Biological carbon capture, Growth kinetics and biomass composition of novel microalgal species

By

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Reg # 00000275895

Session 2018-20

Supervised by

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National University of Sciences and Technology (NUST)

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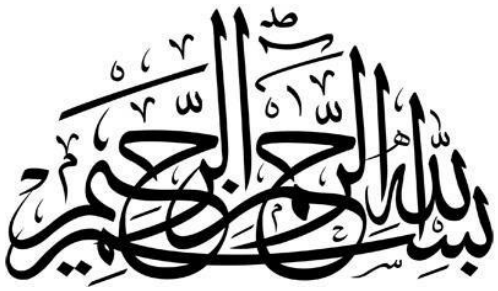

IN THE NAME OF ALLAH, THE BENEFICENT THE MERCIFUL

Read! And thy Lord is Most Honorable and Most Benevolent,

Who taught (to write) by pen, He taught man that which he knew not

(Surah Al-Alaq 30: 3-5)

Al-Quran

THESIS ACCEPTANCE CERTIFICATE

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Dedication

*To **Almighty Allah**, for his infinite blessings.*

*To my **Parents, Grandparents** and **Mentors***

*To my dearest homeland **Pak Sirr Zameen***

Abstract

Carbon sequestration is an important approach for reducing the amount of CO_2 in the atmosphere. Carbon sequestration, unlike carbon emission reduction, allows for the reduction or avoidance of CO_2 emissions by capturing CO_2 from large stationary sources and using it for energy production. Biological carbon sequestration or bio-mitigation via microalgal methods is a potential and sustainable replacement to conventional carbon mitigation strategies. Microalgae are a diverse group of rapidly growing microorganisms that can perform photo-autotrophy, heterotrophy, and mixotrophy. They have a 10–50 times greater CO_2 fixation capacity as compared to the terrestrial plants and may be grown on non-fertile land. The goal of this research is to see how effectively novel *Dictyosphaerium* species fix CO_2, as well as to evaluate their growth kinetics and biomass characterization. Both microalgal strains were supplied with 0.04 %, 2%, 4% carbon dioxide concentration in two various sorts of photobioreactors (Lab-scale photobioreactor and Multi-Cultivator). Results revealed that the growth parameters for both the microalgal strains substantially increased when supplied with 4% CO_2 compared to those provided with 0.04 % and 2% CO_2 in either type of Photobioreactor. Maximum growth was observed in *Dictyosphaerium DHM1* when it was grown in 4% carbon dioxide in the Multi-Cultivator with 2.802 g L^{-1}, and P_{max} = 0.2 g L^{-1} d^{-1}, and the highest carbon dioxide fixation rate was 0.21 g d^{-1}. Furthermore, food, fine chemicals, forage and biofuels made from microalgal biomass can boost the advantages of microalgal-based carbon dioxide fixation even more.

Keywords:

Biological Carbon Capture, *Dictyosphaerium sp.*, Growth kinetics, Microalgae, Multi-Cultivator

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List of Abbreviations

ppm	parts per million
GHGs	Greenhouse gases
CO_2	Carbon Dioxide
EU	European Union
CCUS	Carbon dioxide capture, utilization, and storage
CCS	Carbon capture and storage
UN	United Nations
NASA	National Aeronautics and Space Administration
MOFs	Metal-organic frameworks
CCU	Carbon capture and utilization
BBM	Bold's Basal Medium
UV-Vis	Ultraviolet Visible
EDX	Energy Dispersive X-Ray
SEM	Scanning electron microscopy
FTIR	Fourier-transform infrared spectroscopy
GC/MS	Gas chromatography-mass spectrometry
CHN	Carbon Hydrogen Nitrogen analyzer
RuBisCO	Ribulose 1,5-bisphosphate carboxylase/ oxygenase
kJ	Kilo Joule
DIC	Dissolved inorganic carbon
CCM	Carbon concentration mechanism
CA	Carbonic anhydrase
FCM	Flow cytometer
CFU	Colony-forming units
OD	Optical density
TSS	Total suspended solid
PBR	Photobioreactors
FAME	Fatty acid methyl esters
TAGs	Triacyl glycerides
PUFAs	Polyunsaturated fatty acids
UV	Ultraviolet

Chapter 1

Introduction

1.1 Climate Change

Carbon dioxide, Methane, and oxides of Nitrogen and Sulphur are the most notable greenhouse gases (GHG's) which are accountable for changing in climate and global warming [1]. Since the industrial revolution, the extreme usage of fossil fuels has caused in a steady increase in carbon dioxide concentration, from 200 ppm to 280 ppm, 390 ppm in 2013, and now 400 ppm [2] and according to current data by NASA, CO_2 concentration reaches 417 ppm, as shown in Figure 1.1 [3]. Over 80% of ozone harming substance discharges (i.e., GHG's) in the EU-27 are represented by energy formation, energy usage by industry, administrations, homes, as well as transportation. Emissions related to energy has fallen at a slower rate as compared to overall emissions since 1990 [4].

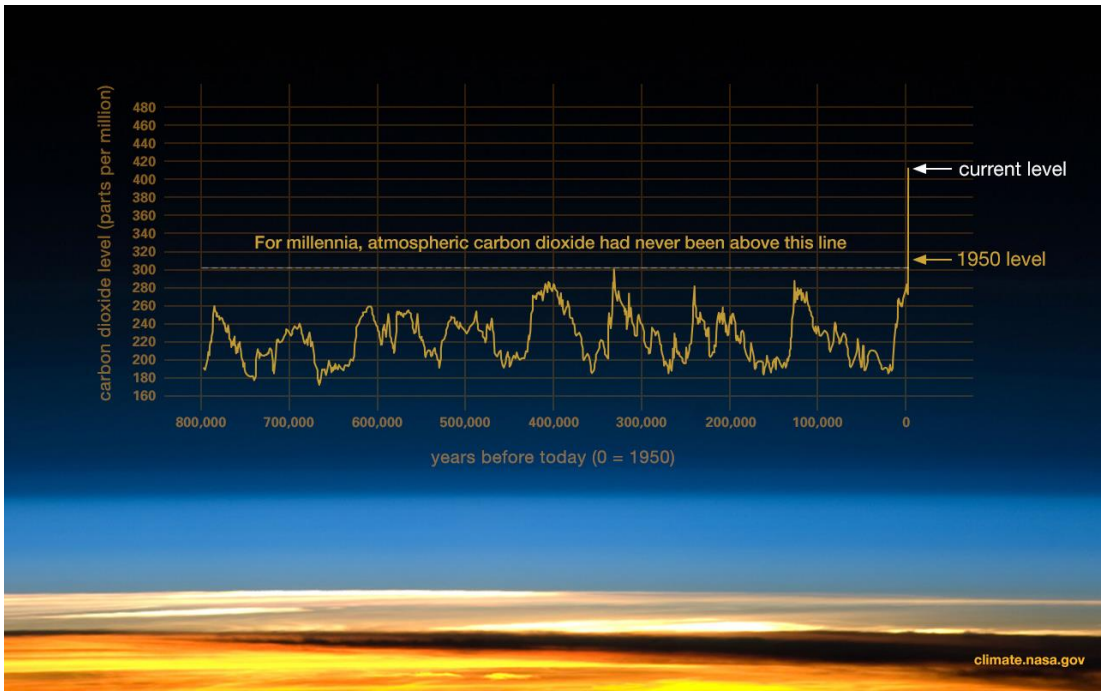

Figure 1.1 The continual rise of Carbon Dioxide concentration in ppm due to excessive fossil fuels burning[3].

Carbon dioxide seems to be the major contributor to climate change. It causes not just an increase in global temperatures, but also a rise in sea level, extreme weather conditions, changes in the farming industry, glacier melting, and species extinction

[5]. Heat waves, temperature variations, and rainfall patterns all have an impact on human health [6]. Because it will have an impact on the country's economy, energy output and consumption cannot be reduced [2]. To address this issue, various measures should be taken, including reduction in fossil fuel usage, shifting towards renewable energy, increased efficiency, recycling, and carbon dioxide capture [7].

1.2 Renewable Energy

The depletion of petrochemical fuels and the continual rise in oil prices have become a global challenge, requiring a global effort to develop new energy sources. Many approaches are now being researched and tested to achieve the sustainability goals outlined in the Kyoto Protocol (1992), with varying degrees of success. Wind, hydropower, geothermal, solar thermal, or solar photovoltaic (PV), carbon sequestration, ocean wave and biofuels energy have all been developed as more environmentally friendly alternatives to burning of fossil fuels.

Solar energy is at the top of the list. Derived from the massive power source that sustains life in our solar system, the heat of the sun, if harnessed, promised the infinite power source science longed for. However, harnessing solar energy is far more difficult. Therefore, research began to investigate other power sources in nature such as, hydropower, wind, geothermal, and biofuel etc.

As per the World Energy Outlook 2017 review, Renewable energy sources are predicted to grab two-thirds of global power plant investment by 2040, and they will soon become the most cost-efficient forms of electricity generation in many nations throughout the world [8].

1.3 Carbon dioxide Capture, Utilization and Storage

It is an successful approach for reducing carbon dioxide emissions and mitigating global warming [9]. There are various CO_2 capturing technologies that have been extensively researched, such as geological, biological, and chemical approaches (absorption, adsorption, cryogenic) [9], [10]. These approaches are technologically complex and expensive which include: pre-combustion, post-combustion (figure 1.2 [11]), and oxygen-fuel combustion capture [12]. In comparison to chemical absorption and geological sequestration and storage, carbon capture via microalgae known as

Biological carbon capture, has recently attracted worldwide attention and has emerged as a promising mitigation approach [13], [14], [15].

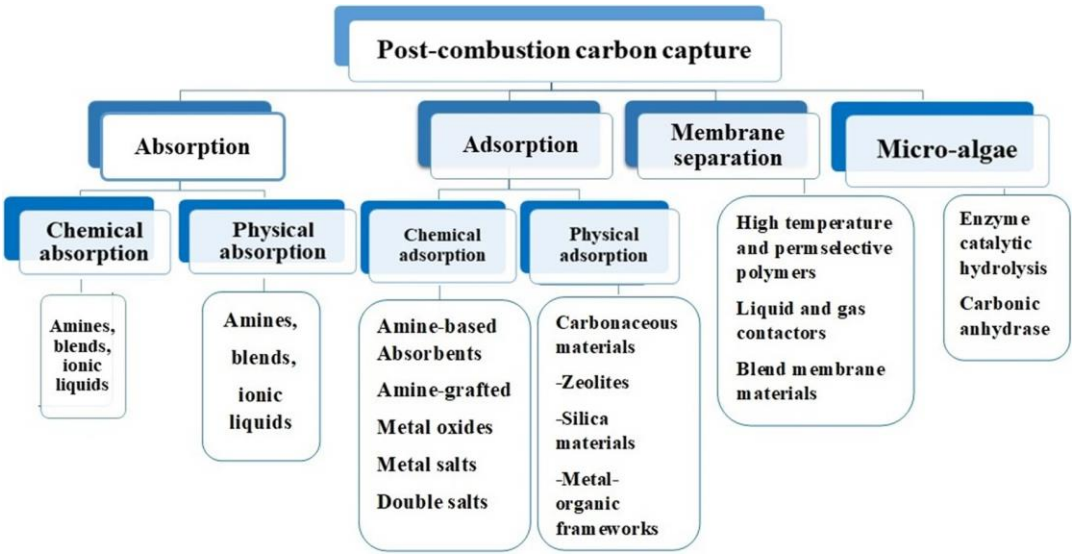

Figure 1.2 Post-combustion carbon capture technologies [11].

1.4 Microalgae

For 3.5 billion years, photosynthetic microorganisms have played a part in substantially all of the planet's ecosystems [16]. “They are 10 to 50 times more efficient than plants at capturing carbon dioxide”, because of their higher growth, great tolerance to extreme environments (pH, temperature), as well as high CO_2 utilization rate [2], [10], [13], [17], [18]. “With a carbon content of 50% in microalgal cells, one ton of microalgae consumes 1.83 tons of carbon dioxide”, accounting for roughly one-third of worldwide CO_2 fixation [19]. Sunlight can be converted, collected, and efficiently stored to meet energy demands sustainably [20]. Under natural and artificial light, microalgae capture dissolved carbon dioxide and convert it to biomass, a valuable organic substance. Carbon dioxide is stored in the form of lipids, carbohydrates, pigments and, proteins, which might also be used for a range of different other purposes (e.g. food, biofuels, feed, pharmaceuticals, etc.) [2], [17], [21], [22]. Microalgae biomass can also be utilized as a third-generation biofuel (biodiesel, biogas, bioethanol, hydrogen) in the transportation and energy sectors, along with other applications [21], [23].

1.5 Biological Carbon Capture

There were very few companies working on algae-based carbon capture and storage (CCS) until a few years ago. And as the use of microalgae for CO_2 sequestration has

grown in popularity in recent years, more corporations have begun to invest in algae-based CO_2 sequestration, and the number of originations in the field has expanded. According to the Oilgae 2011 report, a few of the firms active in this industry are as follows: “1. A2BE Carbon Capture, LLC; 2. Clentueeet Whisky Distillery; 3. RWE Energy; 4. Eni Technologies; 5. Seambiotic; 6. Trident Exploration; 7. NRG Energy; 8. General Electric; 9. Pond Biofuels, Inc.; 10. Cequesta Ltd; 11. Solix Biofuels, Inc.; 12. SarTech; 13. StellarwindBio Energy, LLC; 14. Kent SeaTech Corporation” [24].

International bodies like the OECD International Energy Agency (IEA), which was created in 1978, work to increase collaboration and information exchange between countries with national bioenergy research, deployment and development programs. Pakistan, China, South Africa, India, Brazil the United States, and the European Commission make up the UN International Biofuels Forum. Major research is currently being conducted to produce such biofuel molecules and methodologies are being devised to integrate them into the mainstream fuel use process.

Even though CO_2 fixation by microalgae has been extensively studied in recent decades, there are still many obstacles to overcome before large-scale implementation. Most known microalgal species, are less tolerant of high CO_2 concentrations and might be inhibited in their development [25]. Many microalgal species e.g. “*Chlorella vulgaris, Chlorella sp., Chlorella kessleri, Chlorocuccum littorale, Botryococcus braunii, Scenedesmus obliquus, Chlamydomonas reinhardtii, Tetraselmis sp., Scenedesmus sp.,* and *Spirulina sp.*” have all been researched extensively. [26]. These are promoted as sustainable sources of fuel generation since they are extensively cultivated. However, given the world's growing population and the prevalence of famines and droughts, there is strong opposition to using food crops for fuel generation. Therefore, microalgae is an alternative source of biofuel since it is easy to grow and is widely available around the world. It also has a quick doubling time and doesn't compete for arable land and resources with food crops. Lipids and branched-chain hydrocarbons are abundantly present in algae. This, combined with their ability to fix carbon dioxide in the atmosphere, makes them ideal candidates for biodiesel production.

However, there is still a need to better understand the function of photobioreactors and the discovery of new species and their CO_2 tolerance. New algae species have the possibility to be used in a different array of biotechnological purposes, including; food industry, nutraceuticals, animal fodder, natural food colors, cosmetics, medicinal goods, and 3rd generation biofuels [27].

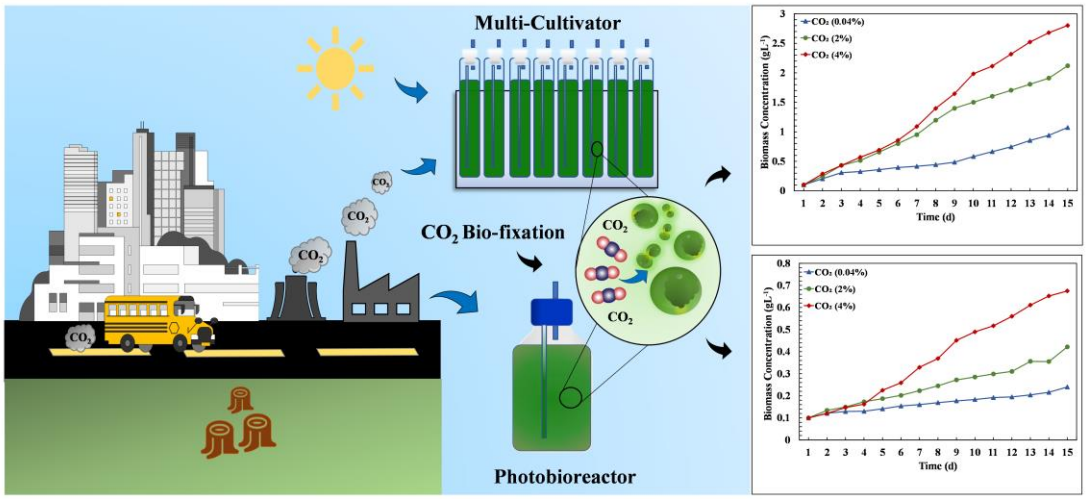

Figure 1.3 Biological Carbon Dioxide capture via microalgae.

