

**Microalgae: Integrated Approach of CO₂
capture, wastewater treatment and**

BY

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A Thesis Presented to the
COLLEGE OF GRADUATE AND INTERDISCIPLINARY STUDIES
KING FAHD UNIVERSITY OF PETROLEUM & MINERALS
DHAHRAN, SAUDI ARABIA

In Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

In

CHEMICAL ENGINEERING

May 2025

DEANSHIP OF GRADUATE STUDIES

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Dedicated To My Parents and Family

ACKNOWLEDGMENTS

All praise is due to Allah, who owns everything in the heavens and on the earth, and to whom belongs the praise of the Hereafter; He is All-Wise and All-Knowing. I am deeply grateful to Allah for granting me the opportunity and capability to complete my master's degree at KFUPM. My heartfelt appreciation goes to my thesis advisor, Dr. Shaikh Abdur Razzak, and my co-advisor, Dr. Mohammad Mozahar Hossain, for their knowledge, compassion, and guidance throughout the past few years, whether in academic matters, my personal life, or my scholarly endeavors. I would like to extend my deep thanks to the members of my thesis committee—Prof. Dr. Mamdouh Ahmed Al-Harthi, Dr. Wasif Farooq, and Dr. Shihab Uddin—for their priceless advice and support during this journey. I am grateful to the Chemical Engineering Department at King Fahd University of Petroleum and Minerals for the chance to enhance my education. Furthermore, I appreciate the cooperation and support from all the faculty, staff, and technicians in the department. A special thank you to my friends in the Chemical Engineering Department for their remarkable support. I also want to express my immense gratitude to my parents and my entire family for their constant love, support, and inspiration, which kept me optimistic and motivated throughout this journey. Lastly, I am thankful to the Pakistani community at KFUPM for their encouragement and steadfast support during my studies.

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LIST OF ABBREVIATIONS

Bold's Basal Medium (BBM)

dry weight of biomass (%_{DWB})

Polyhydroxyalkanoates (PHA)

Maximum Biomass Concentration (mg/L) ($X_{\max}$)

Polyhydroxybutyrate (PHB)

Polyhydroxybutyratevelarate (PHBV)

Photobioreactor (PBR)

Chlorella sorokiniana (*C. sorokiniana*)

Maximum Temperature ($T_{\max}$)

Onset Temperature (T_{onset})

Hydroxyvelartae (HV)

ABSTRACT

Full Name : Fatima Irfan

Thesis Title : Microalgae: Integrated Approach of CO₂ capture, wastewater treatment and Bioplastic production

Major Field : MSc. Chemical Engineering

Date of Degree : May 2025

This study investigates a synergistic approach for sustainable development by utilizing *Chlorella sorokiniana* for the dual purposes of CO₂ capture and wastewater treatment, along with bioplastic production. Synthetic wastewater served as a nutrient source and CO₂-enriched air was employed to simulate industrial flue gas in the controlled growth of microalgae. The study aimed to optimize biomass production and enhance polyhydroxyalkanoate (PHA) accumulation by manipulating salinity, the nitrogen-to-phosphorus (N:P) ratio, and CO₂ concentration. Experimental findings indicate that a balanced N:P ratio (1:1), 4% CO₂, and moderate salinity (2%) significantly improve PHA yields while also aiding in nutrient removal from wastewater. This research highlights the potential of microalgal systems as an integrated solution for wastewater valorization, carbon emission reduction, and the production of biodegradable plastics.

ملخص الرسالة

الاسم الكامل: فاطمة عرفان

عنوان الرسالة: الطحالب الدقيقة: منهج متكامل لاحتجاز ثاني أكسيد الكربون، ومعالجة مياه الصرف الصحي، وإنتاج البلاستيك الحيوي

التخصص: ماجستير في الهندسة الكيميائية

تاريخ الدرجة العلمية: مايو 2025

Chlorella sorokiniana تسعى هذه الدراسة إلى استكشاف نهج متكامل ومستدام يقوم على الاستفادة من الطحالب الدقيقة لتحقيق ثلاثة أهداف بيئية وصناعية رئيسية: احتجاز ثاني أكسيد الكربون، ومعالجة مياه الصرف الصناعي، وإنتاج مواد بلاستيكية حيوية صديقة للبيئة. تم تصميم بيئة تجريبية خاضعة للرقابة حيث تم استخدام مياه صرف صناعية اصطناعية كمصدر للمغذيات، وتغذيتها بهواء غني بثاني أكسيد الكربون لمحاكاة الظروف المنبعثة من مداخل المنشآت الصناعية. ركزت الدراسة على تحسين ، وهي من المواد البلاستيكية القابلة للتحلل الحيوي، (PHA) إنتاج الكتلة الحيوية وزيادة تراكم مادة البولي هيدروكسي ألكانوات CO_2 ، وتركيز غاز (N:P) وذلك من خلال تعديل ثلاثة عوامل رئيسية: تركيز الملوحة، ونسبة النيتروجين إلى الفوسفور عند (1:1)، مع تركيز ثاني أكسيد الكربون بنسبة 4%، ومستوى ملوحة N:P أظهرت النتائج التجريبية أن التوازن في نسبة ، بالإضافة إلى تحسين كفاءة إزالة المغذيات (النيتروجين PHA معتدل عند 2%، يؤدي إلى تعزيز كبير في إنتاجية مادة والفوسفور) من مياه الصرف. توضح هذه النتائج إمكانات نظم زراعة الطحالب الدقيقة في تقديم حلول بيئية متكاملة تجمع بين الاستفادة من مياه الصرف، والحد من انبعاثات الكربون، وإنتاج بدائل مستدامة للبلاستيك التقليدي.

