pH environments, aluminum ions are more soluble and can be toxic to algae, while in high pH environments, phosphorus can become less available to algae. Therefore, controlling the pH is essential to maintain optimal nutrient uptake and avoid toxicity. Additionally, pH affects the efficiency of CO_2 uptake in algae. Most microalgae species grow best at a slightly alkaline pH, which allows for the highest CO_2 uptake rate. However, some species can also grow under acidic

conditions and can utilize bicarbonate as a carbon source. In summary, pH plays a critical role in algal cultivation and must be carefully controlled to optimize growth, productivity, and nutrient uptake.

Salinity: Salinity, or the concentration of dissolved salts in water, is an important factor in algal cultivation. Algae can be grown in a wide range of salinities, from freshwater to seawater, depending on the species of algae being cultivated and the desired end use of the algae. Some algae species are known as halophiles, which means they can grow in high salinity environments such as seawater or brackish water. These types of algae are often used in the production of biofuels, food and feed supplements, and high-value compounds such as omega-3 fatty acids and carotenoids. Other algae species are known as freshwater algae and grow better in lower salinities, such as in freshwater ponds and lakes. These types of algae are often used in the production of food and feed supplements, nutraceuticals, and bioplastics. Salinity affects algae growth in several ways. High salinity levels can increase the osmotic pressure inside the algae cells, causing water to move out of the cells and leading to dehydration and cell death. However, some halophilic algae have adapted to these conditions and have special mechanisms for dealing with high salinity levels, such as the accumulation of compatible solutes that help regulate water balance. In contrast, low salinity levels can limit the availability of essential nutrients, such as nitrogen and phosphorus, which are necessary for algae growth. In freshwater environments, additional nutrients may need to be added to promote optimal algae growth. In summary, the role of salinity in algal cultivation is significant, and it is essential to select the appropriate salinity level based on the type of algae being grown and the intended application.

Carbon dioxide: Carbon dioxide (CO_2) is an essential component for the growth and photosynthesis of algae. Algae use CO_2 to produce organic matter through photosynthesis, which is then utilized as a source of energy for their growth and reproduction. Therefore, providing an adequate supply of CO_2 is crucial for the successful cultivation of algae. In commercial algal cultivation, CO_2 is often supplied through aeration systems that inject CO_2-rich air or flue gas from industrial processes into the culture medium. This approach not only enhances the growth of algae but also provides an opportunity for capturing and utilizing CO_2, which is a greenhouse gas contributing to global warming. Optimizing the CO_2 supply can significantly improve the

productivity of algal cultures. The concentration of CO_2, temperature, light, and nutrient availability are the key factors that influence the growth rate of algae. By controlling these parameters, it is possible to achieve the desired growth rate and biomass yield of algae for various applications, including biofuels, food, and feed. In summary, CO_2 is a vital nutrient for algal growth and plays a critical role in the cultivation of algae. Providing an adequate supply of CO_2 through aeration systems is an essential step towards achieving higher productivity and sustainability in algal cultivation.

Competition: There are presence of some types of contaminations such Eukaryotic grazers (phototrophic Algae, ciliates, flagellates, mesozoplankton), Eukaryotic Parasites (amoeba, vampyrellids, fungi),Pathogens(virus and bacteria) etc. are toxic and unstable for algal culture growth. But there are some solution to reduce or remove those contamination such as capable of surviving at extreme alkaline or saline condition but few contaminations are able to thrive under this condition.

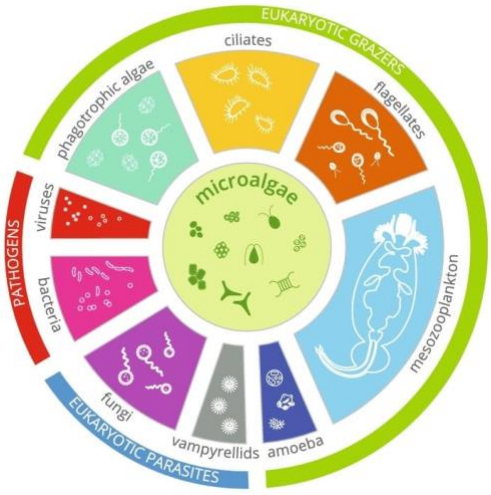


Figure 8. Diverse array of microorganisms are known to feast upon algae

CROP PROTECTION

Most of the algae that have been successfully cultivated at scale to date rely on culture conditions that are inhospitable to most pests (e.g., Dunaliella or Spirulina). Alternatively, for other species some form of batch culture is used to minimize contamination and product loss. Both of these approaches reduce the requirement for crop protection processes. In order to cultivate other desirable strains that require high saline conditions and more neutral pH ranges, more options for crop protection will be required[8].

Some of the oldest and best studied methods for crop protection include the use of relatively simple chemical compounds to treat infected systems. The overall goal is to identify compounds that can be added to production systems at concentrations that kill, inhibit, or mitigate contamination by deleterious species. Ideally, chemical treatments will not be detrimental to algal production, adversely impact downstream use of the biomass, or add excessive additional cost. This balance can be difficult to achieve and limit the utility of these approaches. Although many chemical treatments can both be used in prophylaxis and interdiction, extensive and continued use of chemical agents can lead to desensitization and resistance in the targeted pest species. Here, we discuss the variety of chemical treatments and their effectiveness on prophylaxis and interdiction of algal cultures[9].