Fuel production technologies that are not only eco-friendly and renewable but also efficient in sequestering atmospheric carbon dioxide are required to achieve environmental and economic sustainability, making the development of carbon dioxide neutral fuels one of our society's most pressing tasks. Algae are high-yielding micro-factories that can produce biomass and energy at a rapid rate and also capture carbon at the same time.

1.6 Aims and Objectives

The objective of this research is to study the growth kinetics of two novel microalgal species, *Dictyosphaerium sp. DHM1* and *Dictyosphaerium sp. DHM2*. These strains were further used for the carbon dioxide sequestration in 3 different CO_2 concentrations, amount of carbon dioxide present in air i.e., (0.04%), 2%, and 4%. In addition, two different kinds of photobioreactors having different working volumes (L) were evaluated to achieve high CO_2 removal efficiency in PBR's. And to characterize the algal biomass that how it can be used for energy purposes like biodiesel production, and other industrial and pharmaceutical uses. Their biomass characterization analysis is carried out using: ["(Fourier-transform infrared

spectroscopy (FTIR), Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray Analysis (EDX), and Gas chromatography-mass spectrometry (GC-MS))"].

1.7 Thesis Structure

This thesis is divided into five sections. The thesis's introduction is covered in Chapter 1. This chapter introduces the research problem, defines the study objectives, and discusses the research technique, as well as the research's importance. The full evaluation of the literature on algae growth in photobioreactors, their growth analysis, techniques, and applications are documented in Chapter 2. The Experimental Setup is discussed in Chapter 3. The Experimentation Results and Discussion are presented in Chapter 4. While, Chapter 5 concludes with conclusion and recommendations, as well as a discussion of future study directions for interested researchers.

Summary

In this chapter, global warming and climate change is discussed and techniques to combat climate change are mentioned i.e., shifting towards renewable energy and carbon capture. International focus is rapidly shifting towards renewable energy resources. Wind, solar, Hydral, Geothermal, and biofuel are the chief areas of research these days, and the most demanding area for research is carbon capture and utilization. Photosynthetic carbon-fixing algae are of particular importance in this regard. Because they are widely available, easy to grow, and have a rapid growth cycle.

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Chapter 2

Literature Review

2.1 Carbon Capture

Carbon dioxide capture and storage (CCS) is a method of capturing carbon dioxide from a source and transporting it to storage facility for long term separation. Methane (CH_4), carbon dioxide, nitrous oxide (N_2O), and halocarbons are the main greenhouse gases (GHGs). Even though methane is roughly 20 times highly potent than CO_2, the worrisome rise in carbon concentrations qualifies it as a major producer of GHG, posing significant problems to global pro-environment and sustainability. Numerous technologies or procedures have been actively used over the years for successful CO_2 capture, storage and utilization from point sources [1]. Post-combustion capture, oxyfuel process and pre-combustion capture are three different techniques for CCS.

2.2 Carbon capture technologies

2.2.1 Physical Absorption

Physical absorption is governed by “Henry's Law”. At high pressures and low temperatures, CO_2 is absorbed, then desorbed at lower pressures and higher temperatures. This technology is frequently utilized in a diversity of industrial processes, including the manufacture of natural gas, hydrogen with high CO_2 content and synthesis gas [2].

2.2.2 Chemical Absorption

The traditional chemical absorption method contains of a stripper, and an absorber in which the absorbent is thermally regenerated. In the chemical absorption procedure, carbon dioxide rich flue gas enters from the bottom of a packed bed absorber, touches a CO_2 lean absorbent counter currently, and then flows into a stripper for thermal regeneration. “The carbon dioxide lean absorbent is piped back to the absorber for cyclic usage after regeneration. The carbon dioxide when emitted from the stripper it is then compressed before being moved and stored. And the operational conditions are: (pressure = 1.0 bar, with temperatures in the absorber and stripper ranging from 40˚-60˚ C and 120˚-140˚ C), respectively” [2].

Nevertheless, one significant challenge when using amines for carbon capture and storage is solvent degradation because of the existence of impurities, which results in equipment corrosion, loss of solvent, and other issues [1].

2.2.3 Adsorption

The physical process of a liquid or a gas adhering to a solid surface is known as adsorption. Adsorption is able to help minimize the energy and price of CO_2 capture and separation after burning. The creation of a readily regenerated and durable adsorbent with high CO_2 selectivity and adsorption capabilities, however, is critical to the success of this strategy. "Carbon fibers, activated carbons, zeolites, ion-exchange resins, porous silicates (SBA-15, MCM-41, etc.), metal oxides (CaO, MgO, K_2O, Li_2O), activated alumina, organic-inorganic hybrid sorbents, silica gel, metal-organic frameworks (MOFs), and other surface-modified porous media could all be used to capture CO_2." However, zeolites, MOFs and porous silica show little CO_2 adsorption at high temperatures. Physical and chemical adsorbents are two sorts of adsorbents utilized for CO_2 capture [3].

2.2.4 Membrane separation of carbon dioxide

This is the easiest and most eco-friendly process of CO_2 separation, in which membranes were of ceramic or polymer sieve out carbon dioxide from a combination of gases such as natural gas, flue gas, and other gases under pressure founded on their variance cut off size. Concentration gradient and hydrostatic pressure are the main driving forces for gas separation. The permeability of every gas molecule is influenced by the thickness of the membrane in an indirect manner and vice versa. Sulfonation of polymers increases carbon dioxide separation by enhancing solubility and allowing for more interactions with carbon dioxide molecules among the various membrane types utilized for carbon dioxide separation [4].

2.2.5 Cryogenic separation of carbon dioxide

In this technique CO_2 is separated by condensation and the operating conditions are triple point of CO_2: "-56.6˚C (extremely low temperature) and pressure is 7.4 atm, cooling solvents are used which is separated through distillation." And it works well for separating CO_2 from streams with high CO_2 levels (usually > 90%), and carbon dioxide purity after distillation is normally high (>99.5%). This process produces CO_2 in a liquid condition, which is easier to transport via pipeline and therefore has an advantage as compared to the other CCS technologies [5].

2.3 Biological carbon capture

Plants and microorganisms such as algae, fungi, bacteria and yeast use two methods to sequester carbon, one of which is photosynthesis, and the other is non-photosynthetic processes. CO_2 is incorporated into cellulose, lignocellulose, chitin, hemicellulose, lignin, and other organic carbon products by both autotrophic and heterotrophic organisms. The right use of these organisms for CCU can result in environmentally benign CO_2 sequestration. Different routes, conversion processes, and the potential to produce biomass and bioenergy are present in these organisms [6].

The biological conversion of carbon dioxide by algae is seen as a potential and long-term carbon dioxide mitigation option because it not only lessens atmospheric CO_2 levels but also produces important photosynthetic metabolic products with a wide range of industrial applications. Gaseous carbon dioxide from the point sources, first of all compressed, chilled, and then filtered prior to being fed to algal photobioreactors for effective CO_2 capture [1].

2.4 Microalgae

Algae are photosynthetic organisms that release oxygen and have a simple body design with no roots, stems, or leaves and are frequently found in water. They don't form a single monophyletic group, hence it's difficult to classify them. Individual species of algae occupy particular habitats, although algae as a group are widespread. Some microalgae are connected to a substratum like plants, while others can move like animals (e.g., sloths), while others are just floating in water. Nearly few of them grow roughly on trees, soil, and animals, while others have symbiotic connections with other species (e.g., corals and lichens). Algae have a wide range of intracellular structures. Seaweeds have complex multicellular structures that microalgae lack. Cyanobacteria, often known as blue-green algae, their cell structure are like prokaryotes, which resembles that of bacteria. Eukaryotic algae cells consists of a nucleus and chloroplasts, as well as Golgi bodies, mitochondria, endoplasmic reticulum, and other eukaryotic organelles [7]. Microalgae are microorganisms that can be originate in both aquatic and terrestrial ecosystems. As a result, they signify a diverse spectrum of species that may living in a variety of environments. For microalgae to grow, they require three essential elements: sunshine, water, and a carbon source [8], [9].

2.5 Photosynthesis

"It is a exclusive phenomenon of conversion of sunlight energy in which photoautotrophs convert inorganic molecules and light into organic substances." Almost all species on Earth rely on photosynthesis as the resource of organic matter and energy for their metabolism and development, either directly or indirectly [7]. Plants and algae convert carbon dioxide into carbohydrates or sugars in a multistep photosynthetic process that uses sunlight, or even artificial light and water (H_2O) as energy and electron sources, respectively. Represented by following equation:

$$CO_2 + H_2O + light \rightarrow (CH_2O)_n + O_2$$

The Calvin cycle is the step-in photosynthesis where CO_2 is transformed to sugar using "ATP adenosine-50-triphosphate" and the "carboxylase activity of the enzyme ribulose 1,5-bisphosphate carboxylase/ oxygenase RuBisCO." "A least of 8 mol of photons (quanta) with 218 kJ/mol are required for the synthesis of one mole of CH_2O." Photosynthesis converts "27% of solar energy" is converted into chemical energy, generating 467 kJ per mol of CH_2O versus 1744 kJ required for its development [10]. Represented in Figure 2.1 [11].

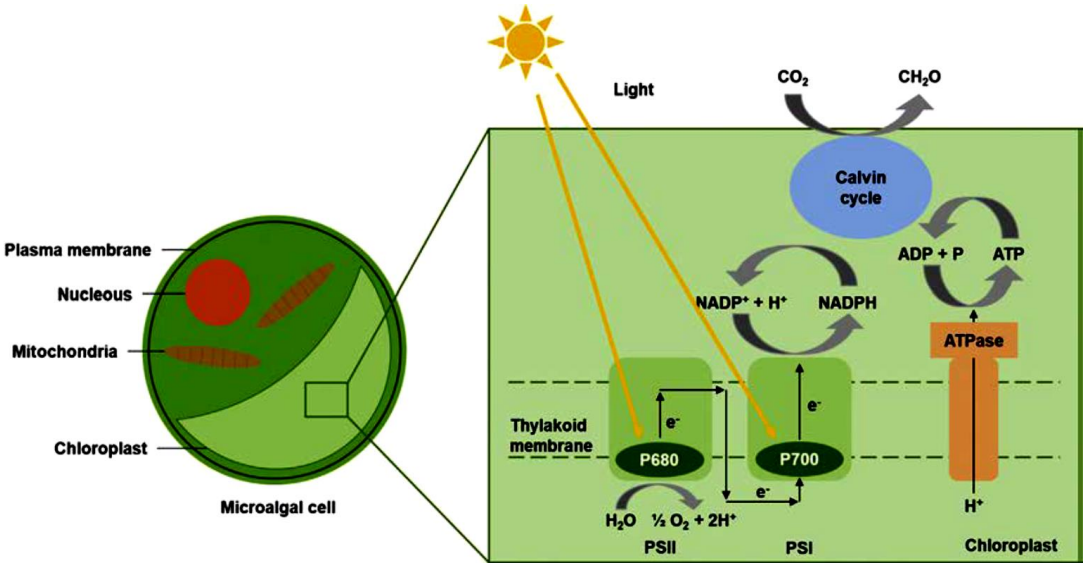

Figure 2.1 Schematic representation of photosynthesis in microalgal cell [11].

2.6 Nutrient's requirements and their effect on the microalgal growth

2.6.1 Carbon

For photosynthesis, microalgae require inorganic carbon, which might be supplied in the form of salts (e.g., bicarbonates) and by air augmentation (because the ambient air does not have adequate carbon dioxide for demanding production of microalgae). CO_2 must be solubilized before microalgae can use it for photosynthesis. Depending on the pH, carbon dioxide dissolves in water in a variety of ways. Microalgae thrive in a pH-neutral environment [12].

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^-$$

2.6.2 Nitrogen

It is the really important element of structural and operating proteins. Microalgae grow at nearly the same pace regardless of the nitrogen source (urea, nitrite, or nitrate). Numerous studies have shown that when there is a nitrogen shortfall, lipid production and storage improve [12].

2.6.3 Phosphorus

The macronutrient phosphorus plays a vital role in cellular metabolism. Pigment buildup in some microalgae may be caused by phosphorus deprivation, but the impact is less than that of nitrogen deficiency [12].

2.7 Factors Affecting Bio-sequestration

2.7.1 Temperature

Microalgal growth is affected by range of parameters, i.e., cellular morphology, temperature, as well as physiology. Higher temperatures accelerate microalgal metabolic rates, but lower temperatures hinder microalgal growth. The ideal temperature for microalgal growth differs by species, ranging from 15˚-26˚ C. If flue gas is to be used to feed the microalgae culture, "thermophilic organisms" are necessary for CO_2 biological sequestration, because flue gas temperatures might reach upto 120˚ C [13]. High temperatures more than optimum temperature can cause CO_2 solubility to drop dramatically, thus lowering photosynthetic efficiency. Low temperatures, on the other hand, limit metabolic activity, reduce chlorophyll as well as protein content, and lengthen the lag phase. In extreme conditions, growth rate is

reduced which causes an increase in lipid, glucose and carotenoid level as defensive strategy to maintain cell metabolism during harsh conditions [1].

2.7.2 Light

Phototrophic microorganisms get their energy from light, so they require optimal light intensity for CO_2 fixation and production of biomass. If the light intensity drops beneath the ideal range, it becomes a restraining factor for microalgal productivity. Also photoinhibition occurs if cells are exposed to strong light intensity for longer time [13].

2.7.3 pH

The major DIC forms, CO_2 and HCO_3 are directly related to the pH value in the growth medium due to reversible chemical equilibriums. Apart from the chemical characteristics of the culture medium, the hydrolysis of carbon dioxide and impurities that are soluble in water e.g., SO_2 from flue gas is responsible for the pH shift in the medium. The effect of pH on microalgal carbon bio fixation and production of biomass are primarily influenced by CO_2 and SO_2 levels [14].

2.7.4 Mixing and aeration

Mixing is required to minimize algal sedimentation, guarantee that the cells in the culture medium are given equal quantity nutrients and light, eliminate heat "stratification (i.e., in outdoor cultures)", and increase gas exchange between the culture medium and the air. It reduces I_O while simultaneously utilizing the flashing light effect. This boosts production by up to 40% in tubular photo-bioreactor [10]. It is feasible to improve mass transfer, establish the uniform supply of nutrients, and light, degass oxygen, and prevent biomass accumulation and sedimentation by mixing properly inside the photobioreactor. Aeration rate and bubble size are considered as crucial factors during mixing of the culture media [1].

2.7.5 Inoculum size and density

As the initial inoculation concentration rises, so does microalgal biomass production and carbon fixation Increased initial biomass concentration in the microalgal development phase can lower CO_2 load and improve CO_2 tolerance [14]. Microalgae cell development and lipid accumulation are substantially influenced by inoculum size, making it most promising biodiesel feedstocks. However, the metabolic process of lipid biosynthesis in response to inoculum size has yet to be extensively studied [15].

2.7.6 Carbon Dioxide concentration

Photosynthesis is a multistep process and requires CO_2 as a major component . Up to a certain point, biomass productivity grows linearly with increasing concentration of carbon dioxide, after which it begins to decline [10]. Carbonic anhydrase (CA) activity is reduced as carbon dioxide concentrations rise, and this is linked to factors like carbon dioxide fixation into cells of microalgae. Algae have a exclusive, innate tendency to accumulate massive amounts of inorganic carbon in their cytoplasm, frequently in higher quantity than outside and this carbon concentration mechanism is called as CCM. High CO_2 concentrations restrict microalgal growth because CO_2 has a decreased affinity for the Rubisco enzyme due to its high Km. For successful CO_2 uptake and biomass development, most microalgae demand low CO_2 concentrations (typically 0.038–10%) [1].

2.7.7 CO_2 mass transfer

In the design and process of carbon dioxide-fed microalgal photobioreactors, mass transport is a critical factor. CO_2 diffusion into water is challenging due to low CO_2 concentrations in the atmosphere (0.039–0.04%) and the high surface tension of water [16]. To improve biomass production and carbon dioxide fixation, microalgal PBR's should have good mass transfer properties. "The key aspects of mass transfer rate in a PBR's: flow velocity, gas hold-up, bubble size, carbon dioxide concentration, and gas-and-liquid contact area" [1].