CHAPTER 1

INTRODUCTION

The Earth is struggling with so many issues right away. But some of the major ones include pollution, either it is being created by humans or animals. We are continually utilizing natural resources and are completely dependent on them without even realizing the threats for survival of humans on this planet in future. We are highly dependent on fossil fuels for our needs. Even now we have confirmed the dangers of these high amount consumption of these synthetic resources, but still, we are unable to find other solutions that can compete with these high value energy sources. One of the products coming out of fossil fuels is conventional plastics and they can be found everywhere in our lifestyle i.e., in food wrapping, razors, bottles, plastic bags, furniture etc. Plastic pollution is a global issue and the most major issue every country is facing right now. Synthetic plastics are not biodegradable, and it takes them more than 200 years to degrade. If dumped into oceans, it starts to convert into microplastic which ultimately damaging aquatic life. According to a study by[1] that, ecosystems have been noticeably harmed by the rising global demand for plastic goods, particularly in marine areas. As microplastics can enter the food chain and cause problems, this urgent issue affects both marine life and human health. If we burn it in the open atmosphere, it causes huge smoke, ultimately a reason for air pollution and plays a huge part in global warming. It seems that methods for reducing plastic pollution, such as landfill disposal, incineration, chemical treatment, and plastic recycling, may not

be the best ones. Many Governments have started campaign and created regulations for the production and usage of the plastic and major institutes all over the world are trying to find out the alternative solution for such a vast product. Also, the other challenge is global warming. The heat from the sun gets trapped on Earth as a result of greenhouse gas production. The temperature of the earth is continuing to increase day by day. The flue gases from industries causing the atmosphere and seasons to be irregular.[2] Please remove the texts in red which is given only as a guide.

1.1 Background and Motivation

A growing global issue is the environmental harm caused by the excessive use of fossil fuels and the accumulation of plastic waste. Annually, more than 400 million tons of plastic is manufactured; most of this non-biodegradable material finds its way into landfills, oceans, and ecosystems, leading to persistent pollution. Simultaneously, nutrient-rich wastewater from both residential and commercial activities results in eutrophication and contamination of freshwater sources, while rising levels of CO₂—exceeding 420 parts per million—accelerate climate change and global warming. To address wastewater pollution, carbon emissions, and plastic waste, we require thorough, long-term strategies.

As photosynthetic microorganisms, microalgae offer a unique opportunity to tackle all three challenges simultaneously. They can thrive in nutrient-rich wastewater while removing excess nitrogen and phosphorus, capture CO₂ from the atmosphere or industrial sources, and under certain stress conditions, they can accumulate polyhydroxyalkanoates (PHAs), which are a type of biodegradable bioplastic. Microalgae are ideal candidates for

large-scale sustainable bioprocesses because, unlike terrestrial crops, they do not need freshwater or arable land and can flourish in a variety of saltwater environments.

1.2 Problem Statement

While microalgae hold significant potential for addressing issues like plastic waste, climate change, and wastewater remediation, there is a noticeable absence of comprehensive experimental studies that integrate all three domains. Most investigations focus primarily on either the synthesis of PHA, carbon dioxide sequestration, or the removal of nutrients. Additionally, research is still lacking on the optimal cultivation of PHA while ensuring effective wastewater treatment and potential carbon dioxide utilization, particularly when using robust strains such as *Chlorella sorokiniana*. Therefore, this study aims to address the requirement for an integrated microalgae-based bioprocess that merges bioplastic production, carbon capture, and wastewater treatment.

1.3 Research Aim

This study aims to investigate and enhance the use of *Chlorella sorokiniana* to produce bioplastics, treatment of wastewater, and capture of CO₂ through stress-induced growth under different nutrient and environmental conditions.

1.4 Research Approach

Chlorella sorokiniana was utilized in five distinct laboratory-scale experiments for this research. The first four experiments focused on inducing stress, adjusting the nitrogen/phosphorus ratio, and managing salinity to enhance PHA production and facilitate

nutrient removal from wastewater without CO₂ enrichment. In the final phase, CO₂ was supplied at various concentrations (2%, 4%, 6%, and 8%) to explore its potential for boosting productivity. Throughout all trials, actual effluent conditions were mimicked using synthetic wastewater.

Optical density (OD₆₈₀), dry biomass, the removal of nitrogen and phosphorus, lipid content (assessed using the SPV methodology), and PHA production were consistently tracked throughout the cultivation process carried out in photobioreactors. FTIR and TGA techniques were employed to characterize the PHAs that were extracted. This multi-faceted approach provided a comprehensive understanding of the separate and combined influences of nutrients, salinity, and CO₂ on the targeted outcomes.