Sr. No.	Chemical Additives	Tested Against	Effective amount	Advantages	Disadvantages
1	Copper Sulphate	*Brachionus calyciflorus* (Rotifers)	0.1-1.5 ppm in 100 mg/l dry weight	High heavy metal concentrations found in many waste water treatment facilities	inhibits algal growth, limits photosynthetic efficiency, inhibits colony formation of S.obliquus, heavy metal pollutan
		Colpoda sp.,(ciliates)	10 mg/L		
2	Peraacetic acid	Grazers	25 ppm	Degradable, several oxidizers, known biocides, ozone microbubbles can spur algal CO_2 uptake	High cost for peracetic acid,EPA warns against use >1.3 ppm for peracetic acid ,50ppm or more than that kill algae
3	Chlorine dioxide	virus and animal plankton	3 mg/l	Degradable , several oxidizer , known biocides	Clo2 is algicides
4	Hydrogen peroxide	Ciliates	150-200 mg/l	Rapid decomposition , light sensitive	Light sensitive
5	Quinine sulfate	*Colpado sp.*	20 mg/l	effective against variety of grazers without harming algae , long lasting in water system	Expensive, toxic to human health , enviornmentally harmful do to developing resistance
		Vorticella sp.	20 mg/l		
		Grazers	10 mg/l		
		Brachionus calyciflorus (Rotifers)	17 µm		
6	Rotenone	*Brachionus calyciflorus (Rotifers)*	0.074 µm	egrrades to nontoxic chemicals, algae are largly insensitive, effective against marine and fresh water	Rapidly degrades in sunlight,extremely toxic to insects and aquatic life , harmul to human in concentrated doses
		Brachinus rotundiformis	0.13 µm		
		Brachionus manjavacas	0.26 µm		
		Oxyrrhis sp.	1.3 µm		
		Euplotes	5 µm		
7	Lugol's iodine	Euplotes ,Oxyrrhis	0.3-1 ml per 100 ml sample	Potent grazer inhibition	Too expensive, too toxic for algae
8	Methylene blue	Euplotes,Oxyrrhis	100 mg/l stock sol. Add 50 ml in 1 gm	Potent grazer inhibition	Too expensive, too toxic for algae
9	Toluidine blue	Inhibit Blue green algae	10 µm p-toluidine per disk	Blue green algae inhubiter,slight inhibit diatoms	expensive, too toxic for algae
10	Ivermectin	Euplotes,Oxyrrhis, Daphnia magna	5.7 mg/l	Potent grazer inhibition	Toxic fot target enviornmental effects

Table 1: Different types of chemical additives there affective amount with advantages and disadvantages

HARVESTING

There are three general methods to grow algae that are across a spectrum.

1. Batch culture
2. Semi continuous batch culture
3. Continuous culture.

BATCH CULTRE

In the batch culture you have a finite volume of media in which the cells grow. You do not add any new media. Over time the biomass looks like this plot. The culture experiences lag-phase, exponential growth, stationary phase, and then decline.

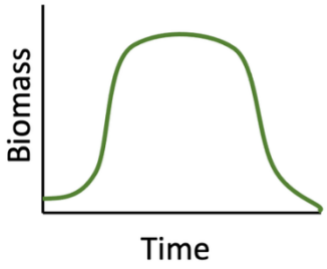


Figure 9. Batch culture

SEMI-CONTINUOUS BATCH CULURE

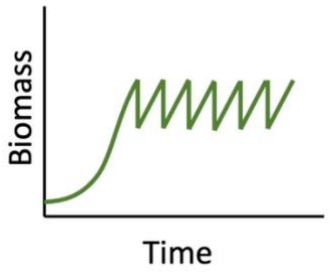


Figure 10. Semi-continuous culture

In the semi-continuous batch culture. The culture is harvested every day to week and new media is added to the container. For the greatest amount of biomass produced, the harvesting is done at the highest biomass possible before the culture enters the stationary phase of growth. The culture experiences the lag phase and exponential phases. If done correctly, it never entered stationary phase or decline. Over time this type of culture method could experience pathogens or weed algae growing in the media, so monitoring of the culture is important.

CONTINOUS CULTURE

Continuous culture is a most efficient way to create biomass. This culture method is also called a chemostat, or a turbidostat (when the density is held constant). In this method, a continuous supply of media is added to the culture. With the culture volume held stationary, every new mL of media yields a new ml of product. You could view this a semi-continuous batch culture, where the harvest phase is every second.

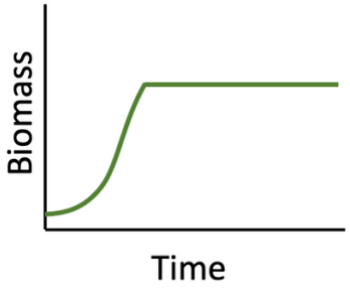


Figure 11. Continuous culture

But here we are follow semi- continuous method for yield higher amount of biomass. Harvesting of microalgae is one of the main parts in microalgae processing. Several studies have suggested that it makes up 20 to 30% of the total production cost due to high energy demand and capital cost [10,11]. In general, all harvesting techniques aim to remove as much culture media from the microalgae biomass to facilitate next downstream processing such as extraction of bioactive compounds. Numerous harvesting methods have been used to collect biomass, including filtration, centrifugation, flocculation and flotation [12].