2.8 Lab Scale Algae Growth Mode

2.8.1 Batch Culture

It is a very popular way of microalgal cell culturing. A little quantity of full culture media and algal inoculum is positioned in a container containing culture and incubated in a promising environment for growth in a simple batch culture technique. To guarantee nutritional and gaseous exchange at the cell and water interface, some type of agitation, such as shaking or impeller mixing, is required. A basic conical flask or an environment-controlled fermenter can be used as the culture vessel. CO_2 is provided to a photosynthetic or mixotrophic culture by purging the bottle or flask with CO_2 enriched air and capping it, by continually supplying the culture with CO_2 enriched air. Natural light and artificial light both can be the sources used to illuminate the culture.

2.8.1.1 Lag phase

It is common to see an initial period where the particular growth rate is sub-maximum. Inclusion of spores or nonviable cells in the inoculum could cause growth. A phase of physiological modification owing to variations in nutrition or culture conditions could also cause a growth lag. When shade-adapted cells are exposed to greater light intensity, for example, growth lag might be noticed. When cells from a later exponential growth phase are employed as inoculum, the lag period may be eliminated.

2.8.1.2 Exponential phase

Cells have attuned to their new environment and are now growing as well as multiplying (accelerating growth phase), ultimately transitioning to the exponential growth phase also known as log phase. The cells develop and then divide exponentially in the latter phase, as long as light energy and mineral substrates are available.

2.8.1.3 Linear Growth phase

Cell concentration, rather than photon flux density, determines the amount of light absorbed by cultural media. That example, most low-flux-density photons might flow through a low-cell-concentration culture, whereas all high-flux-density photons can be collected by a culture having high concentration of cells. Therefore, until all photosynthetically accessible photons impinging on the culture surface are absorbed, the cell culture will continue to grow exponentially.

2.8.1.4 Stationary growth phase

The culture enters the stationary phase when soluble substrate in the culture media get depleted. Photosynthesis is still going on at this point, and storage carbon products like starch and lipids are being built up.

2.9 Parameters of measuring algal growth

The number of algal cells or the increase in microalgal biomass in the form of dry weight, chlorophyll content or ash-free dry weight can be used to determine the growth of algae cultures.

2.9.1 Cell concentration

2.9.1.1 Cell count

Cell counts can be determined using a Neubauer or Sedgwick-Rafter chamber, rely on the size of the algae and whether they are single-celled, chain-forming, or colonial.

The hemocytometer is ideal for cells in blood cell size range (i.e., < 100 µm in diameter) and cell densities of fewer than 10^5 cells m L^{-1}. The Sedgwick-Rafter chamber is excellent for counting cell densities or larger cells more than 10^5 cells m L^{-1} [17].

2.9.1.2 Flow Cytometry and Particle Counters

Cell density can also be measured using flow cytometry. Flow cytometers detect the light scattering and fluorescence physical characteristics of a fluid stream as it passes through light beams at high speeds, up to thousand cells per second. Particle counters have been around for a long time and are extremely useful. They are, however, limited to single cells and may be erroneous for cells that distort quickly, such as *Dunaliella sp.* cells [17]. Although FCM has great potential, it is currently in its early stages of use in microalgae growth. The lack of established techniques for analysis is a significant concern in FCM analysis. [18].

2.9.1.3 Direct cell count with optical light Microscopy

The light microscope is one of the oldest tools for measuring the cell content of microalgae and other microorganisms. It is one of the most precise and reliable ways of determining the number of microorganisms present in mixed cultures. If the scientist is skilled enough, he or she can utilize this method to count distinct microalgal species in same analysis [18].

2.9.1.4 Plate counting method

It is the oldest method. This technique involves distributing the sample on solid medium plate and counting the CFU that result from cell duplication later certain amount of time has passed. To identify colonies formed by single cells, the samples must be sufficiently diluted. Counting colonies on plates assumes that each colony comes from single cell. This hypothesis is false when dealing with cell aggregates or colonies, they must be segregated using optimal pre-treatments that are tough adequate to separate cells while remaining gentle sufficient to keep them alive [18].

2.9.1.5 Biomass Concentration

The amount of dry cell mass in the sample suspension is the goal of this category of analysis. Direct and indirect methods, both can be used to determine biomass. All indirect techniques that measure some factors correlated to dry biomass content are

referred as indirect methods. Using the average dry cell weight, any cell counting method can also be used as an indirect technique to measure the biomass concentration.

2.9.1.6 Optical density

The optical density, which is established on the “Lambert-Beer” linear connection between particle concentration and light absorbance, is the most widely applied indirect method for measuring dry biomass concentration. This method is widely used since it is quick, inexpensive, and only requires a little amount of sample (less than 1 mL). Furthermore, in industrial operations, OD can be measured in real-time. Optical density calculation should be done after a formation of calibration curve, which identifies the mathematical line equation, between dry biomass (DCW) and optical density and evaluates the relationship's range of rationality [18]. Absorbance is a popular way to measure the growth of algae cultures quickly. “To prevent interfering with chlorophyll and other various photosynthetic pigments, conventional wavelengths of 550 or 750 nm are utilized (i.e., where chlorophyll and most other pigments absorb the least amount of light)” [17].

2.9.1.7 Dry Biomass weight

Techniques for measuring TSS in the water samples are the most commonly used direct measure to calculate dry biomass. Biomass is calculated by adding the mass of complete cells in suspension (both alive and dead). Most common direct method to determine biomass concentration is filtration, in which recognized volume solution is filtered having pore size normally ranges from 0.2-1.2 µm and weighed in an oven temperature range from (60–105 °C) until consistent wt. is obtained. Although it is a low-cost procedure, reading the results can take several hours (1–24 hours). Two less common direct procedures for determining entire biomass dry weight include biomass collection by centrifugation from sample, washing with water this is done to remove salts and after that drying at specific temperatures. Centrifugation could be used to distinct populations with various cell sizes because sedimentation rate is substantially determined by particle size. However, compared to filtration, this process needs more work and biomass, is more vulnerable to scientific errors (vial wt. and surface area are greater than filters), and the use of a density gradient frequently required to improve segregation productivity. Centrifugation is seldom employed in laboratory operations for biomass determination for these reasons. [18]. Dry biomass weight is a crucial

parameter for calculating biomass concentration, productivity, and cell component percentages [19].

2.10 Harvesting of algal cultures

Microalgae harvesting methods are classified as physical, chemical, electrical, biological, and entail the removal of microalgal biomass from the growth cultural medium. Sedimentation, flotation, coagulation or flocculation, centrifugation, electrophoresis, and filtering are all typical procedures for harvesting microalgae. Some are discussed below.

2.10.1 Chemical coagulation or flocculation

It is most conventional method for improving the economics of microalgal harvesting procedures. The necessity for these approaches arises mostly from the vast volumes of cultures that should be processed, as well as the need for a universal technique that can be applied to an inclusive range of species. The suspension is concentrated 20–100 times during this harvesting stage. [20].

The target of the ensuing processes determines the right coagulant to use. The application of ferric salts causes the microalgae to turn brown-yellow in color. Nevertheless, there is no influence on cell feasibility. If pigment extraction is desired, ferric salts can't be used in this manner. If the goal is to lower total manufacturing costs, a less expensive and speedier coagulant, such as an aluminum salt, should be used. Cell lysis (10–25%) was found when aluminum salts were used, presumably due to the fast cell aggregation or cell membrane instability [21]. Even though metal coagulants like alum and iron chloride may easily flocculate it, considerable volumes of these salts are mandatory, making it exceedingly expensive [20]. Furthermore, metal contamination may occur in microalgal biomass treated with these chemicals, preventing its use as a biofuel or animal fodder [22].

2.10.2 Centrifugation

It is the fastest procedure of harvesting. In lab centrifugation experiments, 80-90 percent of microalgae can be recovered in 2-5 minutes [23]. The vast majority of microalgae can be harvested using centrifuges [20]. Because it can handle huge volumes of biomass quickly and the biomass remains totally contained during recovery, centrifugal biomass recovery is a viable option for high-value goods [24].

2.10.3 Filtration

It is primarily a dewatering method that is typically used after coagulation or flocculation to increase harvesting efficacy. Its use necessitates the preservation of a pressure drop throughout the system to compel fluid flow through a membrane. During this procedure, microalgae cell layers deposits on the filtration membranes and it becomes thicken, increasing resistance and reducing filtration flow while maintaining a constant pressure drop. This problem is known as fouling or clogging and it is the most significant disadvantage of filtering techniques, increasing their operating costs [20].

2.11 Modality of cultivation

2.11.1 Photoautotrophic

Microalgae in a photoautotrophic culture create chemical compounds using light as an energy source, as well as CO_2 and water. The most typical application for this sort of algal production is for commercial purposes. Because of the potential of photoautotrophic microalgae to absorb waste CO_2, such as that produced by breweries, cogeneration facilities, or biogas plants, it is also a promising approach [25]. Photoautotrophic production is currently the only approach for large-scale algae production that is both technically and commercially possible.

2.11.2 Heterotrophic

This mode of cultivation is defined as, use of organic carbon as a foundation of carbon and energy under dark environments. Microalgae can absorb a broad range of organic substrates, including acetate, glucose, glycerol, sucrose, fructose, lactose, mannose, and galactose which can be generated from residual biomass like wheat straw, corn stover, and sugarcane bagasse hydrolysates. The risk of bacterial contamination is a disadvantage of heterotrophic culture [26].

2.11.3 Mixotrophic

Microalgae that are grown in a mixotrophic environment undertake photosynthesis and utilize both organic molecules and CO_2 as carbon sources. Cultivating a microorganism in a mixotrophic environment indicates the organism can grow in phototrophic, heterotrophic, or both environments. “Photoheterotrophic” cultivation, “photo assimilation”, and “photo metabolism” is another option. Light is required for this type of metabolism to utilize “organic substances” as a “carbon source”. Culturing

microalgae in mixotrophic and photoheterotrophic settings with a wellspring of natural mixtures from squander assets could be a fabulous chance with nothing or even bad fossil fuel byproducts and monetary expenses. [26].

2.12 Production systems

2.12.1 Open ponds

These are the simplest and oldest approach for growing microalgae outdoors, and they are normally set up as shallow ponds with walls. Green microalgae require nutrients, and water, which can be easily provided or distributed by runoff water from neighboring land regions, wastewater from industry or community, and CO_2 from combustion gas from a power plant. Water, fertilizers, and carbon dioxide are constantly delivered into the ponds when the systems are turned on, and biomass is harvested at the end. Raceway and circular ponds are the two extremely common prototypes in use today. The former is frequently employed for large-scale commercial *Dunaliella* and *Spirulina* production, whereas the latter is mostly used for *Chlorella* cultivation in Asian countries. The construction ideas vary when it comes to culture mixing. In practice, paddle wheels are used to agitate the culture in raceway ponds, whereas a rotating pivot in the center of a circular pond is used to mix the culture [27]. CO_2 is injected directly into the culture media in pond system, or it is exchanged with ambient air through mixing and dissolved in the culture media. To optimize the effectiveness of CO_2 fixation in open ponds, a sump near the wheel having a depth of approximately 1.5 m, used to increase CO_2 availability in water when CO_2 is delivered via direct injection [28].

2.12.2 Photobioreactors

Photobioreactors (PBRs) are commonly used because they can address some of the concerns that open ponds have, such as possible contamination, sensitivity to environmental conditions, water loss due to evaporation, appropriateness for just a few strains, and the necessity to occupy substantial land areas. PBR's are typically various types of tanks or closed systems in which microalgae are grown [29], as shown in Figure 2.2 [23]. PBR's are usually closed systems made of translucent or transparent materials like glass or plastic. A PBR can also be defined as a pond surrounded by a greenhouse. Indoor artificial light can be provided by light assortment and distribution devices, or they can directly utilize sunshine. A surge of cleaned water containing every necessary supplement, including CO_2, ought to be taken care of into the

framework consistently all through microalgae improvement. Excess culture overflows and is harvested continuously, along with the increase of microalgal biomass [27].

2.12.2.1 Tubular Photobioreactors

Fully closed tubular PBR's are one of the more ideal forms for outdoor mass culture and could be interesting for the large-scale axenic culture (i.e., without contamination) of microalgae. Tubular bioreactors are made up of a series of transparent tubes that are either straight, coiled, or looped. They are commonly composed of transparent plastic or glass. A pump or airlift technology circulates algae through the tubes. The use of an airlift device appears to have many advantages, including the ability to interchange carbon dioxide and oxygen between the liquid medium and the aeration gas, the reduction of the potential cell damage related with mechanical pumping, and the elimination of moving parts. Floating or immersing the tubes on or in the pool of water has been reported to help manage the temperature of the tubular photobioreactor in some situations [30].

2.12.2.2 Flat plate photobioreactors

Because of the serious level of sun-based light on the plate's surface, the low development of disintegrated oxygen, and the comfort of particular plan for increase, these photobioreactors are appropriate for huge scope creation in both indoor and open air applications. Level plate photobioreactors have a few inconveniences, including the powerlessness to change the temperature, and the creation of algal biofilms on the plates, in addition to the gamble of hydrodynamic pressure, which could hurt explicit microalgae strains. [31].

2.12.2.3 Air lift and bubble-column Photobioreactors:

Simple devices like airlift and bubble-column photobioreactors have remained employed in bioprocessing, the chemical process sector and wastewater treatment. These vertical column PBR's are small, inexpensive, and simple to use. Pneumatically agitated bubble columns and airlift machines achieve the needed mass transfer coefficient and liquid circulation velocity for microalgae production with a low power input. [30].

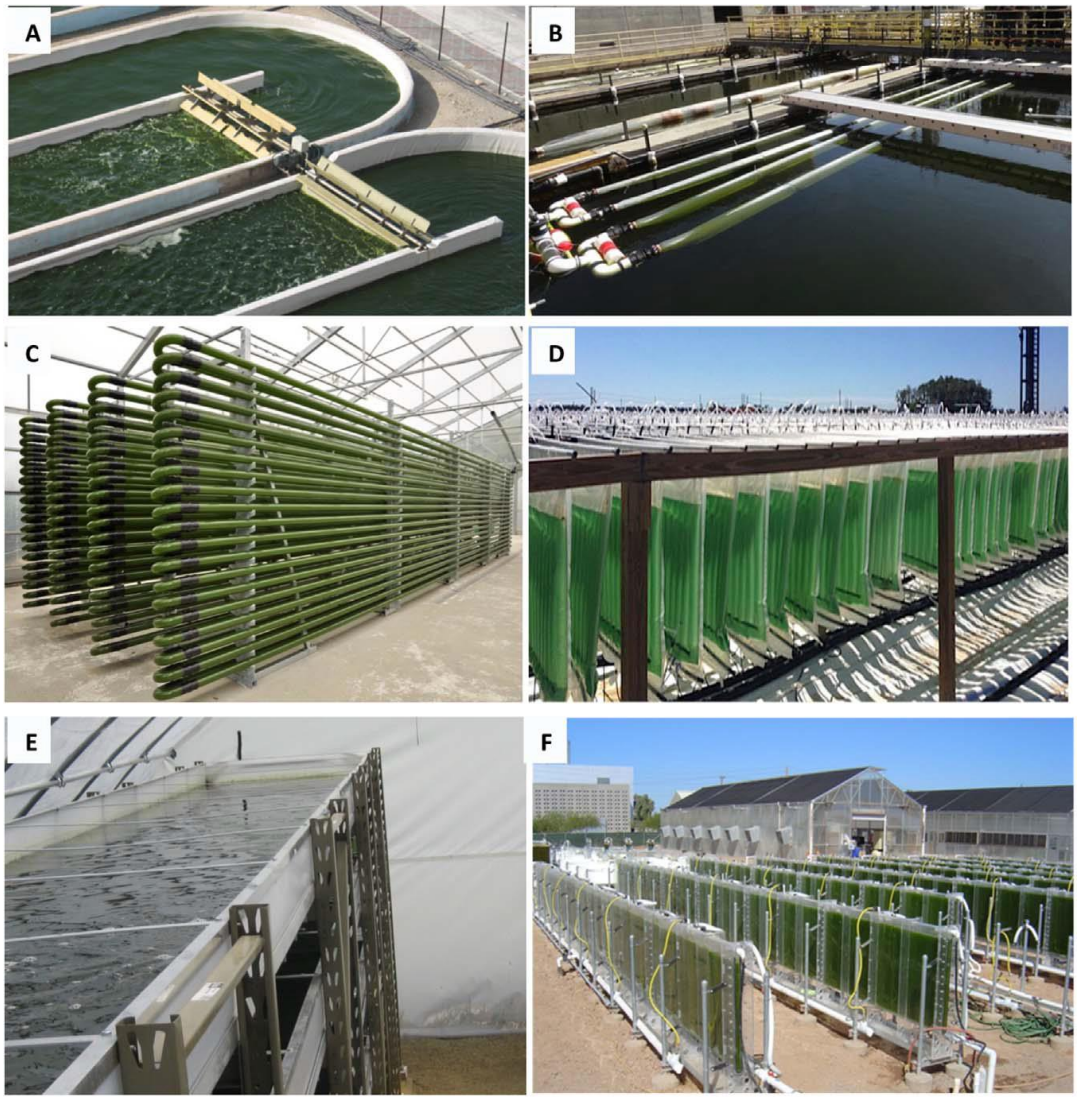

Figure 2.2 Reactors structure for microalgal cultivation [23].