1.5 Significance of Study

This study contributes to the growing field of algae-based biorefinery systems by highlighting *Chlorella sorokiniana's* ability to achieve three vital sustainability goals: wastewater treatment, carbon sequestration, and bioplastic production. The findings promote the development of sustainable, scalable technologies that can simultaneously address wastewater treatment, reduce CO₂ emissions, and provide alternatives to conventional plastics, offering an integrated and circular solution to the most urgent environmental challenges facing the planet.

CHAPTER 2

LITERATURE REVIEW

2.1 Challenges of Global Warming

The primary source of the urgent problem of global warming is human activity-induced carbon dioxide emissions from heavy industries and electricity generation. Coal burning results in a larger output of CO₂—roughly 10-15%—than natural gas combustion, which produces about 5-6% CO₂. [3] Industrial flue gases is released by a number of industrial sectors, such as power generation, refineries, steelworks, cement manufacture, gas processing, and the production of ethylene, hydrogen, and ethanol. According to the International Energy Agency's analysis of these sectors, the energy industry stands out as the one that contributes the most to CO₂ emissions, with China, India, and the US accounting for 85% of the net increase. Coal, natural gas, gas turbine, and fuel oil plants are the sources of emissions in the power industry. These sources release CO₂ into the atmosphere in addition to other components including SO_x, NO_x, N₂, H₂O, O₂, CO, CH₄, H₂, H₂S, and solid particles. [4] According to one latest report the Greenhouse gas emissions all over the world has increased drastically in last 17 years(2005-2022). The major contributor to Global Warming and Climate change in the Industrial Combustion Sector.

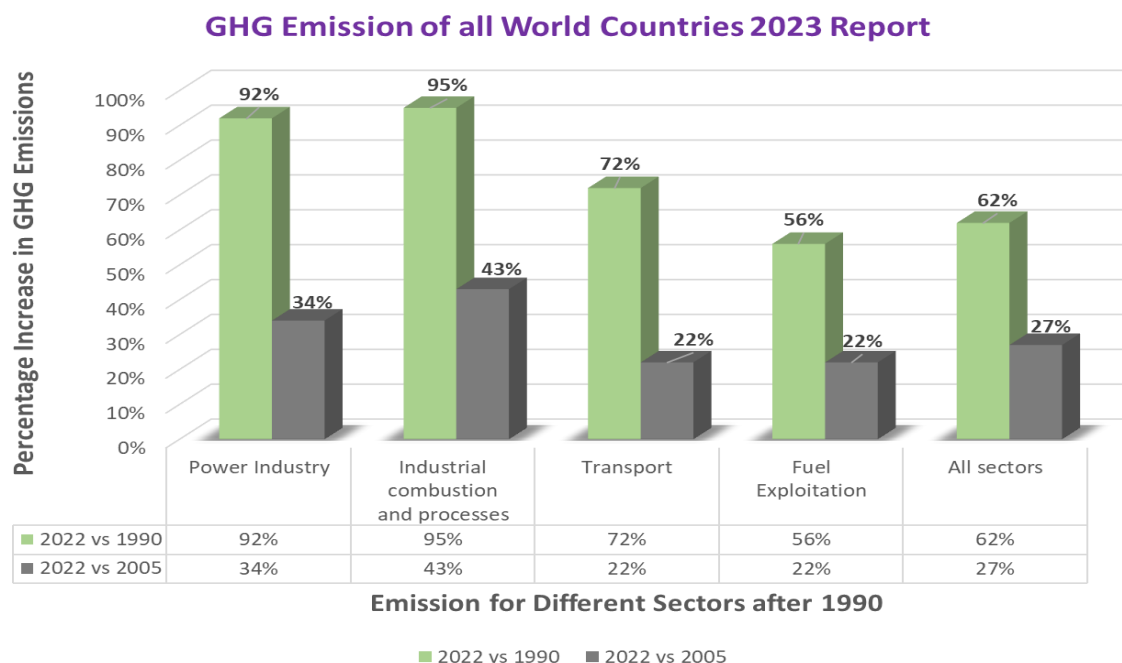


Figure 1: GHG emissions in different industries across the world [22]

The rapidity at which carbon dioxide is naturally extracted from the atmosphere is outpaced by the fast release of CO₂ resulting from the continuous burning of fossil fuels and deforestation. The imbalance causes the amount of CO₂ in the atmosphere to rise up more quickly.[5] Because identifying and renewing adsorbents requires a significant amount of energy, CO₂ removal from industrial gases via chemical and physical processes is typically expensive on a commercial scale.[6] Techniques that are often employed include cryogenic technology, physical and chemical absorption, and membrane separation.[7][8][9][5]