There are 4 types of harvesting method

I. Physical method
II. Chemical method
III. Biological method
IV. Magnetic separation method

SEPRATION METHODS

There are several methods for separation use for biomass production such as

1. Filtration
2. Centrifugation
3. Flocculation
4. Sedimentation
5. Flotation

FILTRATION

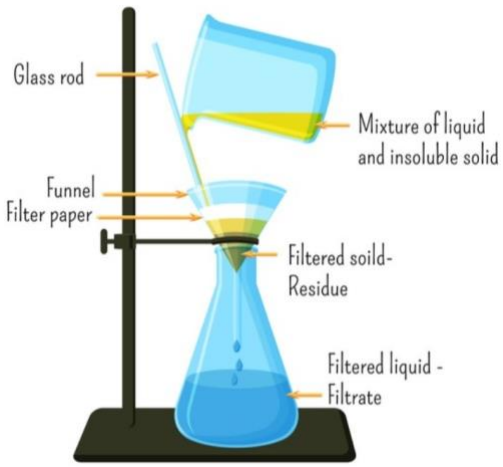


Figure 12. Filtration process

Filtration process utilizes a semipermeable membrane which can retain microalgae on the membrane while allowing the liquid media to pass through, leaving the algae biomass behind to be collected [13]. This method can harvest high concentration of cell from the medium, and the varying pore size of the filter membrane enables the system to suit the need of different microalgae and are able to handle the more delicate species which are prone to damage due to shearing. However, this method is very prone to fouling and clogging and therefore requires frequent change of fresh filter or membrane that might contribute significantly to its processing cost [14].

Filtration is an important method used in algal cultivation to separate algae from their growth medium. There are several types of filtration methods used in algal cultivation, including:

Gravity Filtration: This method involves the use of a filter bed or a filter medium such as sand, gravel, or diatomaceous earth. The algae are allowed to settle out of the growth medium by gravity, and the filtered medium is then collected.

Vacuum Filtration: This method uses a vacuum to draw the growth medium through a filter medium such as a filter paper, membrane or cloth. The algae are retained on the filter medium, while the filtrate is collected.

Microfiltration: This method involves the use of a microfiltration membrane to separate the algae from the growth medium. The membrane has a pore size of 0.1 to 10 micrometres, which allows the algae to pass through while retaining the growth medium.

Ultrafiltration: This method is like microfiltration but uses a membrane with a smaller pore size of 0.001 to 0.1 micrometres. This method can effectively separate small particles and molecules from the growth medium.

Filtration is a crucial step in algal cultivation, as it helps to separate the algae from the growth medium and concentrate them for further processing. The choice of filtration method depends on factors such as the size of the algae, the nature of the growth medium, and the end-use of the algae.

CENTRIFUGATION

Centrifugation operation separates microalgae cells from the culture media based on each component's density and particle size using centrifugal force [15]. This technique has high cell harvesting efficiency, but the process is time consuming and energy intensive [16,17]. Moreover, high gravitational force used in centrifugation might cause cellular damage making it unfavourable for certain applications since the sensitive nutrients might be lost [18, 19]

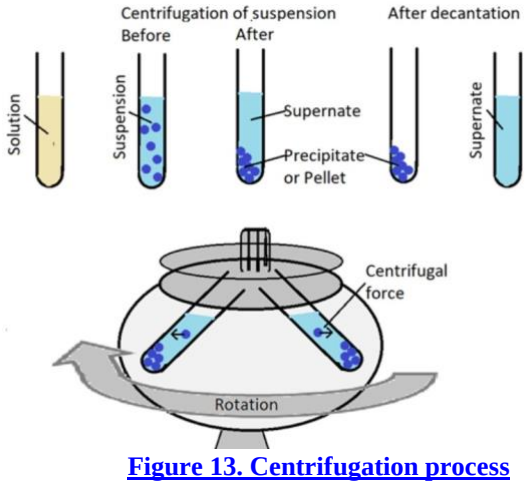


Figure 13. Centrifugation process

There are two types of centrifugal techniques for separating particles:

i. Differential centrifugation
ii. Density gradient centrifugation
    - Rate-zonal centrifugation
    - Isopycnic centrifugation

In differential Centrifugation, the most basic sorting method, also known as differential pelleting a suspension of particles with varying densities or sizes that will sediment at varying rates, with the bigger and denser particles sedimenting more quickly. The use of centrifugal force can accelerate these sedimentation speeds. Differential pelleting is frequently used to extract cells from tissue homogenate or to create crude subcellular sections. Differential centrifugation experiences contamination and subpar recoveries because of the variability in biological particles. Resuspension and repeating the centrifugation procedures can be used to handle contamination by various particle types. (i.e., washing the pellet)

Centrifugation based on the rate of settling is used in density gradient centrifugation. The two kinds of density gradient centrifugation are rate zonal and isopycnic, respectively. By layering the sample as a narrow zone on top of a density gradient, the issue of cross-contamination of particles with varying sedimentation rates can be avoided in rate-zonal centrifugation. In this manner, unlike in differential centrifugation, the faster sedimenting particles are not contaminated by the slower ones. The gradient offers a medium with rising density and viscosity while stabilizing the bands. A narrow zone of sample is placed on top of a density gradient.. articles move under centrifugal force at varying speeds based on their mass. Instead of density, size and mass play a major role in how quickly particles settle. If centrifuged for a long enough time, all of the particles will ultimately form a pellet because the particle density is higher than the gradient density. Particles are only divided based on their densities in isopycnic separation, also known as buoyant or equilibrium separation. When a particle's density matches that of the gradient medium around it, the particle size no longer influences how quickly it moves. The gradient medium's density must be higher than that of the particles to be divided.