2.13 Applications of microalgae

2.13.1 Biodiesel production

Algae are good preliminary materials to produce biodiesel, which can be use up to replace non-renewable fossil fuel-based transport fuels due to their elevated lipid content and easiness of growing. Triacyl glycerides (TAGs) are a type of lipid that serves as a storage task in the cell, allowing microalgae to withstand harsh environmental situations. Transesterification reactions (e.g., with the use of methanol) can convert lipid into biodiesel and fatty acid methyl esters (FAME) once it has been extracted. FAME has physical properties that are similar to fossil fuels-based diesels. It's also non-lethal and biodegradable, which is a bonus. The usage of biodiesel as a

low-blend component in transportation fuel (up and around 7%) does not necessitate any distribution system upgrades, preventing costly infrastructure variations [32].

2.13.2 Bioethanol production

Apart from biodiesel, algae can also be used as a fermentation feedstock to create bio-ethanol, albeit there is little research on the subject. Carbohydrates in microalgae can be degraded to sugars, which are subsequently fermented by yeast to produce bioethanol. After the distillation process, the ultimate product, ethanol, is obtained. Microalgae offer several unique features that are favorable to bioethanol production when compared to woody biomass [27].

2.13.3 CO_2 fixation

The release of CO_2 into the atmosphere because of the burning of fossil fuels is a severe environmental concern. Thus, to sequester CO_2, many strategies (such as filtration or additional mechanical or chemical procedures) have been established. However, biological methods are being explored as a viable option for reducing CO_2 emissions. Photosynthetically, algae are capable of fixing carbon dioxide. The usage of microalgae to absorb carbon dioxide from power plant exhaust gas appears to be an effective technique for avoiding GHG's emissions into the atmosphere. If microalgae are employed for biofuel production, carbon dioxide emissions from power plants might be offset by CO_2 fixation via the photosynthetic process, resulting in a net CO_2 release of zero [8]. Beside carbon dioxide capture microalgae can also be used for many other purposes, as shown in Figure 2.3 [11].

2.13.4 Hydrogen gas

Biohydrogen production has gotten a lot of interest as a result of the need for alternative energy sources. Despite numerous efforts, large-scale biohydrogen production has failed due to high production costs, the usage of particular machinery, and low biomass fixation. Many studies have reported producing biodiesel by exposing particular green microalgal species to several stress conditions, e.g., light deprivation, can result in an evident yield of hydrogen gas. There are three basic paths for hydrogen production. Indirect photolysis, ATP-driven photolysis and direct photolysis are the three types of photolysis. Algae convert water into hydrogen and oxygen through photosynthetic ability or direct photolysis [33].

2.13.5 Wastewater treatment

Algae culture in the wastewater is a hybrid technique to wastewater treatment, carbon dioxide utilization, and biomass production for bioenergy and biofuels. Wastewater treatment is a multi-step method that refines wastewater before it is discharged into a natural water body. Through algae culture, the goal of treatment is to eradicate or at least optimize the decrease of nutrients, organic matter, sediments, and other contaminants. There is currently a trend in Pakistan to discharge unprocessed wastewater (from municipal, industrial and agricultural sources) directly into surrounding water bodies, substantially altering their chemical, physical, and biological characteristics [34].

Microalgae is a good competitor for biofuel production since it can thrive in a variety of synthetic environments as well as many types of wastewaters. Microalgae cultivation can likewise be utilized to treat wastewater. Past exploration has shown that microalgae use supplements from wastewater to harvest biomass, which may then be used to make an assortment of helpful items such ethanol, biodiesel, methanol, hydrogen, manure, biomolecules, and carotenoids. As a result, using microalgae to treat wastewater is a zero-waste, ecologically beneficial, and cost-efficient technique, as valuable by-products might possibly compensate the cost [35].

Many algal species may effectively grow in wastewater due to their capacity to consume copious inorganic phosphate and nitrogen. Microalgae, specifically, are incredibly compelling at eliminating these supplements from sewage-based wastewater, either in suspension or immobilized structure. [36].

2.13.6 Phycoremediation

Contamination of the environment is one of the most pressing issues facing the entire planet. Heavy metals (e.g., Ni, Cd, Cu, As, Hg, and Pb) are significant polluters of soil and aquatic ecosystems since they are non-degradable in nature. Although a variety of techniques have been used to eliminate harmful metals from polluted sites, more effective and environmentally friendly ways are still needed. Phycoremediation, or the use of algae species to remove heavy metals and other toxins such as dyes, nutrients, ions, and other ions from water and wastewater, has been proven to be eco-friendly, ecologically sound, and a value-added technology [37].

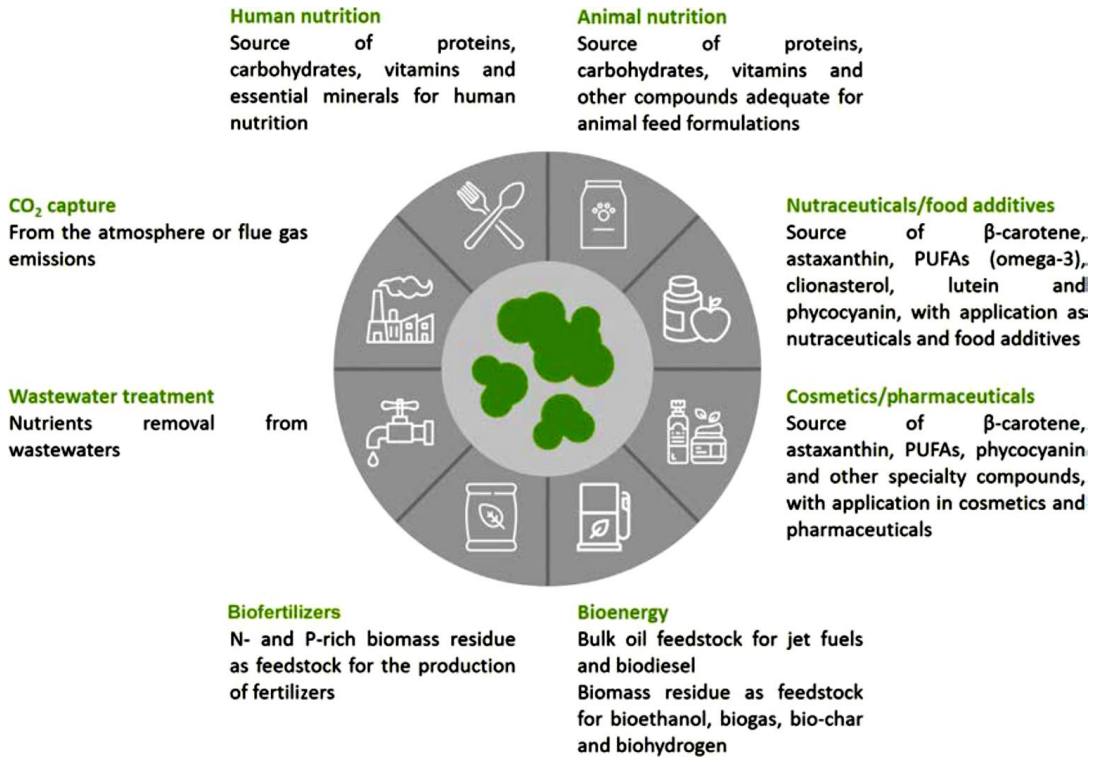

Figure 2.3 Microalgal uses and its applications in different fields[11].

2.13.7 Alka(e)nes

The main components of diesel, gasoline, and jet fuel are alka(e)nes. A diverse spectrum of bacteria can create them naturally. “Bio-alka(e)nes” can be utilized as a replacement for conventional biofuels. To date, five microbial processes have been found or reconstituted that convert “fatty acids or fatty acid derivatives into alka(e)nes.” The findings pave the way for high-efficiency microbial production of alka(e)nes. Synthetic biology technologies might be used to enhance metabolic routes and enhance alka(e)ne synthesis by assembling modules generated from these alka(e)ne “biosynthetic pathways” as biological pieces. The odd number n-alkanes,

often at C17, C15 or C21, dominated the alka(e)ne outline of algae. *Botryococcus braunii* is a green microalga with a high-level concentration of alka(e)nes [38].

2.13.8 Alkenes Production

Light alkenes (e.g., propene, ethene, and butenes) were formed by "thermal catalytic cracking" of microalgal species, which can aid in as inexhaustible stage molecules for a perhaps more practical substance producing industry. Catalytic production of fuels and chemicals, as well as light olefins, from whole microalgal cells (rather than lipids extraction) has recently received attention to avoid existing energy-demanding and expensive recovery and lipid extraction, though cost-efficacious cell mass drying stays a task. The synthesis of light olefins was discovered by Dong and his team, they used: "modified ZSM-5 catalyst and a catalyst-to-cell mass ratio of 20:1 to thermochemically treat microalgae cells at temperatures of 500–700 °C" [39]

2.13.9 High value-added products

2.13.9.1 Fatty acids

The enzymes required to produce "Polyunsaturated fatty acids with more than 18 carbons" are absent in higher plants and animals. As a result, they must obtain them from their diet. "Long-chain PUFAs" are commonly obtained from fish and fish oil, although safety concerns have been increased due to the potential buildup of toxins in fish. Furthermore, the use of fish oil as a food additive is restricted due to issues such as its characteristic fishy odor, bad taste, as well as low oxidative stability. Fish oil is not suited for various purposes due to the presence of mixed fatty acids. Microalgae should also be considered a possible source of PUFAs because polyunsaturated fatty acids have been observed in fish due to the microalgae found in marine habitat. [40].

2.13.9.2 Phenols

Algae's antioxidant and antibacterial capabilities are due to the phenolics they contain. Chloroform, hexane, ethanol, methanol, and water were used sequentially in the extractions. The levels of phenolics identified in different species using these different solvents varied [41].

2.13.9.3 Proteins and amino acids

The high protein content of microalgae biomass, such as glycoprotein from *Chlorella vulgaris*, phycoerthrin from *P. cruentum*, and C-phycocyanin from *S. platensis* is well known. Microalgae like *C. minutissima, Chlorella sorokiniana, C. luteoviridis, C.*

sphaerica, *Scenedesmus* species, and *Stichococcus* species and *Aphanizomenon flos-aquae*, generate mycosporine (e.g., amino acids), which are the strongest UV absorbing chemicals in nature. Phytases, protease, galactosidase, laccases, lipases, amylolytic enzymes, cellulases, carbonic anhydrase and antioxidant enzymes are just a few of the enzymes that microalgae are able to produce [42].

Summary

Climate change caused by global warming is endangering humanity's existence and raising doubts about its long-term survival. The implementation of numerous technologies for CCS has become critical in this chapter as a sustainable ways for reducing carbon dioxide amount in the atmosphere as well as at CO_2 emission point sources. Biological Carbon Capture by microalgal cell factories have emerged as an essential carbon dioxide sequestration platform among many CCS techniques. It also looks at the impact of main physicochemical parameters such microalgal strains, light, pH, inoculum size, temperature, carbon dioxide concentration, mass transfer, and flow and mixing in CO_2 fixation. It may be used to not only capture CO_2, but also to produce bio-fuels and other valuable products from biomass, all of which contribute to the establishment of a sustainable ecosystem.

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Chapter 3

Materials and Methods

3.1 Microalgal cultures

The *Dictyosphaerium sp. Strain DHM1* (NCBI: txid1860073) and *Dictyosphaerium sp. Strain DHM2* (NCBI: txid1860074) has been collected from the microalgae stock cultures of the Atta-ur-Rahman School of Applied Biosciences (ASAB), school of National University of Sciences and Technology (NUST) H-12, Islamabad. Both species belong to the phylum Chlorophyta (green algae). Stock Cultures were maintained in a shaking incubator (figure 3.1), with an agitation speed of 120 rpm at 26˚C, under continuous illumination.

Figure 3.1 Stock Algal Cultures in shaking incubator.

3.2 Bold's Basal Medium (BBM)

"Bold's Basal Medium (BBM)" was used as a growth medium. The chemicals used in this experiment were of analytical grade from Sigma Aldrich and Merck. The chemical composition of the media is presented in the table 3.1. The media was autoclaved at: "temperature of 121˚C for 15 minutes and the final pH was 6.4-6.6"

Table 3.1 BBM media chemical composition.

S. no.	Chemical Name	Chemical formula	Weight (g/L)
1.	Sodium nitrate	$NaNO_3$	5
2.	Calcium chloride	$CaCl_2.2H_2O$	0.5
3.	Magnesium sulphate	$MgSO_4.7H_2O$	1.5
4.	di-Potassium hydrogen, orthophosphate	K_2HPO_4	1.5
5.	Potassium di-hydrogen, orthophosphate	KH_2PO_4	3.5
6.	Sodium chloride	NaCl	0.5
7.	Ethylenediamine tetra acetic acid	EDTA	1
8.	Ferrous sulfate	$FeSO_4.7H_2O$	0.0996
9.	Boric acid	H_3BO_3	0.2284
10.	Zinc sulfate	$ZnSO_4.\ 7H_2O$	0.1764
11.	Maganese chloride	$MnCl_2.4H_2O$	0.0288
12.	Molybdneum oxide	MoO_3	0.0142
13.	Copper sulphate	$CuSO_4.5H_2O$	0.0324
14.	Cobalt nitrate	$Co(NO_3)_2.6H_2O$	0.0098
15.	Potassium hydroxide	KOH	Pellets
16.	Sulphuric acid	H_2SO_4	1.84 (mL)

3.3 Algal Cultivation

In a sterile glass tube, a pure colony of microalgae was used to inoculate 10mL of liquid BBM. The culture volume was raised up to 100mL and subsequently to 250mL when the cell number increased. Finally, sterile cultures were grown in Pyrex glass bottles of 1 L and 2 L capacity. Each culture bottle contained 20-40mL inoculum, 50-100mL BBM, and the total volume was made upto 1L with distilled water. At a temperature of 22-23°C, culture bottles were kept under direct white fluorescent light. Aquarium air pumps (AP-35) were employed to maintain a constant 18.5 psi of aeration, figure 3.2.

Figure 3.2 Algal Cultures in laminar flow.

3.4 Experimental set-up

Two types of photobioreactors were used in this experiment. The schematic diagram is demonstrated in figure 3.4. Initially, all the reactors were seeded with a suspension that have an inoculum density of 0.1 g L^{-1} in dry weight. “Three different carbon dioxide concentrations were used CO_2 v/v i.e., 0.04%, 2%, 4% at an average flow rate of 50 ml min^{-1}.” Each strain was cultivated for 15 days, and the experiments were carried out in duplicates figure 3.3.

3.4.1 Lab-scale Photobioreactor

It consisted of Pyrex reagent glass bottles having a capacity of 500 ml and working volume was 450 ml. The bottle diameter × height was 81 mm × 175 mm. Culture bottles were kept under white light in a laminar flow chamber at 24˚-32˚C, as same as the lab temperature, with a 24-hour light cycle.

3.4.2 Multi-Cultivator

Multi-Cultivator MC 1000-OD was used, which contains 8 cultivation tubes of 100 ml with a working volume of 70 ml. Each tube was 3 cm in diameter and 20 cm in length. All the tubes were immersed in a "temperature-controlled water bath at 26˚±0.5˚C" Continuous illumination was provided via a Light Emitting Diode (LED) panel behind the cultivation tubes. With the help of ODView software, growth was continuously monitored by measuring "Optical density (OD) at two wavelengths of 680 nm and 720 nm." To maintain a sterile gas stream 0.2 µm filter was used. The light was adjusted to 60 µmol (photon) $m^{-2}s^{-1}$ having a static light regime throughout the experiment.