The utilization of microalgae to biofix CO₂ from flue gases has the promise to mitigate greenhouse gas emissions and yield valuable products. Microalgae do not compete with agricultural crops and fix CO₂ at a rate far quicker than terrestrial plants. The specific strain of microalgae used and the compounds/concentrations in the flue gas are key factors in the capture of carbon dioxide from the gas.[10] The requirement for flue gas pretreatment may

be neglected if microalgae were able to collect flue gas without any prior preparation. However, employing microalgae to directly capture CO₂ from industrial exhaust gas is usually a challenging operation.[11]In a study presented by [12], upgradation of biogas was done coming out of industrial source by reducing the CO₂ content when aerated by desulfurized biogas(H₂S less than 50 ppm) produced from anaerobic digestion of swine wastewater, CO₂ being utilized to grow microalgal strain *Chlorella* sp. (MB-9). For instance, a high amount of CO₂ fixation rate was achieved around 1.2g per liter per day by another strain of *Chlorella* sp. Cv against a simulated flue gas(10% CO₂, 200 ppm NO_x and 100 ppm SO_x).[13]

2.1.1 Harmful impacts of Flue gases on Environment; A need for Carbon Capturing and Utilization

Flue gases, emitted from the combustion of fossil fuels, have harmful impacts on the environment. These gases, primarily composed of carbon dioxide and other greenhouse gases, contribute to global warming and climate change. [14] The increasing concentration of these gases in the atmosphere leads to the greenhouse effect, trapping heat and causing the Earth's temperature to rise. This rise in temperature is associated with various detrimental effects, such as the melting of polar ice caps, sea-level rise, extreme weather events, and disruptions. [15] Furthermore, flue gases also contain pollutants such as sulfur dioxide and nitrogen oxide, which contribute to air pollution and the formation of smog. [16] Exposure to high levels of greenhouse gases, particularly carbon dioxide and other pollutants present in flue gases, can have adverse effects on human health. These effects can range from respiratory issues, such as asthma and chronic obstructive pulmonary disease, to cardiovascular problems like heart disease and stroke. [17]

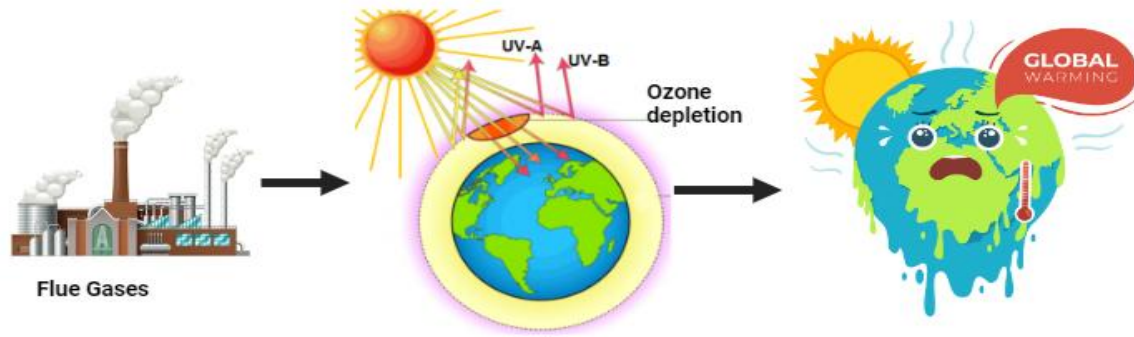


Figure 2: Impact of Flue Gases on Environment

To mitigate the harmful impacts of flue gases on the environment, carbon capture and utilization technologies have gained significant attention.[18] Carbon capture and utilization technologies involve capturing the carbon dioxide emissions from flue gases and finding ways to utilize or store them. This can be done through various methods, such as carbon capture and storage, where the captured carbon dioxide is stored underground, preventing its release into the atmosphere.[19] Additionally, carbon capture and utilization technologies can involve converting captured carbon dioxide into useful products such as biofuels, chemicals, or building materials. These technologies not only help reduce greenhouse gas emissions but also contribute to the development of a circular carbon economy, where carbon dioxide is viewed as a resource rather than a waste product. [20] Microalgae synthesize organic compounds from CO_2 and water through photosynthesis; they do not release any contaminants or require additional energy beyond what they receive from sunlight.[21] As microalgae absorb CO_2 more quickly than terrestrial plants, it effectively use flue gas CO_2 to produce biomass. For this reason, aquatic microalgae are especially well-suited because of their quick growth, high biomass output, and flexibility in terms of possible product generation.[22] On top of that, the biomass produced by the collaboration of flue gases and microalgae may be processed into useful metabolites,

biofuels, and animal feed.[5] In the cement industry, using microalgae for off-gas treatment instead of traditional methods has two benefits: it produces biofuels and other biorefinery products and reduces the toxicity of flue gas.[23]

2.1.2 Potential Nutrient Source for Microalgae

Microalgae synthesize organic compounds from CO₂ and water through photosynthesis; they do not release any contaminants or require additional energy beyond what they receive from sunlight. As microalgae absorb CO₂ more quickly than terrestrial plants, it effectively uses flue gas CO₂ to produce biomass. For this reason, aquatic microalgae are especially well-suited because of their quick growth, high biomass output, and flexibility in terms of possible product generation.[40] On top of that, the biomass produced by the collaboration of flue gases and microalgae may be processed into useful metabolites, biofuels, and animal feed.[23] In the cement industry, using microalgae for off-gas treatment instead of traditional methods has two benefits: it produces biofuels and other biorefinery products and reduces the toxicity of flue gas.[24]