FLOCCULATION

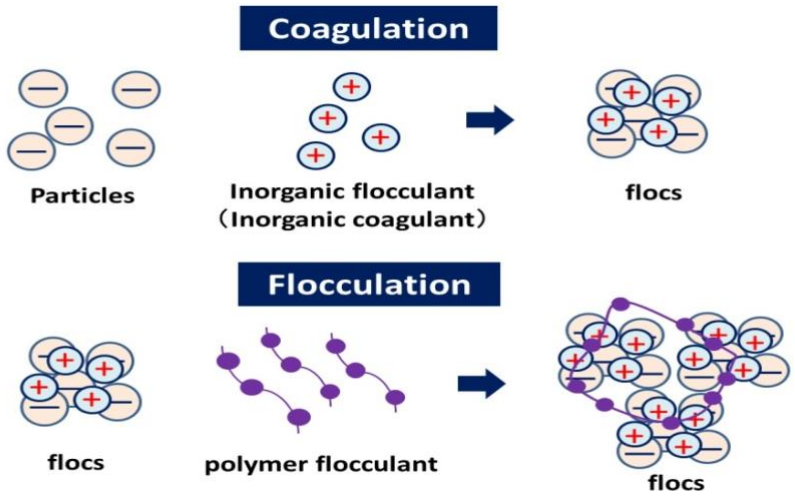


Figure 15.Coagulation and Flocculation basic principle

Flocculation is a process where free floating unicellular microalgae cells aggregate together to form a larger particle known as floc by the addition of flocculating agent to remove the surface charge of cells [20]. This can be beneficial for various reasons such as increasing the sedimentation rate of the algal biomass, reducing the energy required for harvesting, and improving the efficiency of downstream processing.

There are several methods for inducing flocculation in algal cultivation, including chemical flocculation, pH adjustment, and biological flocculation. Chemical flocculation involves adding chemicals such as aluminium sulphate or ferric chloride to the culture medium, which results in the formation of insoluble compounds that bind with algal cells and cause them to settle. However, the chemicals are not eco-friendly due to their high toxicity, and they must be removed by additional treatment processes which add to the production cost [21].pH adjustment involves changing the pH of the culture medium to a level that is optimal for the formation of flocs. Biological flocculation involves the use of microorganisms that produce extracellular polymers that bind with algal cells and cause them to aggregate. Bio-flocculants on the other hand are much safer and eco-friendly when compared to their chemical counter parts. They are also cheaper to be used, and typically there is no pre-treatment required before further downstream processing of microalgae and recycling of culture media [22,23]. Most of the bio-flocculants used are biopolymers such as acrylic acid and chitosan which exist naturally or produce artificially [24].

The choice of flocculation method will depend on various factors such as the type of algae being cultivated, the desired final product, and the scale of production. It is important to carefully select and optimize the flocculation method to ensure that it is effective, cost-efficient, and does not negatively impact the quality of the final product.

SEDIMENTATION

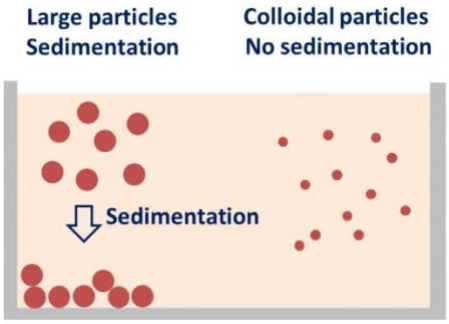


Figure 14. Sedimentation principle

Sedimentation refers to the settling of particles, such as algae cells, in a liquid. In algal cultivation, sedimentation can be a useful process for separating algae from the growth medium. Sedimentation can occur naturally due to gravity or can be facilitated by centrifugation or filtration.

Algae can be separated from the growth medium by allowing the culture to settle and then removing the liquid above the settled algae. This method is commonly used for large-scale cultivation of algae for biofuels or other industrial applications.

There are several factors that can affect the sedimentation rate of algae, including the size and shape of the algae cells, the density of the growth medium, and the strength of the gravitational field. Some algae species may be more prone to sedimentation than others, and the sedimentation rate can also be influenced by the conditions in which the algae are grown, such as light intensity, nutrient availability, and temperature.

To maximize the efficiency of sedimentation, it is important to maintain optimal growth conditions for the algae and to carefully control the rate of sedimentation to avoid damage to the cells. In addition, other methods such as centrifugation or filtration may be used in conjunction with sedimentation to achieve a higher degree of separation and purification of the algae.

FLOTATION

Flotation is a common technique used in algal cultivation to harvest algae cells from the cultivation medium. In flotation, air bubbles are introduced into the culture, which adhere to the surface of the algae cells and make them buoyant. This allows the cells to float to the surface of the culture where they can be collected and removed.

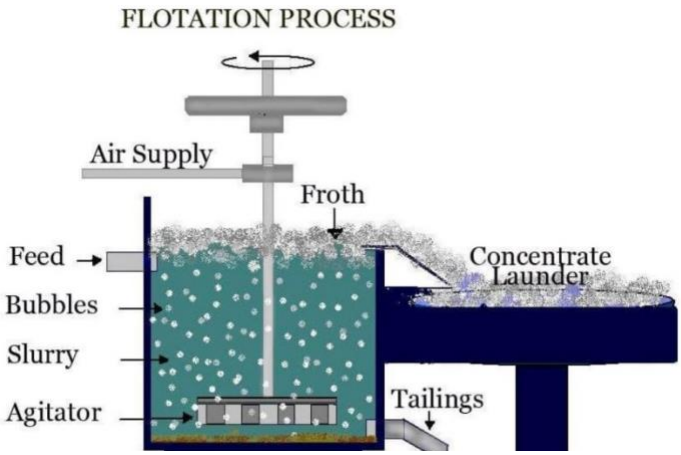


Figure 15. Flotation process

There are two main types of flotation methods used in algal cultivation: dissolved air flotation (DAF) and foam fractionation. In DAF, air is dissolved in the culture under pressure and then released, creating bubbles that adhere to the algae cells. In foam fractionation, a surfactant is added to the culture to create a stable foam that traps the algae cells.