Figure 3.3 Algal cultivation in Multi-Cultivator (a) and PBR (b) for Growth kinetics study.

3.5 Analytical Methods

3.5.1 Optical Density (OD)

The OD of microalgae cultures in batch PBR were measured manually at regular intervals of time (24 h) by taking absorbance at 680 nm and 720 nm with the help of a spectrophotometer (T92+ Spectrophotometer). While Optical density was periodically measured at selected time intervals in Multi-Cultivator MC 1000-OD at two wavelengths of 680 nm and 720 nm after every 24 hours.

3.5.2 Dry biomass

Microalgal dry biomass was obtained by centrifugation of the 10 ml sample which was further washed twice with distilled water at 4000 rpm for 10 minutes and was placed in the oven at 105˚C for 16 h. The correlation between the dry biomass and optical density weight was determined by linear regression analysis [1].

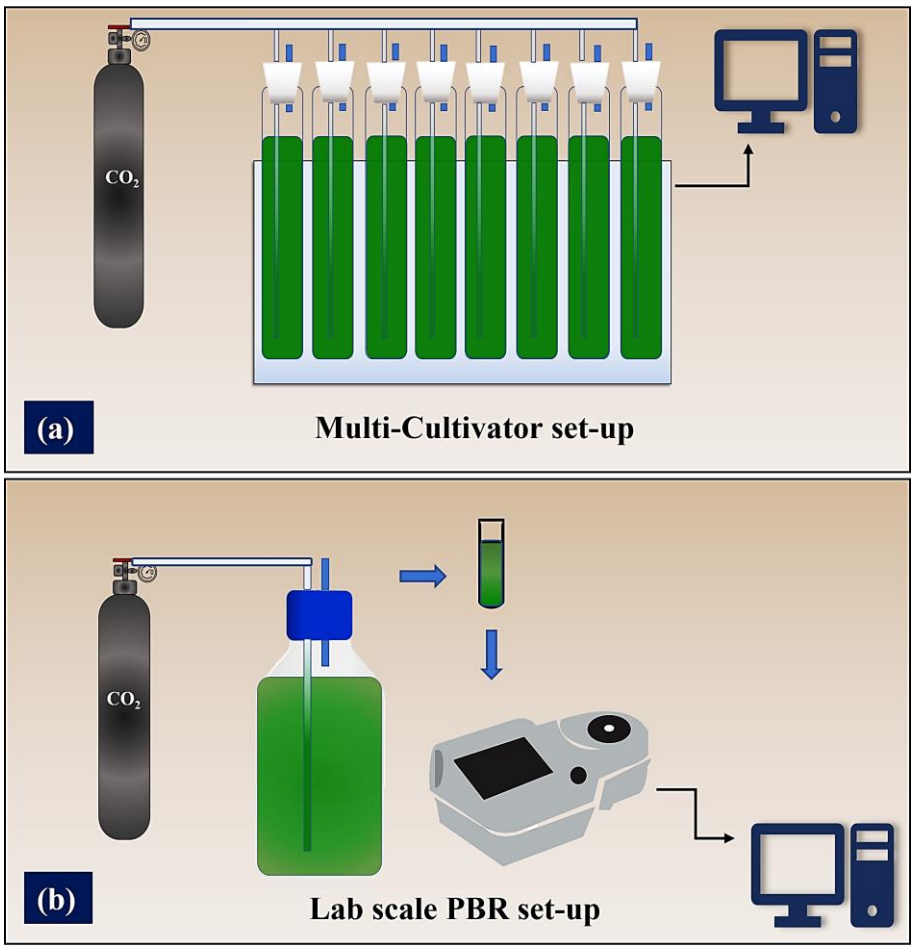

Figure 3.4 Schematic diagram of *Dictyosphaerium sp.* in (a) Multi-Cultivator and (b) Lab scale Batch Photobioreactor (PBR).

3.6 Growth Kinetic Parameters

For the growth kinetics assessment, dry biomass values (g L^{-1}) were used. The specific growth rate and cell doubling time were determined using the method [2]. The "specific growth rate (μ, day^{-1})" was determined by applying Eq. 1

$$\mu = \ln X_2 - \ln X_1 / t_2 - t_1 \ldots\ldots\ldots (1)$$

In equation 1 "X_2" and "X_1" represent the dry biomass weight (g L^{-1}) at time "t_2" and "t_1", respectively. The maximum biomass obtained was designated as X_{max} (g L^{-1}). Equation 2 was used to calculate the Cell doubling time (t_d, day)

$$t_d(d) = \ln 2 / \mu_{max} \text{ …….. (2)}$$

while productivity (P_{max}, g $L^{-1}d^{-1}$) by [3], was calculated using Eq. 3

$$P_{max} = (X_t - X_o) / (t_t - t_o) \text{ …….. (3)}$$

where, P_{max} (g $L^{-1}d^{-1}$) is the maximal productivity, and "X_t" is the biomass concentration and "X_o" is the initial biomass concentration at time "t" and at inoculation time respectively.

3.7 Carbon dioxide Fixation by microalgae

CO_2 bio-fixation rates (g d^{-1}) [4] were calculated using equation 4.

$$R_c = Cc \times P \times (MCO_2/MC) \times V \text{ …….. (4)}$$

where, "Cc is the mass fraction of carbon in dry microalgae biomass (%w/w)", which was determined using elemental analyzer, "MCO_2 is 44 g mol^{-1}", "MC is 12 g mol^{-1}", "P is Biomass productivity (g $L^{-1}d^{-1}$)" and "V is working volume"

3.8 Harvesting

Every 15 days, cultures were taken from the growth rack and laid aside to allow biomass to settle gravitationally. The upper clear supernatant was decanted after a day, and the thick liquid culture at the bottom was centrifuged at 6000 rpm to recover biomass. The supernatant carrying salt media was removed after each cycle. Distilled water was used to wash the recovered biomass in the form of pellets twice. The harvested biomass was placed in sterile petri plates and dried for 24 hours at 45-55 degrees Celsius in a hot air oven, Figure 3.5. Dry biomass was collected and ground

into a fine powder in a sterile pestle mortar. The algal biomass was then preserved in glass vials at -20°C for future investigation.

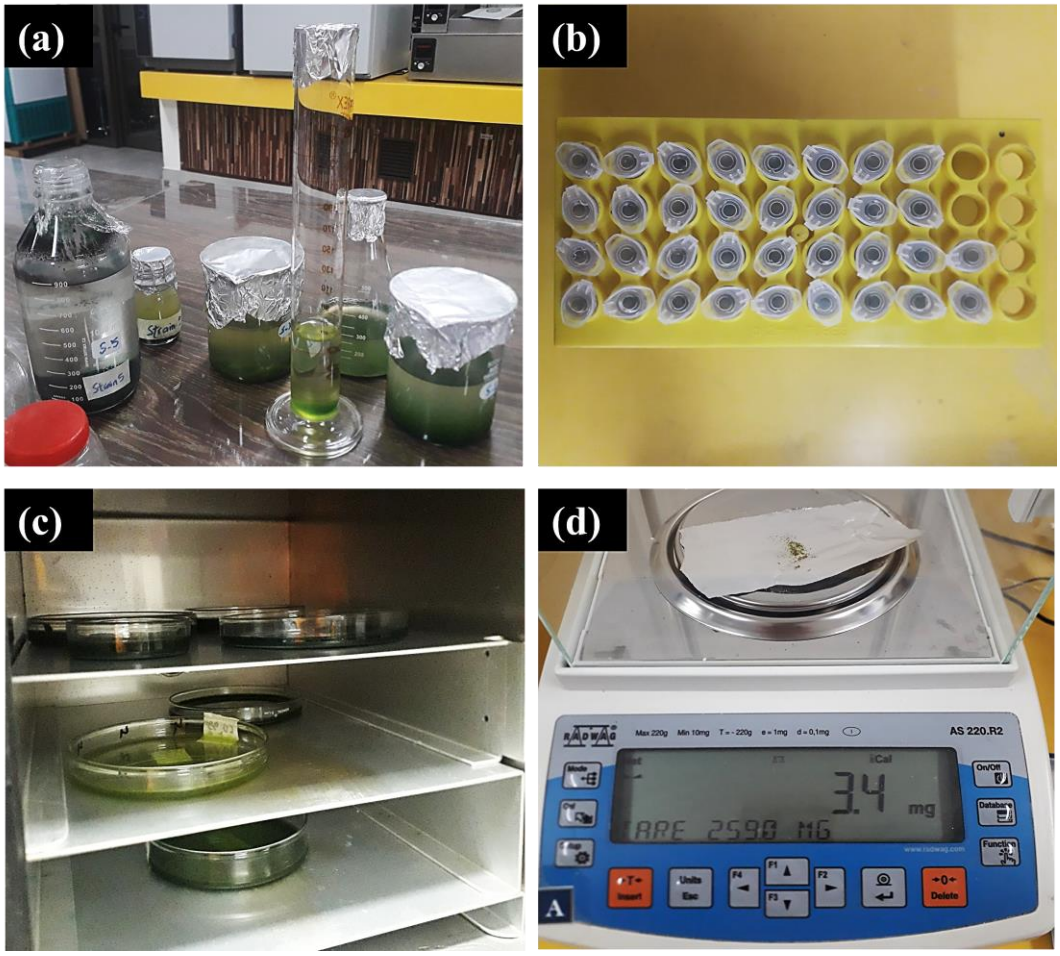

Figure 3.5 Sedimentation of algal cultures (a), washing and centrifugation (b), drying in oven (c), dry biomass for weight calculation (d).

3.9 Biomass Characterization

3.9.1 The morphological study by Scanning electron microscopy (SEM)

For morphological analysis, Microalgal samples were washed by physiological medium to remove any mucilage or any other contamination. “2.5% glutaraldehyde in 0.1M phosphate buffer” was used to fix the samples having pH 7.2-7.4 for 24-48h. Algal samples were washed further with “0.1M phosphate buffer” 4 times for 15 min. and then rinsed with distilled water 3 times each for 5 min. Dehydrated with increasing concentration of ethanol (i.e.) 50%, 70%, 80%, 90%, 95% and 100% for 20 minutes figure 3.6 (a). Following that, these samples were placed in a Critical point drying CPD, and after drying, they were mounted on a stub over carbon foil figure 3.6 (b) and sputter-coated with gold figure 3.6 (c and d) for improved and better visibility, for SEM examination figure 3.6 (e) (Oxford Instruments, VEGA3 TESCAN).

3.9.2 Fourier-transform infrared spectroscopy FTIR analysis

The Cary 630 FTIR Spectrometer, Agilent Technologies, USA, was used to analyze dried algal biomass. The dried algal powder was mixed with potassium bromide (KBr) powder and formed into tablets before being analyzed in the 4000–650 cm^{-1} wavelength range [5].

3.9.3 Elemental analysis through CHN elemental analyzer

Dry pulverized microalgal biomass powder was used for elemental analysis of carbon, hydrogen, and nitrogen content in the biomass. Nitrogen, Helium, and Oxygen were used as carrier gases and 80 mg samples of dry biomass were used. The conditions are: "furnace temperature = 950˚C, whereas the oven temperature = 50˚C"

3.9.4 Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray (EDX) Analysis

The "EDX" was used to determine the elemental composition of dry algal biomass. The samples were attached to the EDX sample holder and carefully placed in the analyzing chamber, where the machine controlled the pressure. To determine the true elemental composition using EDX, samples were not carbon coated. The machine determined the atomic and weight percentage of the dry algal biomass elements.

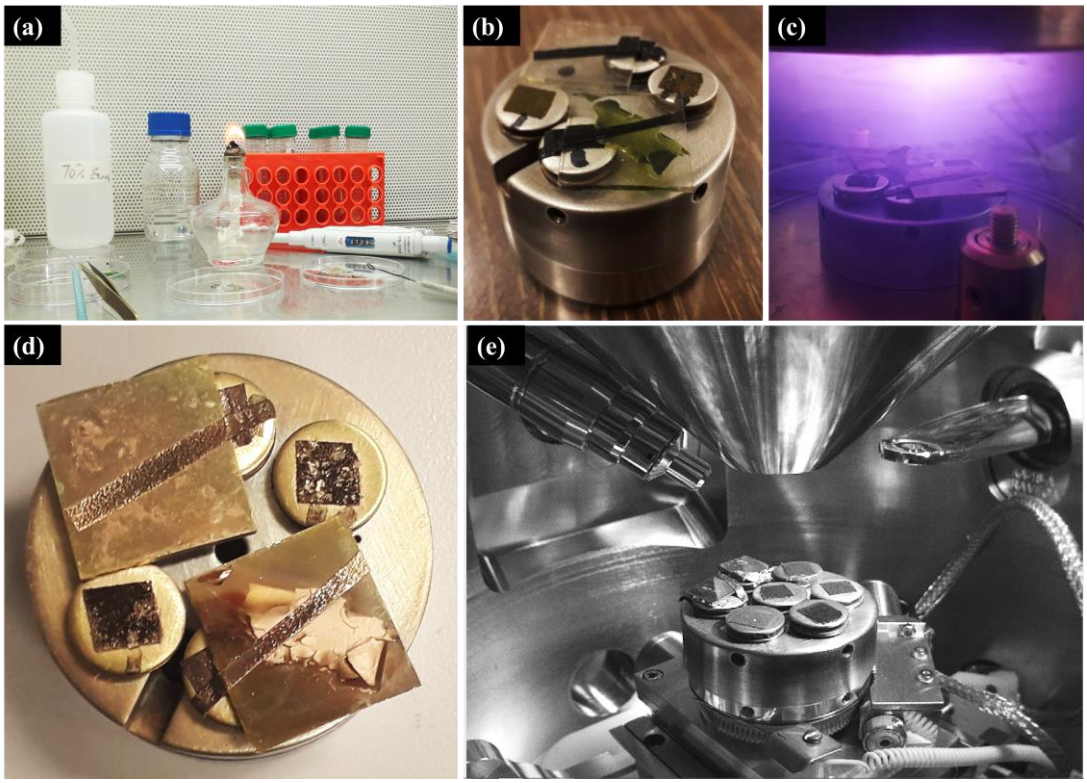

Figure 3.6 Microalgal step-by-step sample preparation for SEM examination.

3.9.5 Gas chromatography-mass spectrometry (GC-MS) analysis

Using a mortar and pestle, the dried algae were pulverized. The extract was made by mixing 1 g of powdered algae with 10 ml of methanol. After that, the mixture was incubated at 32˚C overnight on an orbital shaker. A funnel and No. 1 Whatmann filter paper were used to filter the extract. In a 100 ml conical flask, the extract was collected. To get the crude extract of the algae, the collected extract was dried using a rotating vacuum evaporator to remove the solvent. The extracted substance was examined using a Shimadzu GCMS-QP2020. "The column utilized was a Shimadzu SH-Rxi-5Sil MS Capillary Column having length 30 m, internal diameter 0.25 mm and film thickness 0.25 µm. During the run, the injector temperature was kept at 260˚C. One µL of sample extract was injected into the GC-MS instrument, using the following oven temperature settings: 60˚C for 2 minutes, then 300˚C at a rate of 10˚C per minute; and held for 6 minutes at that temperature. The mass detector method included a 240˚C transfer line temperature, a 240˚C ion source temperature, and a 70-eV electron ionization impact. The scanning interval was set to 0.1 seconds, and the scan time was set to 0.2 seconds, with a scan range of 50 to 600 Daltons". The GC-MS spectra were compared to the standard library database of the "National Institute of Standards and Technology (NIST)" [6].

Summary

This chapter discloses CO_2 fixation through green approach using novel microalgal species (*Dictyosphaerium sp.* strain *DHM1* & strain *DHM2*). Both microalgal strains were supplied with 0.04 %, 2%, and 4% CO_2 concentration in two different sorts of photobioreactors (Lab-scale photobioreactor and Multi-Cultivator). Their biomass characterization analysis is carried out using "Fourier-transform infrared spectroscopy (FTIR)", "Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray Analysis (EDX)", and "Gas chromatography-mass spectrometry (GC-MS)".