2.1.3 Overview of the carbon capture and storage methods

The concentration of CO₂, the most important GHG and the major contributor to global warming, has reached formidable levels. Corresponding to a 32% increase, from around 280 ppm to 400 ppm, since the industrial revolution.[24] In addition to causing global warming, the increased CO₂ concentration in the atmosphere has also contributed to a 30% increase in ocean acidity. Marine ecosystem biodiversity is being negatively impacted by this rise in acidity.[25] Reducing CO₂ emissions through reduced use of fossil fuels and improved carbon capture and sequestration methods is the main requirement.[26]

A detailed description of the CO₂ emissions, Separation and Capturing Techniques and Utilization is presented by [27] recently. CO₂ capture, transit, and storage are the three main phases of CCS operation. At important point sources like power plants and cement manufacturing facilities, CO₂ collection is carried out.[19] The goal of post-combustion CO₂ capture is to collect and capture CO₂ from exhaust gases, sometimes referred to as flue gases, prior to their discharge into the atmosphere. With this technology, CO₂ is extracted after the combustion process.[28] When nitrogen (N₂) and oxygen (O₂) are present in the mixed air composition, the fuel burns.[29] Different methods are used to separate and extract CO₂ from other components in exhaust gases, such as membrane separation, physical adsorption, chemical absorption, and cryogenic distillation. After being captured, ships or pipes are used to move the compressed CO₂ to storage facilities.[18] Although CCS has a large amount of storage capacity, it faces obstacles such as high shipping and operating costs, environmental risks from possible long-term CO₂ leakage, and doubts about its efficacy.[24] Carbon may be separated and captured using a variety of techniques, including membrane separation, physical adsorption, chemical absorption, and cryogenic distillation. These techniques are particularly useful for large emission sources such as cement factories, power plants, and different exhaust systems.[30][31] After the process of separation and capture, the highly concentrated carbon is compressed and transported by pipelines and ships.[27] Although there are several main ways to store energy, including geological storage, oceanic storage, in-situ carbonation, saline aquifers, and biological storage, each has its own set of challenges that must be overcome before it can be used on a large scale. Repurposed oil and gas reservoirs, unmineable coal seams, and salinized aquifers are some of the geological storage methods

that are employed in operations. A notable impediment to the transportation of CO₂ across long distances concerns the costs involved in setting up the facilities and infrastructure required for the process.[32][33] While saline aquifers are not valuable commercially, they are useful for storing injected CO₂ from CCS systems. Compared to other geological locations such as coal seams and oil fields, comparatively little is known about the storage properties of CO₂ in saline aquifers, despite their enormous capability for CO₂ storage.[34] As a result, deep ocean storage appears to be a promising location for large amounts of CO₂ produced by humans. The direct injection of significant volumes of CO₂ into the ocean, which may alter the chemistry of seawater by decreasing its pH and causing ocean acidification, makes this approach controversial.[34] Although in situ carbonation holds great potential for storing CO₂, finding appropriate injection locations and comprehending their effective CO₂ reactivity remain difficult, which limits its application.[35] Forestation, oceanic fertilization, and microalgae-based capture are examples of biological capture techniques. Of these, microalgae are particularly noteworthy as a potentially effective solution to the drawbacks of conventional CCS technology. Their outstanding CO₂ fixation efficiency is a noteworthy advantage, providing a more environmentally friendly way to convert CO₂ waste into biomass. Microalgae are an attractive technology for capturing carbon since studies show that they are more capable of capturing carbon than both terrestrial plants and seas.[36] As the forestation needs much land, long time requirement and affect biological diversity.[37] Also, the oceanic fertilization is cost intensive process and may affect marine biodiversity.[38]