Flotation has several advantages over other harvesting methods, such as centrifugation or filtration. It is relatively simple and inexpensive to implement, requires less energy than other methods, and can be used with a wide range of algal species. However, flotation may not be suitable for harvesting algae at large scale, and the collected algae may require further processing to remove excess water and other impurities.

Sr. No.	Process	Advantages	Disadvantages
1.	Filtration	Less expensive, wide variety of filters and membrane available	Require frequent backwashing, time consuming, highly dependent on algal species; best suited to large algal cells. Clogging or fouling is an issue.
2.	Flotation	Cost efficient and more rapid than sedimentation	Use of chemical depend on suspended particles, less reliable, micro algal species specific, high consumption and high capital cost
3.	Centrifugation	Quick, High efficient, Good recovery	Expensive due to high energy consumption and high capital costs
4.	Sedimentation	Low cost, potential for use as a first stage to reduce energy input and cost of subsequent stages	Slow separation, final concentration may be low, may not be suitable for Nanochloropsis cells

5.	Microfiltration/ Ultrafiltration	Capable to handle Nanochloropsis cells, very efficient and can reach up 98% dewatering Can be used as pre-treatment prior to centrifugation	High operating costs and membrane fouling

Table 2: Different types of harvesting methods and their advantages and disadvatages

EXTRACTION AND PURIFICATION

PRODUCT EXTRACTION

Extraction is the first step to separate the desired natural products from the raw materials

Methods for product extraction:

There are 6 methods for product extraction

1. Soxhlet extraction
2. Solid liquid extraction
3. Pressurized liquid extraction
4. Super critical extraction
5. Ultrasonic assisted extraction
6. Microwave assisted extraction
7. Hydrothermal liquefaction

Soxhlet extraction

Modern conventional extraction methods include soxhlet extraction. We use modified versions of the soxhlet extractor by taking into account how helpful they are in order to remove obstacles. The modified soxhlet apparatus contains some of the following:

- Ultrasound-assisted Soxhlet extractors
- Microwave-assisted Soxhlet extractors.
- Extractor with electrically heated distillation chamber
- Extractor having Condenser with solvent distribution nozzle to spray condensed solvent into the powder bed
- Fluidized-bed soxhlet extraction
- High-pressure soxhlet extraction

Soxhlet extraction	
Advantages	Disadvantages
Efficient and continuous extraction.	Extraction by Soxhlet is only possible with boiling solvents or azeotropes.
It needs less solvent to yield concentrated extract.	The desired components must be soluble in the solvent at a high temperature.
We can continue the process until the powder gets completely exhausted. Due to which extraction efficiency is much greater than the traditional extractor.	We cannot extract from more than one sample at a time.
We can use modified Soxhlet extractors to meet different needs and increase efficiency further.	

By modifying certain things, we can use the Soxhlet extractor on the industry level.	

Table 3: Advantages and Disadvantages of soxhlet extraction

Solid Liquid extraction

A sort of extraction known as solid-liquid extraction is carried out between the solid and liquid phases. The solvent (liquid) and the solute phase (solid) are mixed while being distributed in the liquid. Or, to put it another way, the substance is moved from a solid to a liquid phase.

Solid Liquid extraction	
Advantages	Disadvantages
commonly used	chances of impurities
Easiest method	Introduction of Analytical errors

Table 4: Advantages and Disadvantages of Solid liquid extraction

Pressurized liquid extraction

One of the sample preparation techniques is pressurized fluid extraction (PFE), which can be used to separate specific analytes from a sample matrix into a solvent to enable further analysis. PFE uses a pressurized cell that resembles a liquid chromatographic column to separate materials with solvent.

Pressurized liquid extraction	
Advantages	Disadvantage
less solvent	not suitable for thermo labile compound
Less time of extraction extraction	

Table 5: Advantages and Disadvantages of Pressurized liquid extraction

Super critical extraction

Using supercritical fluids as the extracting solvent, supercritical fluid extraction (SFE) is the process of isolating one component (the extractant) from another (the matrix). Although it is frequently from a solid material, extraction can also be done from liquids.

Super critical extraction	
Advantages	Disadvantages

friendly method	high capacity investment
Can be used for thermolabile compounds	Requirement of high pressure

Table 6: Advantages and Disadvantages of Super critical extraction

Ultrasonic assisted extraction

One of the changes is called ultrasound-assisted extraction (UAE), in which ultrasound waves are used to aid the extraction procedure. Cell disruption and efficient mass transfer are two important aspects that boost the effectiveness of using ultrasound vibrations.

Ultrasonic assisted extraction	
Advantages	Disadvantages
eco friendly	lack of uniformity in the distribution of ultrasound energy
Can replace the solvents with GRAS solvents	Decline of power with time
High extraction efficiency	
Reduced extraction time	
Good for thermo labile compounds	

Table **7 :** Advantages and Disadvantages of Ultrasound assited extraction

Microwave assisted extraction

Extraction Assisted by Microwave (MAE) by heating solvents holding samples with microwave energy and partitioning analytes from a sample matrix into the solvent, MAE is a traditional method for the extraction of active components from medicinal plants.

Microwave assisted extraction	
Advantages	Disadvantages
reduced solvent usage	high capital cost
Highly extraction rate	
Improved extraction yield	

Table **8:** Advantages and Disadvantages of Microwave assisted extraction

Hydrothermal liquefaction

Hydrothermal liquefaction processes biomass in water at high/subcritical temperatures (below 374 °C) and high pressure (above water vapour pressure) with or without a catalyst. HTL can process 5-20% algae concentration as input. In the HTL process, microalgae biomass is broken down in water to small molecules which then can repolymerize into oily compounds. HTL therefore allows wet conversion of biomass, thus reducing the energy consumption in drying process.