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Chapter 4

Results and Discussion

4.1 Morphological analysis

Dictyosphaerium is found in a wide range of freshwater environments and is likely global. Green water blooms can occur in eutrophic reservoirs and fishponds. Colonies break off at the end of the growth season, creating individual cells. Free-floating, spherical to irregular colonies with spherical to oval to ellipsoid to cylindrical cells, 1-10 m in diameter or length, figure 4.1. Mother cells divide in two planes that are

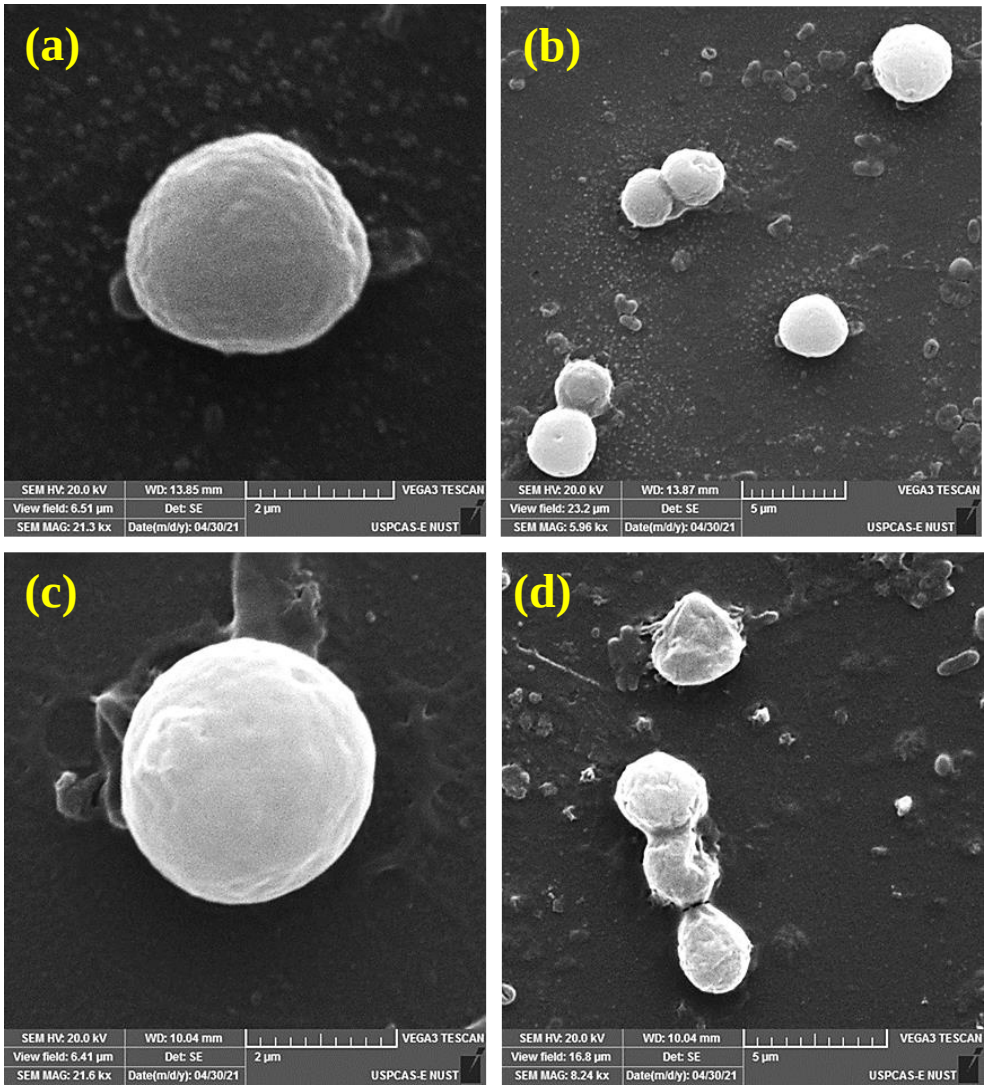

Figure 4.1 Morphological structure of microalgal cells a: *Dictyosphaerium DHM1* (2 µm), b: *Dictyosphaerium DHM1* (5 µm) and c: *Dictyosphaerium DHM2* (2 µm), d: *Dictyosphaerium DHM2* (5 µm).

perpendicular to each other. Spores are released once the parental cell ruptures and attach to the cell wall remnants, which grow into mucilaginous stalks [1].

4.2 Linear Regression

All experimental values of R-square are above 0.98 representing linear data, in table 4.1.

Table 4.1 Linear Regression.

PBR type	**Strain**	**CO_2 conc.**	**Linear regression equation**	**Coefficient of determination**
MC-OD	*DHM1*	0.04%	Y = 0.4862x + 0.0013	R^2 = 0.9994
MC-OD	*DHM1*	2%	Y = 0.8846x – 0.0252	R^2 = 0.9998
MC-OD	*DHM1*	4%	Y = 0.889x – 0.0342	R^2 = 0.9997
PBR	*DHM1*	0.04%	Y = 0.4712x + 0.006	R^2 = 0.9969
PBR	*DHM1*	2%	Y = 0.3614x + 0.0067	R^2 = 0.985
PBR	*DHM1*	4%	Y = 0.5053x – 0.0101	R^2 = 0.9999
MC-OD	*DHM2*	0.04%	Y = 0.5092x – 0.0618	R^2 = 0.9937
MC-OD	*DHM2*	2%	Y = 0.7608x + 0.0266	R^2 = 0.997
MC-OD	*DHM2*	4%	Y = 0.8066x – 0.0046	R^2 = 0.9999
PBR	*DHM2*	0.04%	Y = 0.5265x – 0.0172	R^2 = 0.9953
PBR	*DHM2*	2%	Y = 0.4977x – 0.0064	R^2 = 0.9997
PBR	*DHM2*	4%	Y = 0.5498x – 0.0193	R^2 = 0.9998

4.3 Effect of carbon dioxide on growth rate of microalgal species

Photosynthetic microalgae growth depends upon various factors e.g., Temperature, pH, nutrients availability, the concentration of carbon dioxide, light, and proper aeration. Extreme high and low values of any of these factors cause a decline in algal growth [2]. Two novel species of microalgae have been tested under different Carbon dioxide concentrations i.e., air (0.04%), 2%, 4%. The highest μ_{max} in *DHM1* and *DHM2* was observed under 4% carbon dioxide concentration in Multi-cultivator i.e., 1.05779 (d^{-1}) and 0.916291 (d^{-1}) respectively. And in lab-scale PBR their values were 0.328504 (d^{-1}) and 0.548873 (d^{-1}) for *DHM1* and *DHM2* respectively. While overall lowest μ_{max} was observed under normal air concentration of carbon dioxide in lab-scale PBR for both species. Cell doubling time was extremely noticeable in both

strains. The maximum time which was required to double their cell numbers is 87.27 hours or 3.63 days in PBR under uncontrolled laboratory temperature and 0.04% concentration of CO_2 which is the normal concentration of CO_2 in the air and its value is 400 ppm. The minimum time required was 15.7 hours to double its number under 4% concentration of carbon dioxide in a Multi-cultivator. For *DHM1* the biomass productivity (P_{max}) and dry cell weight (DCW) in g L^{-1} were also highest and clearly observed under 4% CO_2 concentration, in Multi-Cultivator their values were 0.2093 g L^{-1} d^{-1} for P_{max} and DCW was 2.802 g L^{-1} as demonstrated in figure 4.2 (a) for biomass in (g L^{-1}). And for *DHM2* the highest values of biomass productivity and overall dry cell weight (DCW) were 0.164 g $L^{-1}d^{-1}$ and 2.4 g L^{-1} respectively in Multi-cultivator under 4% CO_2 concentration. Figure 4.2 shows, increase in carbon dioxide concentration causes increase in biomass g L^{-1} production. The most of the studies have found that raising CO_2 concentrations in microalgae cultures increases biomass productivity [3], [4]. At 0.04% (air) concentration of carbon dioxide the *Dictyosphaerium DHM1* and *Dictyosphaerium DHM2* have shown better specific growth rate (d^{-1}) and biomass productivity (g L^{-1} d^{-1}) in Multi- cultivator as compared to the species i.e. "*C. vulgaris, S. obliquus", "Psammothidium sp.", "M. contortum"* when grown in "vertical column photobioreactor" [5].

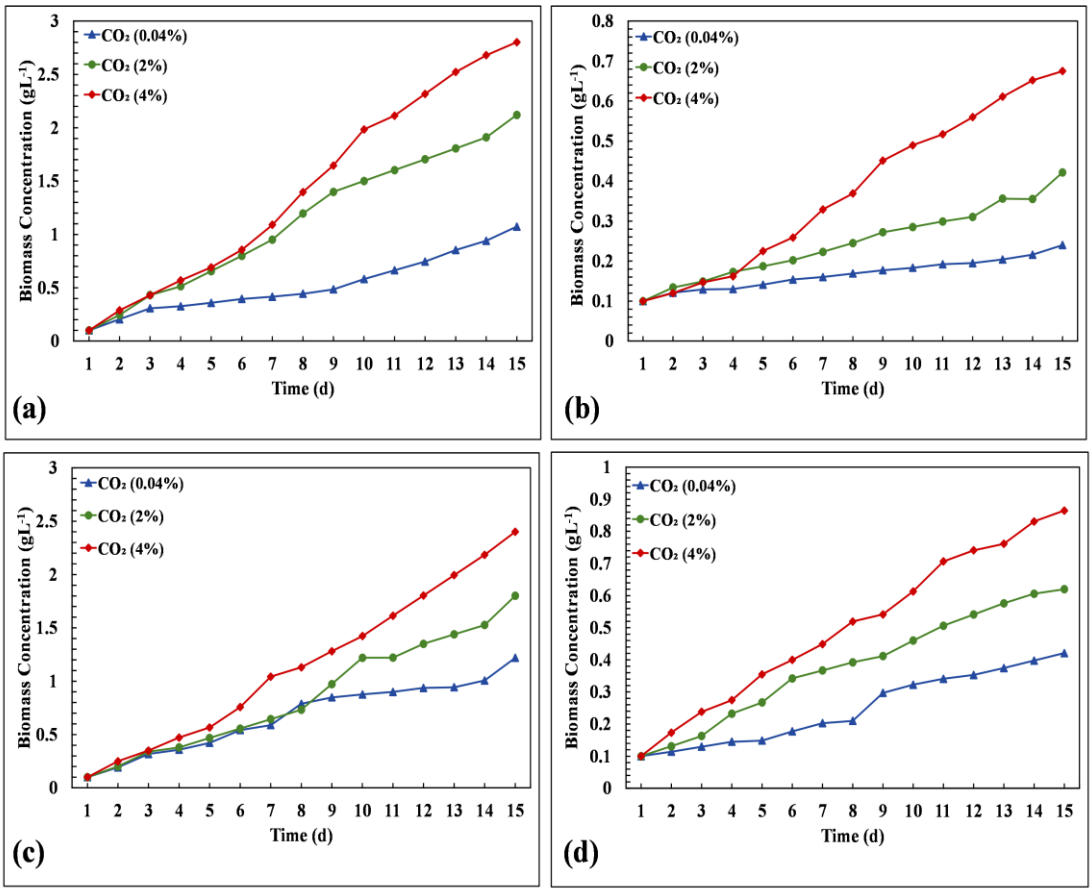

Figure 4.2 Biomass Concentration (DCW g L^{-1}) (a) *Dictyosphaerium DHM1* MC-OD, (b) *Dictyosphaerium DHM1* PBR, (c) *Dictyosphaerium DHM2* MC-OD and (d) *Dictyosphaerium DHM2*.

Table 4.2 Mean values of Growth kinetics and CO_2 fixation rate.

Strain	**Bioreactor type**	**CO_2 Conc.**	**Specific Growth rate μ_{max} (d^{-1})**	**Cell doubling time (d)**	**Max. productivity P_{max} ($g\ L^{-1}\ d^{-1}$)**	**CO_2 Fixation rate (max) ($g\ d^{-1}$)**	**X_{max} (gL^{-1})**
DHM 1							
	MC-OD	0.04%	0.717	0.965	0.105	0.106	1.073
	MC-OD	2%	0.896	0.773	0.165	0.167	2.12
	MC-OD	4%	1.057	0.655	0.209	0.211	2.802
	PBR	0.04%	0.190	3.636	0.021	0.030	0.24
	PBR	2%	0.292	2.368	0.034	0.049	0.421
	PBR	4%	0.328	2.110	0.044	0.063	0.675
DHM 2							
	MC-OD	0.04%	0.652	1.062	0.109	0.093	1.219
	MC-OD	2%	0.698	0.992	0.124	0.106	1.801
	MC-OD	4%	0.916	0.756	0.164	0.141	2.4
	PBR	0.04%	0.347	1.997	0.025	0.030	0.421
	PBR	2%	0.356	1.945	0.048	0.059	0.62
	PBR	4%	0.548	1.262	0.073	0.089	0.865

4.4 Comparison between Multi cultivator and Lab-scale PBR

Biomass productivity is one of the most crucial parameters affecting carbon dioxide bio-capture rate because it tells us about the growth of microalgae [5]. The geometry and structure of photobioreactors are important for the growth of these small eukaryotic organisms. A noticeable difference was observed when the same *DHM1* strain was grown at the same time, under the same carbon dioxide levels (0.04%, 2%, 4%) in Multi-Cultivator and Lab-scale PBR. The values for *DHM1* and *DHM2* were mentioned in Table 4.2.

The higher efficiency in Multi-Cultivator is due to its unique design and various features, i.e., temperature-controlled bath unit, the 8 cultivation tubes were immersed in the water bath and the temperature was maintained at 26˚C. While in Lab-scale PBR no such temperature-controlled unit was present, the strains grown in PBR was at lab temperature, as the lab temperature fluctuates from 26˚C to 33˚C it affected the growth of microalgae. The other possible reason for the higher efficiency in Multi-Cultivator is that its lower volume and diameter of cultivation tubes as compared to the PBR [5], [6]. Due to this the light penetration is better in Multi-Cultivator as well as mixing of cells plus gas-liquid transfer is efficient too. In Lab-scale PBR due to higher volume, the light penetration to all the cells became difficult, and thus not all the cells get the equal number of light. Because of light absorption and dispersion, light intensity at the depth of dense algal solution is considerably diminished. The wavelength, cell concentration, light penetration distance, and photobioreactor geometry all influence light intensity attenuation [7]. Increasing photons causes a decrease in the growth rate of microalgae, this effect is known as photoinhibition [8]. Figure 4.3 (a) and (b) shows specific growth rate for both the strains in Multi-cultivator as well as in PBR. This study shows that the species used in this experiment is highly affected by light. Equal number of light to all microalgal cells are very important for its growth and better performance.

4.5 Carbon dioxide fixation rate

The results show that the Multi-cultivator has a greater CO_2 bio-fixation rate, which is mostly owing to a faster growth rate, as demonstrated in figure 4.3 (c) and (d) for *DHM1* and *DHM2* respectively. Additionally, because the mixing conditions of Multi-Cultivator encouraged greater CO_2 removal by limiting growth on the walls of culture tubes and due to the smaller diameter than PBR. CO_2 fixation increases with the

increasing concentrations of carbon dioxide levels. And thus, the highest CO_2 fixation is observed when 4% carbon dioxide is supplied and the lowest is observed when

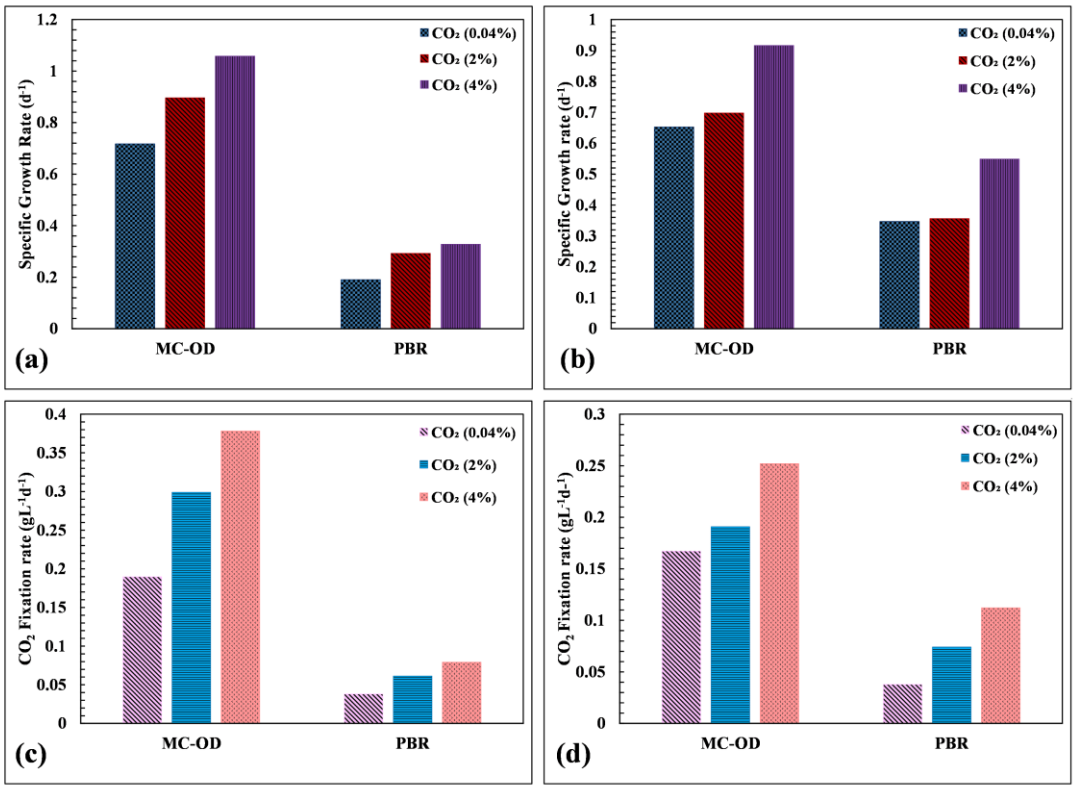

Figure 4.3 Specific Growth rate (d^{-1}) (a) *Dictyosphaerium DHM1*, (b) *Dictyosphaerium DHM2*, CO_2 fixation rate (c) *Dictyosphaerium DHM1* and (d) *Dictyosphaerium DHM2*.