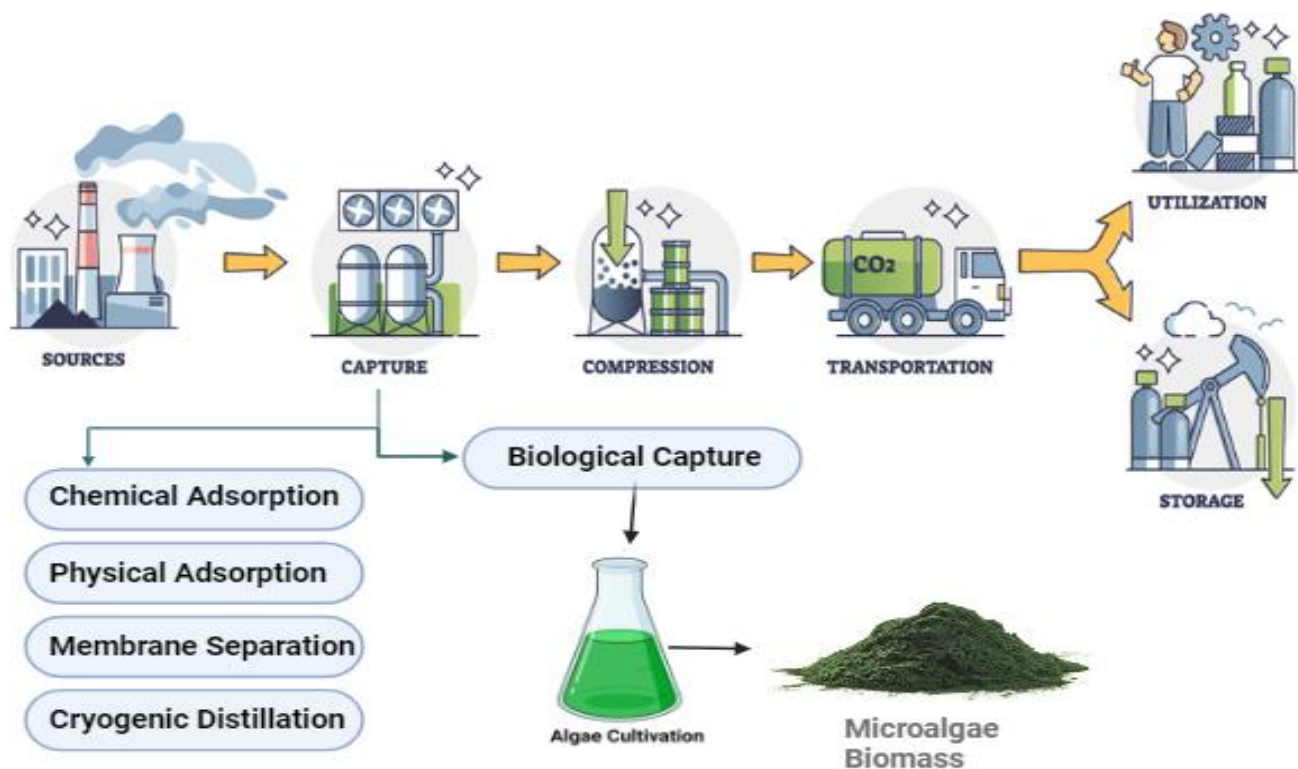


Figure 3: Carbon Capturing, Utilization, Storage loop and methods

2.1.4 Microalgae as a potential carbon capture agent (Biological system and technique of carbon capturing)

According to a recent study, using microalgae for biological carbon dioxide (CO_2) capture is seen to be a convincing way to use the excess CO_2 that is created by cars, power plants, volcanic activity, decomposition of organic materials, and wildfires. Three main methods are used to collect carbon dioxide from power plants: oxy-fuel combustion, post-combustion capture, and pre-combustion capture. The post-combustion method's advantage is that it may be used to power plants that already exist.[39] While pre-combustion capture is generally more efficient and enables the use of generated hydrogen as a transportable fuel or product, the fundamental gasification process frequently has higher initial investment costs than traditional pulverized coal power plants. Both systems require large equipment sizes because of the low partial pressure of CO_2 and the enormous

volume of flue gases. In addition, they need expensive capture costs, energy-intensive cooling systems, and pre-treatment to remove pollutants.[40] On the other hand, carbonaceous fuel must be burned in pure oxygen to use oxy-fuel combustion technology instead of air. The relatively simple separation of CO₂ is a significant benefit, but there are drawbacks as well, such the requirement for pre-treatment, the potentially expensive cost of providing oxygen, which might prevent commercialization, and the high temperatures required in the combustion process.[41] An alternate strategy is the chemical looping combustion method, in which CO₂ is the principal combustion product and is maintained apart from N₂, avoiding the energy-consuming air separation procedure. Still in its early phases of development, this approach lacks adequate large-scale operating experience.[34] Comparing microalgae cultivation's CO₂ collection capabilities with those of other post-combustion CO₂ capture techniques is crucial. The species of the microalgae, the growing methods, and the environmental parameters—temperature, pH, turbidity, salinity, light intensity, trophic modes, and available nutrients in the culture—all effect how effective microalgae are at capturing CO₂. [42] Through a process called bio-sequestration, microalgae may sequester industrial pollutants, most notably carbon dioxide. Microalgal cells take in CO₂ and use photosynthesis to turn it into biomass throughout this phase. The biomass that is produced has the potential to be used in the manufacture of bioenergy and other goods with added value.[43] The polysaccharides found in microalgae, which are plentiful in our ecosystems, may be harvested and used to produce biopolymers,[44] resulting in a double benefit of reducing CO₂ emissions and producing sustainable alternatives to traditional plastics.[45] Three different mechanisms allow microalgae to take up inorganic carbon: (i) bicarbonates are converted by external carbonic anhydrase

into CO_2 , which easily and unobstructedly passes through cell membranes; (ii) CO_2 is absorbed directly through the plasma membrane; and (iii) certain membrane carriers, also known as dissolved inorganic carbon (DIC) pumps, facilitate the direct uptake of bicarbonates.[46]