HTL	
Advantages	Disadvantages
lower amount of organic carbon found in the water phase following gasification leading to high carbon efficiencies	In Particular acidic and oxidizing conditions can cause rapid corrosion

Table 9: Advantages and Disadvantages of HTL

PRODUCT PURIFICATION

Purification is done to separate those contaminants that resemble the product very closely in physical and chemical properties

Product purification done by following 3 methods

i. Chromatography
ii. Precipitation
iii. Membrane Filtration

Chromatography:

Chromatography is a widely used method for the purification of different compounds, including those derived from algal cultivation. In algal cultivation, chromatography can be used to purify pigments, lipids, and other valuable compounds from algal biomass.

One example of chromatography used in algal cultivation is high-performance liquid chromatography (HPLC), which is commonly used for the separation and purification of algal pigments such as chlorophylls, carotenoids, and phycobiliproteins. These pigments are important for various applications, including food colorants, cosmetics, and pharmaceuticals.

Another example is preparative thin-layer chromatography (TLC), which can be used to separate and purify lipids from algal biomass. Algae are known to be a rich source of omega-3 fatty acids,

which have important health benefits. TLC can be used to isolate specific types of lipids from algal biomass, such as triglycerides or phospholipids, which can be further processed and used in various applications.

Overall, chromatography is an important tool for the purification of valuable compounds derived from algal cultivation, and it can help to increase the efficiency and productivity of algal bioprocessing.

Precipitation:

Precipitation can be a useful technique for purifying algae culture in certain situations. Algae cells can be easily separated from the liquid medium they grow in by forming aggregates, which can then be removed by precipitation.

One common method for precipitating algae cells is by adding a chemical coagulant such as aluminum sulfate (alum) or ferric chloride to the culture. The coagulant interacts with the organic matter and minerals in the culture, causing the algae cells to aggregate and settle to the bottom of the container. This process is known as coagulation-flocculation.

Another method for precipitation is by adjusting the pH of the culture to promote aggregation. For example, increasing the pH can cause the cells to form larger aggregates, which can then be removed by settling or filtration.

Precipitation can be particularly useful for removing unwanted contaminants from the culture, such as bacteria or other microorganisms. However, it is important to note that precipitation can also remove nutrients that the algae need to grow. Therefore, it is important to optimize the process to ensure that the algae culture is not depleted of essential nutrients.

Overall, precipitation can be a useful tool for purifying algae culture, but it should be used carefully and with consideration for the specific needs of the culture being grown.

Membrane filtration:

Membrane filtration is an effective method for the purification of algal cultures because it is a gentle and non-destructive process that retains the viability of the algal cells. It is also a scalable and cost-effective method that can be used for both laboratory-scale and industrial-scale algal cultivation. Additionally, the use of membrane filtration can improve the quality and consistency of algal products, and reduce the need for chemical treatments or other harsh purification methods.

DRYING

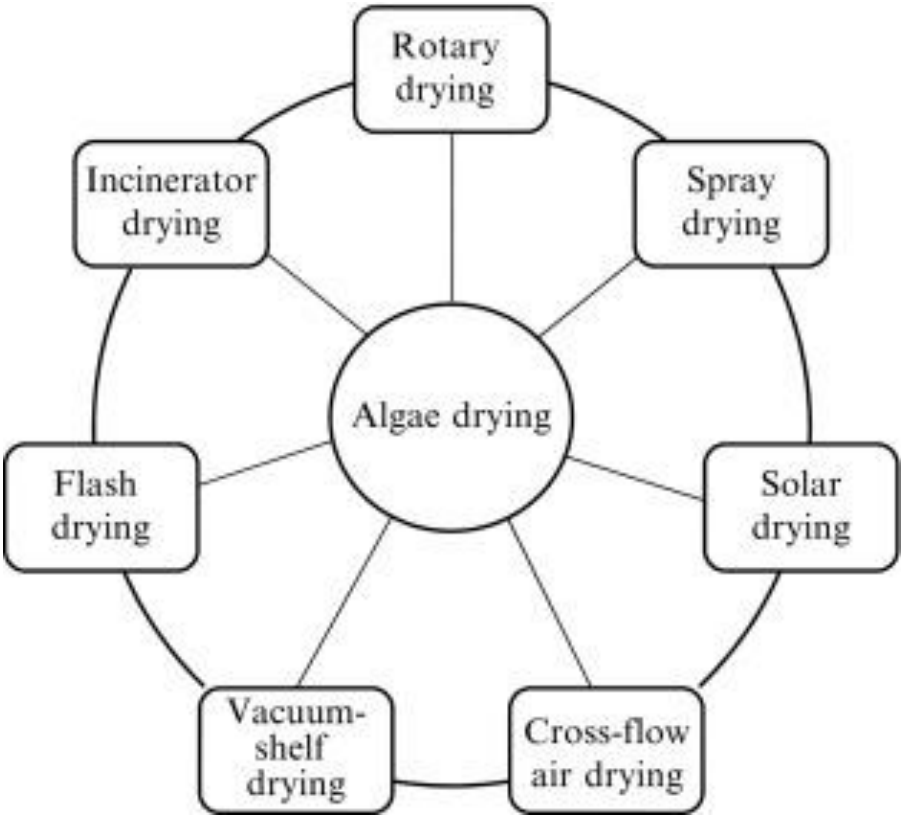


Figure 16. Methods for drying

Drying is a common method used in algal cultivation for several purposes, including:

Preservation: Drying is an effective method of preserving algal biomass for extended periods. By removing water from the algae, the growth and reproduction of microorganisms that can spoil the biomass are inhibited, thus allowing for longer-term storage.