0.04% carbon dioxide is supplied. When carbon dioxide is supplied as same as present in the air the *Dictyosphaerium DHM1* and *Dictyosphaerium DHM2* have shown better carbon dioxide fixation results when compared to the study performed by [9] the value was 0.075 (g L^{-1} d^{-1}) for "*C. vulgaris"*, while in our study the *Dictyosphaerium DHM1* and *Dictyosphaerium DHM2* values are 0.106 (g d^{-1}) and 0.093 (g d^{-1}) respectively for Multi-Cultivator, as represented in Table 4.2. *Dictyosphaerium DHM1* fixed more CO_2 than *Dictyosphaerium DHM2*.

4.6 Fourier-transform infrared spectroscopy (FTIR)

The algal samples were examined using the "Fourier Transform Infrared (FTIR)" technique. FTIR spectroscopy has shown to be a useful tool for examining biological material throughout the previous decade [10]. The use of FTIR spectroscopy in algal biomass analysis has proven to be effective in defining and monitoring the chemical composition of a complex material like microalgae. The Table 4.3 reveals the presence of organic compounds. The absorption spectra revealed distinct absorption bands that

matched various bio components across the wavenumber range 4000-650 cm^{-1} [11]. Both species of *Dictyosphaerium DHM1* shown in figure 4.4 (a) and *Dictyosphaerium DHM2* figure 4.4 (b) shows bands that reveal the presence of carboxylic acids, alkanes, alkynes, allenes, amides, aromatic nitro compounds, alcohols, and ethers. The only difference is in two regions: one in the range of 885-870, which indicates the presence of aromatics in *Dictyosphaerium DHM1*, and the other in the range of 700-600, which indicates the presence of aliphatic Bromo compounds, which are exclusively found in *Dictyosphaerium DHM2*. FTIR spectroscopy appears to be a promising analytical method for determining the biofuel potential of algae since it allows for fast screening of microalgal cells. Specific macromolecular groups, such as proteins, carbohydrates, and lipids are present. Each macromolecule has its own set of functional groups i.e aldehydes, ketones, esters, ethers, alcohols and carboxylic acids which reveals that Both microalgal species can be beneficial raw materials for sustainable biofuel production.

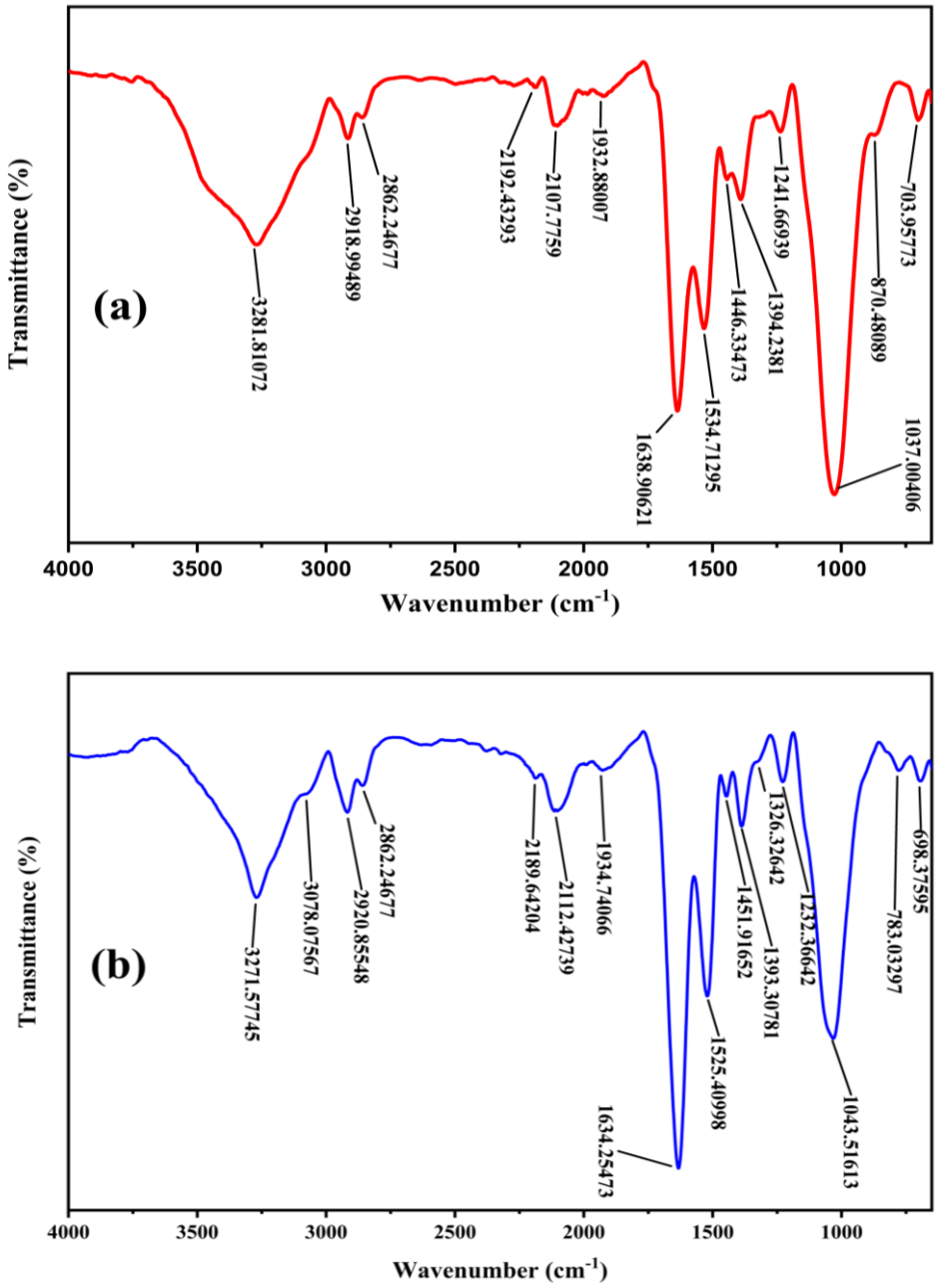

Figure 4.4 FTIR Functional groups of (a) *Dictyosphaerium DHM1* and (b) *Dictyosphaerium DHM2*.

Table 4.3 FTIR functional groups of *Dictyosphaerium DHM1* and *Dictyosphaerium DHM2*.

DHM1 **Main peaks (cm^{-1})**	***DHM2*** **Main peaks (cm^{-1})**	**Frequency range (cm^{-1})**	**Functional groups**	**Compound class**
3281	3271	3300 - 2500	O-H stretching	Carboxylic acids
	3078	3300 - 2500	O-H stretching	Carboxylic acids
2918	2920	3000 - 2850	C-H stretching	Alkanes
2862	2862	3000 - 2850	C-H stretching	Alkanes
2192	2190	2260 - 2190	C≡C stretching	Alkyne
2107	2112	2140 - 2100	C≡C stretching	Alkyne
1932	1934	2000 - 1900	C=C=C stretching	Allenes
1638	1634	1640 - 1600	N-H out of plane	Amides
1534	1525	1555 - 1485	N–O stretching	Aromatic nitro compounds
1446	1451	1470 - 1350	CH_2, CH_3 deformation	Alkanes
1394	1393	1410 - 1310	O-H bend	Phenol or tertiary alcohol
	1326	1350 - 1260	O-H in-plane bend	Primary or secondary
1241	1232	1275 - 1200	C–O stretching	Alkyl aryl ether
1037	1043	1075 - 1020	C–O stretching	Alkyl aryl ether
870		885 - 870	C–H out of plane	Aromatics
703	783	800 – 700	C-Cl stretch	Aliphatic chloro compounds
	698	700 - 600	C-Br stretch	Aliphatic bromo compounds

4.7 Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray Analysis (EDX)

"SEM" with electron stimulated X-ray microanalysis employing "Energy dispersive X-ray" is one of the most fundamental, detailed, and genuine technologies for describing the microstructure of any inorganic or organic substance [12]. The experiment produced a quantitative and qualitative elemental analysis of all major (C, N, O) and minor elements (Mg, Al, Si, P, K, Ca, S,) of dry biomass of microalgae, using SEM-EDX in a complete vacuum atmosphere. To evaluate the elemental, chemical, and physical characteristics of algal biomass, this technique is commonly used. Elemental analysis of biomass is regarded as one of the most efficient characterizations for any type of potential energy production from biomass [13]. Both the strains contain these elements mentioned in the Figure 4.5 but have different percentage compositions.

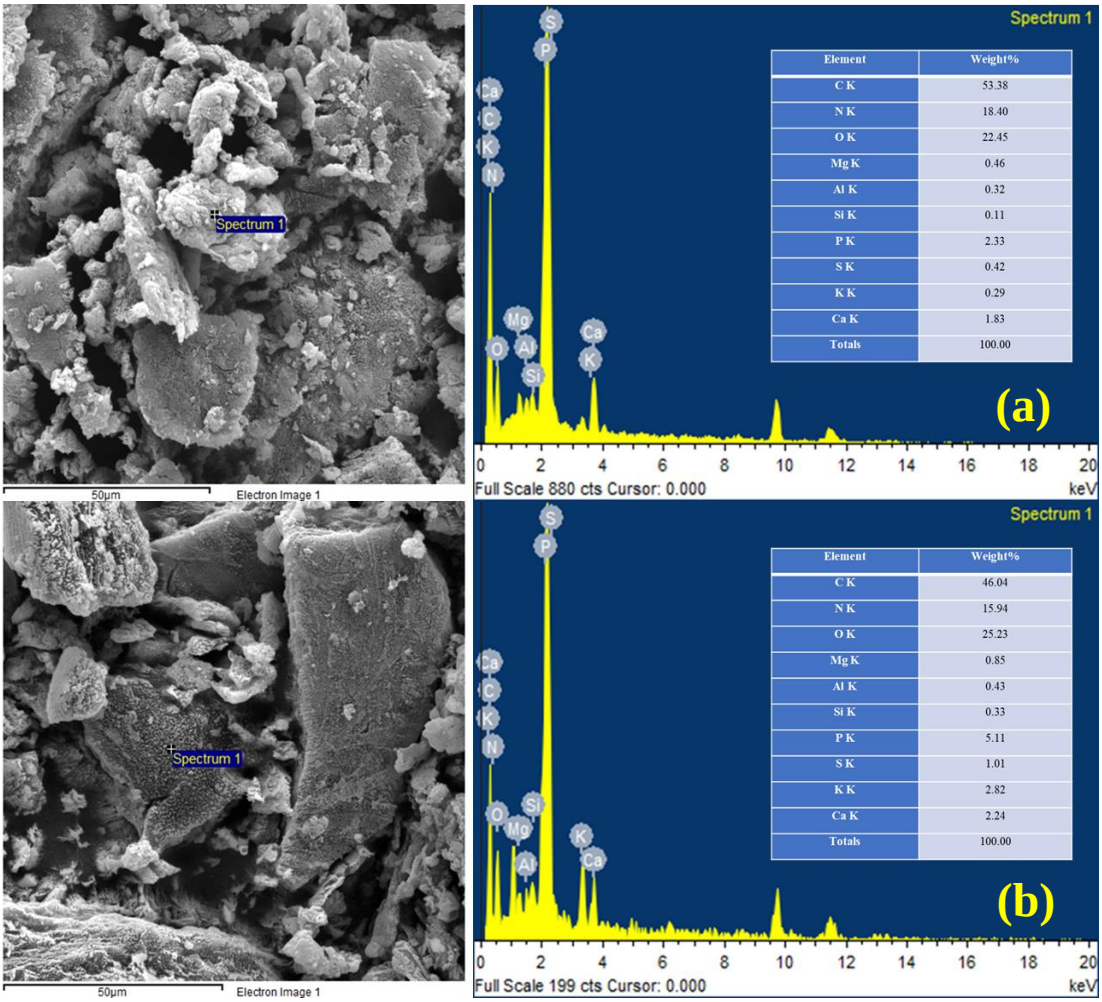

Figure 4.5 Dry Biomass and Elemental composition of (a) *Dictyosphaerium DHM1* and (b) *Dictyosphaerium DHM2*.

4.8 Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography aids in the qualitative identification of chemicals present using the mass spectrum of analytes. In the current investigation, a total of 22 peaks in *Dictyosphaerium DHM1* and 20 peaks in *Dictyosphaerium DHM2* were discovered. A list of detected chemicals is tabulated, along with their retention time, name, organic compounds class, and area percentage (%), in table 4.4 and 4.5.

In *Dictyosphaerium DHM1* 22 different compounds were revealed with their area percentage (%) are as follows; [“(2,2-Dimethoxybutane (4.04 %), Undecane (2.46 %), Carbamoselenothioic acid, dimethyl-, S-methyl ester (4.53 %), Tetracontane (7.99%), Thymol, TMS derivative (2.90 %), Eicosane (7.34 %), 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl), ester (12.52 %), Ethyl homovanillate, TMS derivative (2.52 %), Heptacosane (6.85 %), 2,6-Dihydroxyacetophenone, 2TMS derivative (2.39 %), 3-Methylsalicylic acid, 2TMS derivative (3.78 %), 2-Amino-3-cyano-4-phenyl-5-carbo-

ethoxy-6-methyl-4H-pyran (3.92 %), 1,4-Bis(trimethylsilyl)benzene (3.48 %), Thieno [2,3-c], furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl- (2.22 %), 2,4,6-Cycloheptatrien-1-one,3,5-bis-trimethylsilyl- (3.78 %), 1,2-Bis(trimethylsilyl)benzene (3.03 %), 1,2,4-Triazol-3-amine,5-(1,3,5-trimethyl-4-pyrazolyl)amino (3.82 %), 2-Nonanol, 5-ethyl- (4.65 %), 7-Hydroxy-7,8,9,10-tetramethyl-7,8-dihydrocyclohepta[d,e],naphthalene (5.69 %), Carbonic acid, monoamide, N-(2-ethylphenyl)-,propyl ester (4.65 %), Hexestrol, 2TMS derivative (4.56 %), Methyl[2-(1-methylpyrazol-4-yl)ethyl]amine (2.89 %))"] Alkanes, Esters, Phenols, Ketones, Benzoic acid and derivatives, Alcohols and Aromatic hydrocarbons are some classes of compounds that are observed in *Dictyosphaerium DHM1* strain compounds demonstrated in figure 4.6.