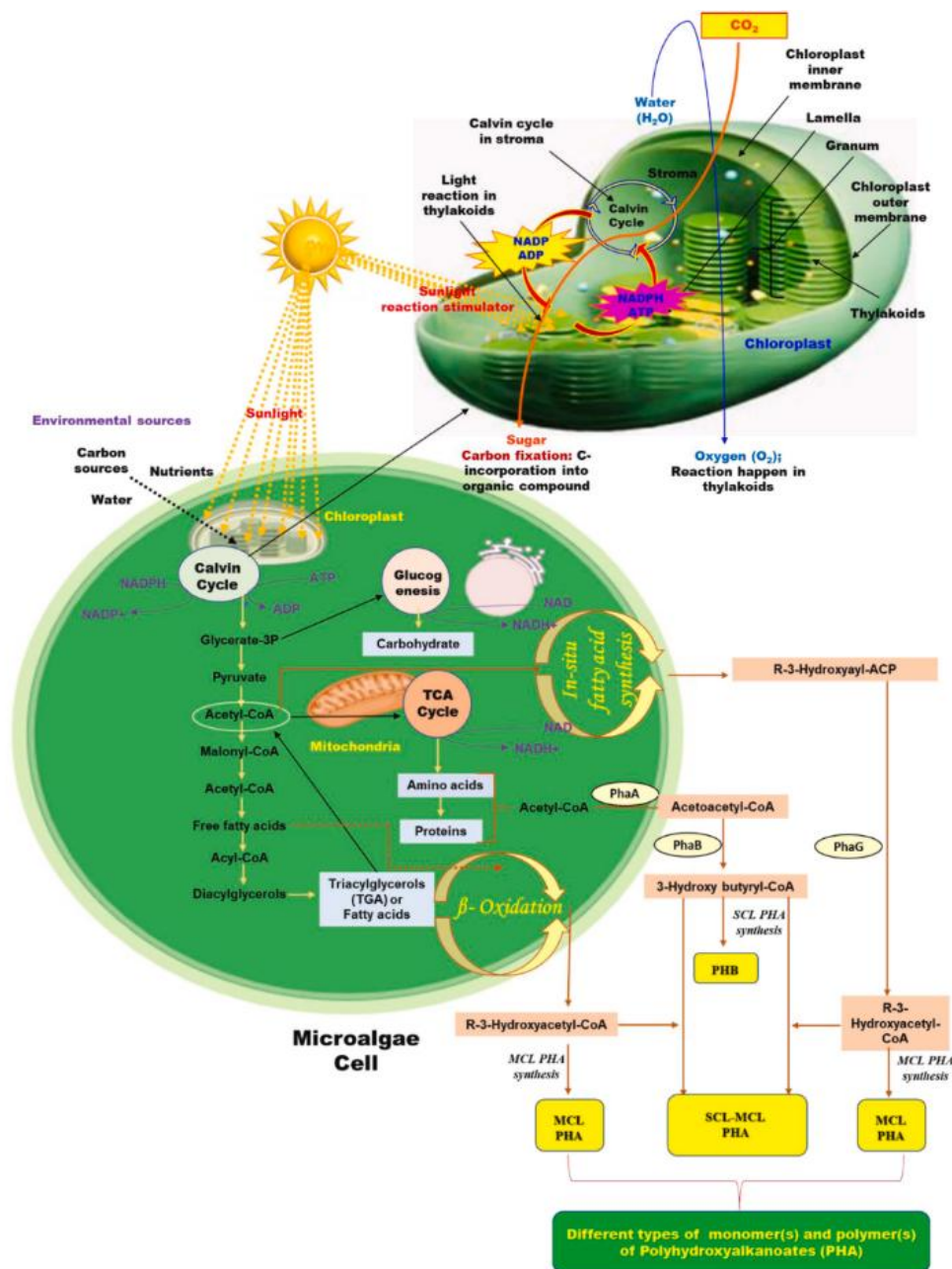


Figure 4: Mechanism of PHA accumulation in microalgae cell.

2.2 Microalgae based wastewater treatment

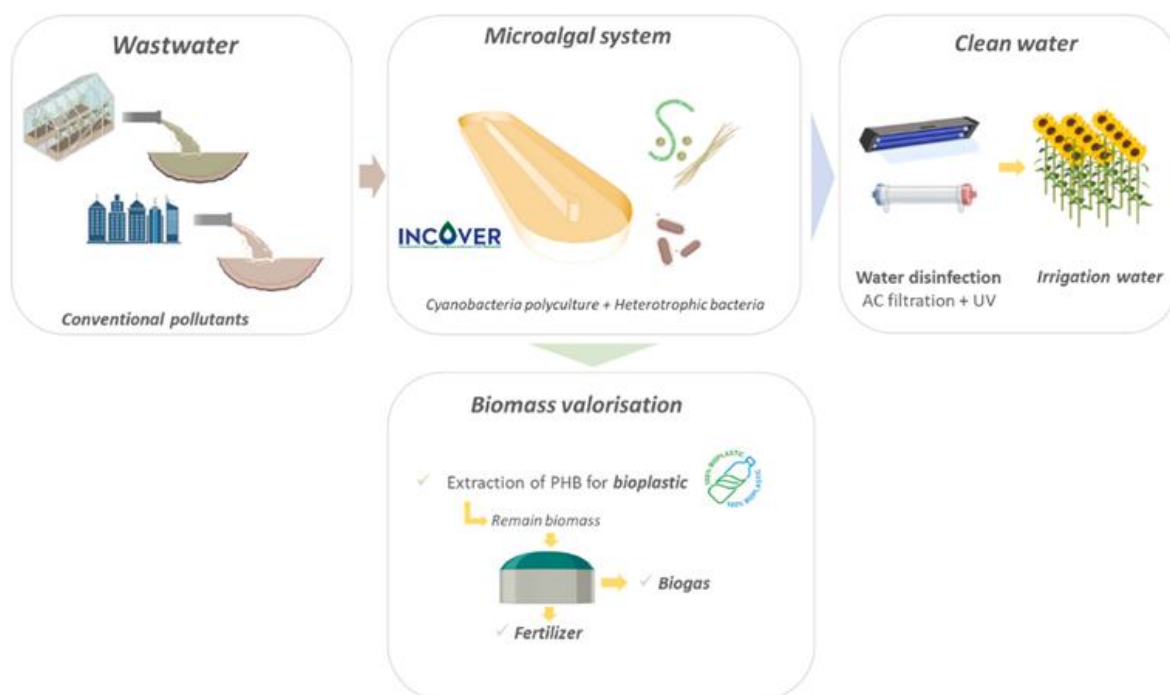
The use of microalgae in wastewater treatment has garnered significant interest as a sustainable and cost-effective alternative to conventional methods. Wastewater from domestic, agricultural, and industrial sources is abundant in nutrients such as phosphorus and nitrogen, which are primary contributors to eutrophication if left untreated. Microalgae can absorb these nutrients during their growth and convert them into biomass, which can be utilized to produce valuable products like animal feed, bioplastics, and biofuels. Therefore, microalgal systems offer benefits in both biomass valorization and bioremediation.