Concentration: Drying also allows for the concentration of algal biomass. By removing water from the biomass, the dry weight of the biomass increases, which can be advantageous for

downstream processing and extraction of valuable components such as lipids, pigments, and proteins.

Value addition: Dried algal biomass can be used for various applications, such as animal feed, fertilizer, and biofuels. Drying the biomass can increase its value by making it more accessible for these applications. There are 2 types of drying instrument present in the field

1. Ring dryer
2. Spray dryer

Both ring dryers and spray dryers have advantages and disadvantages, and the choice of which one to use depends on the specific requirements of the algal cultivation process. Ring dryers are better suited for larger volumes of biomass, while spray dryers are better suited for producing a finer powder. Additionally, ring dryers are more energy-intensive than spray dryers, but they can achieve higher drying rates. Ultimately, the choice of which dryer to use will depend on the specific needs and constraints of the algal cultivation process.

RESULT & DISCUSSION

- The optical density vs ash-free dry weight in large scale algal cultivation can provide insights into the growth and productivity of algae. Optical density is a measure of the amount of light absorbed by a culture of algae, which is proportional to the concentration of cells in the culture. Ash-free dry weight, on the other hand, is a measure of the biomass of the algae culture that remains after burning off any organic material. In general, as the culture of algae grows and the concentration of cells increases, the optical density will also increase. At the same time, the ash-free dry weight will also increase as the algae accumulate more biomass. The relationship between optical density and ash-free dry weight can provide useful information about the health and productivity of the algal culture. For example, if the optical density is increasing rapidly but the ash-free dry weight is not keeping pace, it could indicate that the algae are not growing efficiently and may be experiencing stress or nutrient limitations. Alternatively, if the ash-free dry weight is increasing rapidly but the optical density is not increasing at the same rate, it could indicate that the algae are becoming more denser or clumped together, which could affect their ability to absorb nutrients and light. In general, a healthy and productive algal culture will show a strong correlation between optical density and ash-free dry weight, indicating that the algae are growing efficiently and accumulating biomass at a consistent rate.
- The light vs aerial productivity in large scale algal cultivation shows the relationship between the amount of light that is being supplied to the algae culture and the amount of biomass that is being produced per unit area or volume of the culture. In general, there is a positive correlation between light supply and biomass production up to a certain point. Beyond that point, however, the rate of biomass production will begin to level off, and

eventually may even decrease, even if more light is supplied. This is because there are other factors besides light that can limit the productivity of the algae culture, such as nutrient availability, pH levels, temperature, and CO_2 concentration. In addition, excessive light can actually be detrimental to the algae if it causes photo inhibition or other forms of stress. Therefore, the optimal level of light supply for maximum aerial productivity will depend on a variety of factors, including the specific strain of algae being cultivated, the environmental conditions, and the cultivation method being used. It is important for algal cultivators to carefully monitor and adjust the light supply to ensure that it is not causing any negative effects and is optimized for maximum biomass production.

Quality control: Quality control is an essential aspect of large-scale algal cultivation. This includes monitoring the culture for contaminants, measuring the nutrient content of the medium, and ensuring that the final product meets the required specifications.

In summary, successful inoculum scale-up and cultivation of algae on a large scale requires careful planning, monitoring, and optimization. By following these key steps, it is possible to achieve high yields and consistent growth patterns, leading to a reliable and sustainable source of algal biomass for various applications

CONCLUSION

Algal cultivation in open ponds is a promising approach for large-scale outdoor production of algae. The process involves growing algae in open ponds, which can be natural or artificial, exposed to sunlight and nutrients to promote growth. Open pond systems offer several advantages, such as low capital and operating costs, ease of maintenance, and high productivity. However, they also have some limitations, including susceptibility to contamination, variable productivity, and potential water loss due to evaporation.

Overall, the success of large-scale outdoor algal cultivation in open pond systems depends on various factors, such as the choice of algal strain, pond design, water quality management, and harvesting techniques. With the right conditions and careful management, open pond systems can be a cost-effective and sustainable solution for producing algae-based products such as biofuels, food and feed supplements, and pharmaceuticals.

REFERENCE

1. Zeng, J., Wang, Z., & Chen, G. (2021). Biological characteristics of energy conversion in carbon fixation by microalgae. In *Renewable and Sustainable Energy Reviews* (Vol. 152). Elsevier Ltd. https://doi.org/10.1016/j.rser.2021.111661
2. Levasseur, W., Perré, P., & Pozzobon, V. (2020). A review of high value-added molecules production by microalgae in light of the classification. In *Biotechnology Advances* (Vol. 41). Elsevier Inc. https://doi.org/10.1016/j.biotechadv.2020.107545
3. Randrianarison, G. and M.A. Ashraf, Microalgae: a potential plant for energy production. Geology, Ecology, and Landscapes, 2017. 1(2): p. 104-120.
4. Khan, M. I., Shin, J. H., & Kim, J. D. (2018). The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. In *Microbial Cell Factories* (Vol. 17, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s12934-018-0879-x
5. Selmani, N., M.E. Mirghani, and M.Z. Alam. Study the growth of microalgae in palm oil mill effluent waste water. In IOP Conference series: earth and environmental science. 2013. IOP Publishing
6. Posadas, E., et al., Microalgae cultivation in wastewater, in Microalgae-Based Biofuels and Bioproducts. 2017, Elsevier. P. 67-91.
7. Jegathese, S. J. P., & Farid, M. (2014). Microalgae as a Renewable Source of Energy: A Niche Opportunity. *Journal of Renewable Energy*, *2014*, 1–10. https://doi.org/10.1155/2014/430203