In *Dictyosphaerium DHM2* 20 different compounds that were identified with their area percentage (%) were; ["(2-Methoxy-3-methyl-butyric acid,methyl ester (4.858419 %), Pterin-6-carboxylic acid (3.519504 %), 2,5-Dihydroxyacetophenone, 2TMS derivative (3.761605 %), 4-tert-Butylphenol, TMS derivative (4.26576 %), Ginsenol (3.744813 %), Patchouli alcohol (3.565731 %), 4-tert-Butylphenol, TMS derivative (3.682288 %), 1,2-Bis(trimethylsilyl)benzene (3.53916 %), [1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-,ethyl ester (4.943367 %), Octadecanoic acid, 16-oxo-, methyl ester (9.120919 %), 2-Ethylacridine (5.410283 %), 1H-indene-5-carboxylic acid, 3-(4-carboxycyclohexyl)octahydro-1,1,3-trimethyl- (7.564696 %), 2'-Hydroxypropiophenone, TMS derivative (6.889855 %), 1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)- (6.999102 %), Methyl 16-acetoxyheptadecanoate (4.629553718 %), 1H-Pyrazole,1-ethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)- (4.435359206 %), Purine-2,6-dione, 8-(3-ethoxypropylamino)-1,3-dimethyl-3,9-dihydro- (5.893793579 %), Brallobarbital (5.723898075 %), Propanoic acid, 2-[(5,7-dimethyl[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)thio]-, ethyl ester (3.590524177 %), 1H-isoindole-1,3(2H)-dione, 5-(ethylthio)- (3.861369219 %))"] Alkenes, Esters, Phenols, Ketones, Alcohols and Aromatic hydrocarbons, Amino acids and Fatty acids are observed in *Dictyosphaerium DHM2* strain shown in figure 4.6.

Table 4.4 GCMS *DHM1* Methanolic extract.

Compound	**Classification**	**Retention Time**	**Area percentage (%)**
2,2-Dimethoxybutane	Alkane Hydrocarbon	2.680	4.04
Undecane	Alkane Hydrocarbon	6.245	2.46
Carbamoselenothioic acid, dimethyl-, S-methyl ester	Alkyl ester	21.490	4.53
Tetracontane	long chain n-alkane	25.240	7.99
Thymol, TMS derivative	Phenol	25.398	2.90
Eicosane	straight chain alkane	26.042	7.34
1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester	Ester	26.503	12.52
Ethyl homovanillate, TMS derivative	Ethyl ester	26.810	2.52
Heptacosane	Straight chain alkane	26.908	6.85
2,6-Dihydroxyacetophenone, 2TMS derivative	Alkyl phenyl ketones	28.270	2.39
3-Methylsalicylic acid, 2TMS derivative	Benzoic acids and derivatives	28.335	3.78
2-Amino-3-cyano-4-phenyl-5-carboethoxy-6-methyl-4H-pyran	Pyrans	28.480	3.92
1,4-Bis(trimethylsilyl)benzene	Aromatic hydrocarbon	28.605	3.48
Thieno[2,3-c] furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl-	Derivative of Furan/ derivative of thieno	28.905	2.22

2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	Ketone	28.995	3.78
1,2-Bis(trimethylsilyl)benzene	Aromatic hydrocarbon	29.250	3.03
1,2,4-Triazol-3-amine, 5-(1,3,5-trimethyl-4-pyrazolyl)amino	Triazoles	29.532	3.82
2-Nonanol, 5-ethyl-	Alcohol	29.620	4.65
7-Hydroxy-7,8,9,10-tetramethyl-7,8-dihydrocyclohepta[d,e]naphthalene	Aromatics hydrocarbon	29.726	5.69
Carbonic acid, monoamide, N-(2-ethylphenyl)-,propyl ester	Ester	29.857	4.65
Hexestrol, 2TMS derivative	Stilbenes	30.699	4.56
Methyl[2-(1-methylpyrazol-4-yl)ethyl]amine	Amine	31.155	2.89

Table 4.5 GCMS *DHM2* Methanolic extract.

Compound	**Classification**	**Retention Time**	**Area percentage (%)**
2-Methoxy-3-methyl-butyric acid,methyl ester	Fatty acid methyl esters	2.678	4.858419
Pterin-6-carboxylic acid	It derives from an alpha-amino acid.	22.825	3.519504
2,5-Dihydroxyacetophenone, 2TMS derivative	Phenol	23.085	3.761605
4-tert-Butylphenol, TMS derivative	Phenol	24.320	4.26576
Ginsenol	Alcohol	25.485	3.744813
Patchouli alcohol	Alcohol	25.570	3.565731
4-tert-Butylphenol, TMS derivative	Phenol	26.310	3.682288
1,2-Bis(trimethylsilyl)benzene	Aromatic Hydrocarbon	26.405	3.53916
[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid,4,7-dihydro-7-imino-, ethyl ester	Ester	26.455	4.943367
Octadecanoic acid, 16-oxo-, methyl ester	Fatty Acid	26.540	9.120919
2-Ethylacridine	Aromatic hydrocarbon	26.695	5.410283
1H-indene-5-carboxylic acid, 3-(4-carboxycyclohexyl), octahydro-1,1,3-trimethyl-	Alkene	26.730	7.564696
2'-Hydroxypropiophenone, TMS derivative	Ketone	26.827	6.889855
1,4-Benzenediol,2,5-bis(1,1-dimethylethyl)-	phenol derivative	26.925	6.999102

Methyl 16-acetoxyheptadecanoate	Fatty acid	27.118	4.629553718
1H-Pyrazole,1-ethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-	Derivative of pyrazole	27.410	4.435359206
Purine-2,6-dione,8-(3-ethoxypropylamino)-1,3-dimethyl-3,9-dihydro-	Amino acid	27.867	5.893793579
Brallobarbital	Barbiturates	28.820	5.723898075
Propanoic acid,2-[(5,7-dimethyl[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)thio]-,ethyl ester	Ester	29.515	3.590524177
1H-isoindole-1,3(2H)-dione, 5-(ethylthio)-	derivative of isoindole	31.285	3.861369219

Alkanes are important chemical raw materials and the primary constituent of gasoline and lubricating oils. Eicosane belongs to the higher alkane group and is used to make candles. Undecane is an 11-carbon straight-chain alkane that is used in diesel and aviation fuel (kerosene). Heptacosane is a 27-carbon alkane with a straight chain. Lubricating oil is mostly made up of alkanes with 17-35 carbon atoms. They are anti-corrosive due to their hydrophobic nature, which prevents water from coming into contact with the metal surface [14]. Microalgae have just begun to emerge as possible feedstocks to produce light olefins. Light alkenes like ethene, propene, and butene were produced by thermal catalytic cracking of microalgae, which can serve as renewable platform molecules for a possibly more sustainable chemical manufacturing industry [15]. One of the most promising approaches to manufacture biofuels is by microbial biosynthesis of fatty alkanes or alkenes[16]. *Synechocystis* modified strains have also created alkanes and alkenes, including isoprene, which are the basic units of synthetic rubber [17]. Esters are used in fragrances, cosmetics, culinary flavors, and surfactants, such as soap and detergents [18]. Benzoic acid and its derivatives are usually utilized in cosmetics, food industry, sanitary goods, oral, parenteral, and topical medications as antibacterial and antifungal preservatives [19]. The phenolics

in algae are responsible for their antioxidant and antibacterial properties [20]. Hydrocarbons are used in the petrochemical sector to make candles, biopesticides (insect pheromones), paraffin wax, and other petroleum by-products. Microalgae, being photosynthetic organisms with high biomass productivity, would be perfect for harnessing the synthesis of hydrocarbons from fatty acid precursors among microorganisms [17]. For biodiesel generation, the fatty acid composition of microalgal species has been studied extensively. Microalgae-based biofuel has less sulfur or nitrogen than fossil fuel, therefore studying the volatile compounds of different microalgae is essential for reducing greenhouse gas emissions [21]. These algal species used as an alternate fuel source, plus it has medical and petrochemical worth, well-illustrated in figure 4.7

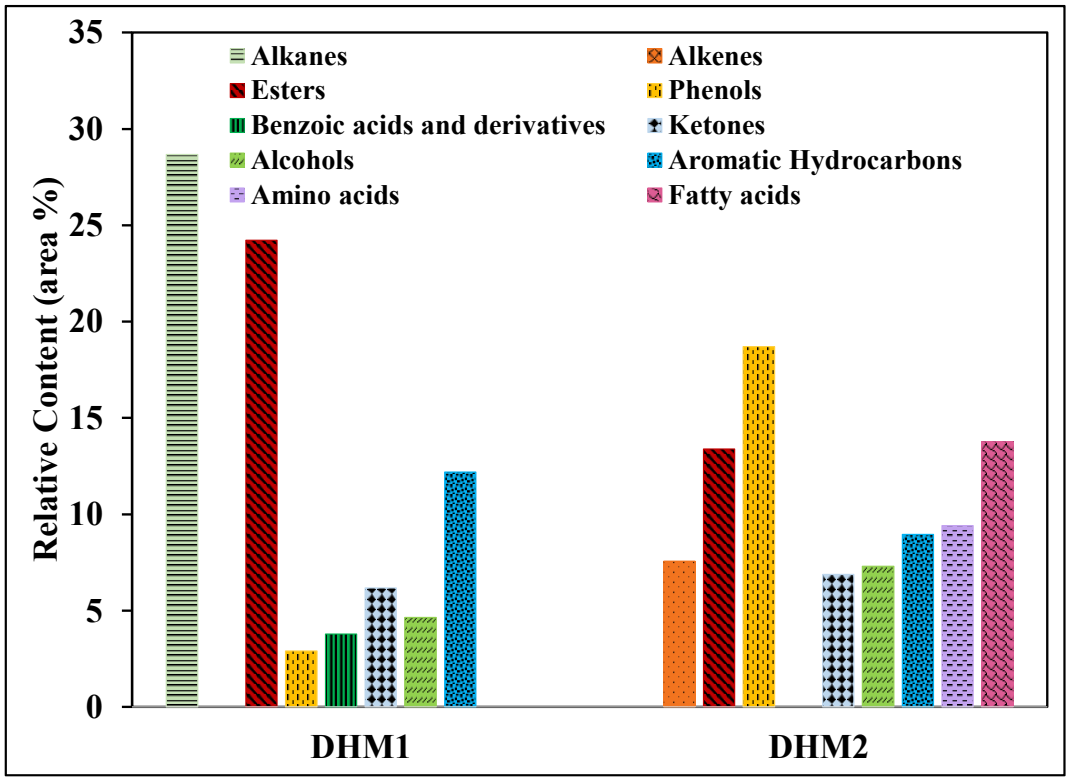

Figure 4.6 Overall chemical composition (GC/MS) of *Dictyosphaerium DHM1* and *Dictyosphaerium DHM2*.

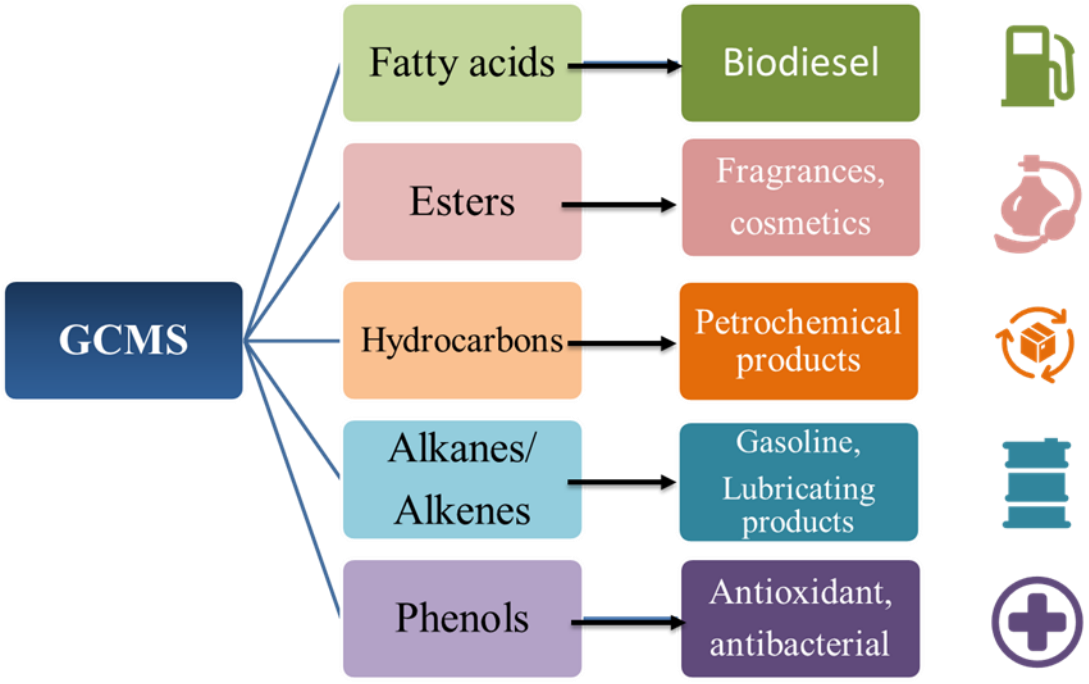

Figure 4.7 Biomass applications of novel microalgal species *Dictyosphaerium DHM1* and *Dictyosphaerium DHM2*.

Summary

In this chapter, Results showed that the growth parameters for both microalgal species substantially increased when supplied with 4% CO_2 compared to when supplied with 0.04 % and 2% CO_2 in either type of Photobioreactor. Maximum growth was noted for *Dictyosphaerium DHM1* when it was grown in 4% carbon dioxide in the Multi-Cultivator with 2.802 g L^{-1} (X_{max}), 0.2 g L^{-1} d^{-1} (P_{max}), and highest carbon dioxide fixation rate was 0.21 g L^{-1} d^{-1}. The biomass characterization of microalgae shows that these strains have the ability to not just capture carbon dioxide but also can be further used for energy purposes like biodiesel production, and other industrial and pharmaceutical uses.

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Chapter 5

Conclusions and Recommendations

5.1 Conclusion

Algal cultivation has two main benefits to combat climate change. Firstly, carbon dioxide is being removed from the atmosphere which is the primary contributor to global warming, and it helps to slow down climate change. Secondly, its biomass can be used as an alternative fuel. Biofuel production from algae is more environmental friendly than the best-performing conventional biofuels, and it may be the most sustainable alternative available to replace liquid fossil fuels used in transportation, the petrochemical sector, and remote fuel supply. In any event, despite the high investment costs, several algal biofuel companies have recently arisen, and the world is waiting for the first company to commercialize algae fuel.

- In this work, two novel strains of *Dictyosphaerium DHM1* and *Dictyosphaerium DHM2* species were cultivated under three different CO_2 concentrations, 0.04%, 2%, and 4%.
- The maximum CO_2 fixation rate was obtained at 4% CO_2 by *Dictyosphaerium DHM1* in Multi-Cultivator.
- This research also demonstrates the possibility of exploiting these algal species as an alternate fuel source along with, medical and petrochemical worth.
- The production of biomass can be increased by feeding these species with CO_2.
- The findings suggest that these Microalgal species, which have the potential for CO_2 bio-fixation have never been studied in CO_2 capture investigations before.
- *Dictyosphaerium* can withstand a wide variety of temperatures and can easily grow in Pakistan's moderate and mild environment.
- The ideal temperature for *Dictyosphaerium* growth is 25-35 C˚.
- Under ideal conditions, *Dictyosphaerium* produces a high yield in a short period and has a high rate of carbon dioxide fixation.

5.2 Recommendations

Even though the algae were cultivated in ideal conditions, temperatures in Pakistan can reach to alarmingly high levels during the summer, requiring the use of temperature control systems.

The following areas should be the focus of algal research and development for CO_2 mitigation:

- Two novel *Dictyosphaerium DHM1* and *Dictyosphaerium DHM2* species should also be cultivated and tested under 10%-15% CO_2 concentrations or even higher concentrations of carbon dioxide.
- Lipids should be extracted from algae for future production of biodiesel.
- Genetically modified species should be used which are more tolerant to high temperatures and high concentrations of carbon dioxide.
- Lowering capital expenses and reducing the need for raw materials when these species are scaled up to the commercial level.

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Appendix-1 Publication

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Biological carbon capture, growth kinetics and biomass composition of novel microalgal species

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ABSTRACT

Microalgae have been identified as a promising solution for capturing carbon dioxide (CO_2) and have been proven to be one of the most effective methods for CO_2 reduction. The present research discloses CO_2 fixation through green approach using novel microalgal species (*Dictyosphaerium* sp. strain *DHM1* & strain *DHM2*) and evaluation of growth kinetics along with biomass characterization. Both microalgal strains were supplied with 0.04%, 2%, and 4% CO_2 concentration in two different sorts of photobioreactors (Lab-scale photobioreactor and Multi-Cultivator). Results showed that the growth parameters for both microalgal species substantially increased when supplied with 4% CO_2 compared to when supplied with 0.04% and 2% CO_2 in either type of Photo-bioreactor. Maximum growth was observed for *Dictyosphaerium DHM1* when it was grown in 4% carbon dioxide in the Multi-Cultivator with 2.802 g L^{-1} (X_{max}), 0.2 g L^{-1} d^{-1} (P_{max}), and highest carbon dioxide fixation rate was 0.21 g L^{-1} d^{-1}.