8. Pugazhendhi, A., Shobana, S., Bakonyi, P., Nemestóthy, N., Xia, A., & Banu J, R. (2019). A review on chemical mechanism of microalgae flocculation via polymers. In *Biotechnology Reports* (Vol. 21). Elsevier B.V. https://doi.org/10.1016/j.btre.2018.e00302
9. Springer Nature Switzerland. (2019). *Agronomic Practices for Photoautotrophic Production of Algae Biomass*. www.springer.com. 2019, from https://doi.org/10.1007/978-3-030-25233-5-4
10. Uduman, N., et al., Dewatering of microalgal cultures: a major bottleneck to algae based fuels. Journal of renewable and sustainable energy, 2010. 2(1): p. 012701
11. Barros, A. I., Gonçalves, A. L., Simões, M., & Pires, J. C. M. (2015). Harvesting techniques applied to microalgae: A review. In *Renewable and Sustainable Energy Reviews* (Vol. 41, pp. 1489–1500). Elsevier Ltd. https://doi.org/10.1016/j.rser.2014.09.037
12. Singh, G., & Patidar, S. K. (2018). Microalgae harvesting techniques: A review. In *Journal of Environmental Management* (Vol. 217, pp. 499–508). Academic Press. https://doi.org/10.1016/j.jenvman.2018.04.010
13. hattab, M. al. (2015). Microalgae Harvesting Methods for Industrial Production of Biodiesel: Critical Review and Comparative Analysis. *Journal of Fundamentals of Renewable Energy and Applications*, *05*(02). https://doi.org/10.4172/2090-4541.100015
14. J Fundam Renew Energy Appl, 2015. 5(2): p. 1000154. Milledge, J.J. and S. Heaven, A review of the harvesting of micro-algae for biofuel production. Reviews in Environmental Science and Bio/Technology, 2013. 12(2): p. 165-178.
15. Soomro, R. R., Ndikubwimana, T., Zeng, X., Lu, Y., Lin, L., & Danquah, M. K. (2016). Development of a two-stage microalgae dewatering process – A life cycle assessment approach. *Frontiers in Plant Science*, *7*(FEB2016). https://doi.org/10.3389/fpls.2016.00113
16. Dassey, A.J. and C.S. Theegala, Harvesting economics and strategies using centrifugation for cost effective separation of microalgae cells for biodiesel applications. Bioresource technology, 2013. 128: p. 241-245.
17. Rawat, I., et al., Biodiesel from microalgae: a critical evaluation from laboratory to large scale production. Applied energy, 2013. 103: p. 444-467.

18. Heasman, M., et al., Development of extended shelflife microalgae concentrate diets harvested by centrifugation for bivalve molluscs–a summary. Aquaculture Research, 2000. 31(89): p.
19. Knuckey, R.M., et al., Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. Aquacultural Engineering, 2006. 35(3): p. 300313. Muylaer
20. Muylaert, K., et al., Harvesting of microalgae: overview of process options and their strengths and drawbacks, in Microalgae-based biofuels and bioproducts. 2017, Elsevier. P. 113-132.
21. Rinanti, A. and R. Purwadi. Harvesting of freshwater microalgae biomass by Scenedesmus sp. As bioflocculant. In IOP Conference Series: Earth and Environmental Science. 2018. IOP Publishing.
22. Salim, S., et al., Harvesting of microalgae by bio-flocculation. Journal of applied phycology, 2011. 23(5): p. 849-855.
23. Nguyen, T. D. P., Le, T. V. A., Show, P. L., Nguyen, T. T., Tran, M. H., Tran, T. N. T., & Lee, S. Y. (2019). Bioflocculation formation of microalgae-bacteria in enhancing microalgae harvesting and nutrient removal from wastewater effluent. *Bioresource Technology*, *272*, 34–39. https://doi.org/10.1016/j.biortech.2018.09.146
24. Pugazhendhi, A., Shobana, S., Bakonyi, P., Nemestóthy, N., Xia, A., & Banu J, R. (2019). A review on chemical mechanism of microalgae flocculation via polymers. In *Biotechnology Reports* (Vol. 21). Elsevier B.V. https://doi.org/10.1016/j.btre.2018.e00302

FIGURE REFERENCE

Figure no.	Reference
1.	https://theconversation.com/microalgae-is-natures-green-gold-our-pioneering-project-to-feed-the-world-more-sustainably-170158
4.	https://ars.els-cdn.com/content/image/1-s2.0-S030626191500272X-fx1_lrg.jpg
5.	https://www.semanticscholar.org/paper/DEFINING-THE-PROBLEMS-IN-INTEGRATION-OF-MICROALGAE-Kerestecio%C4%9Flu-Pekmezci/def4a05975fcbfd4d635dc398c24b52ad567a48c
8.	https://link.springer.com/chapter/10.1007/978-3-030-25233-5_2

9.,10.,11.	https://algaeresearchsupply.com/blogs/news/batch-semi-continuous-and-continuous-cultures
12.	https://www.yaclass.in/p/science-cbse/class-9/is-matter-around-us-pure-6984/separation-techniques-5978/re-2e001de7-d904-416d-a52a-6a3b55c5def5
13.	https://datprep.com/general-chemistry-lab-techniques-cheat-sheet-all-you-need-to-know/
14.	https://khangngoc.com/van-de-ve-nuoc/do-duc/xu-ly-nuoc-bang-keo-tu.html
15.	https://kenkidryer.com/2020/05/10/flocculation-treatment/
16.	https://www.911metallurgist.com/blog/tag/froth-flotation-process
17.	https://www.sciencedirect.com/science/article/pii/B978044464192200007X