2.2.1 Nutrient Removal Potential of Microalgae

One feasible strategy for establishing circular and sustainable biorefineries involves integrating microalgae-based wastewater treatment with the production of valuable bioproducts such as PHA. Wastewater significantly reduces the operational expenses of cultivating microalgae by serving as a growth medium and providing a cost-free source of essential nutrients. By adjusting growth conditions (for instance, through nutrient limitation or salinity stress), algal cells are prompted to store compounds instead of reproducing, a process that is leveraged for accumulating polymers.

For instance, it was observed that using microalgae cultivated in agro-industrial effluent successfully combined PHA production with nutrient removal.[47] Similarly, another investigation cultivated *Chlorella vulgaris* under nitrogen-deficient conditions with synthetic urban wastewater, achieving substantial PHA accumulation alongside high phosphate removal (87%). These studies demonstrate that wastewater can serve as a dual-functional substrate for biopolymer production and treatment.[48]

A



B

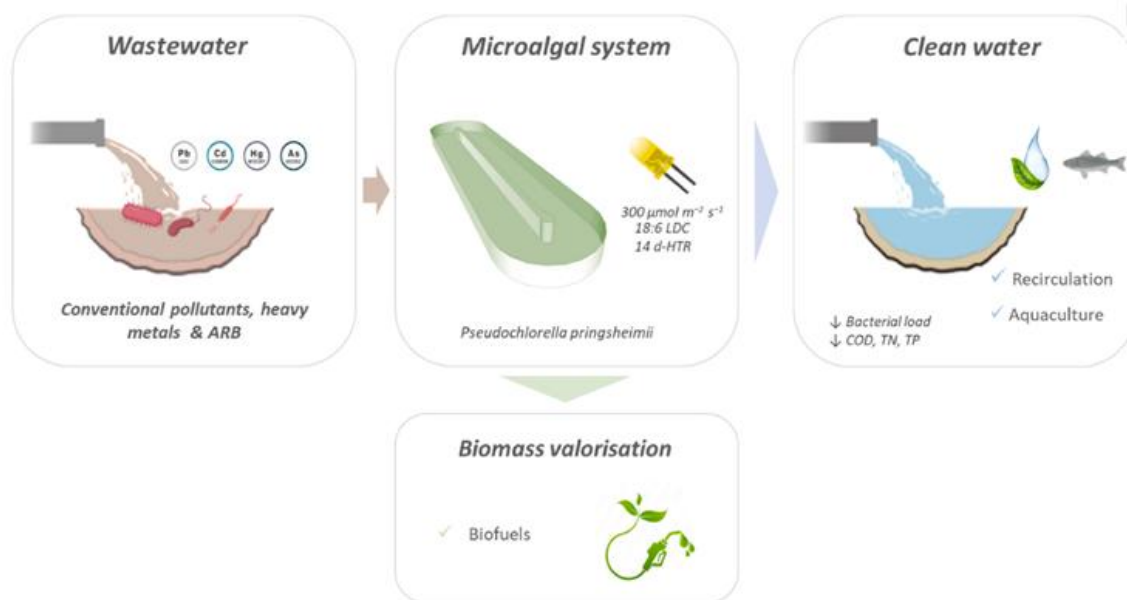


Figure 5: Microalgal based wastewater treatment [49]

2.3 Common Methods of Microalgal Cultivation

"Algal culture," which emphasizes the true potential of growing algae, must be acknowledged on a worldwide scale as a workable substitute for burning fossil fuels, which have long been regarded as the "black gold" of the modern era.[50] The first step is cultivation followed by harvesting.[51] The cultivation method mostly makes use of photobioreactors (PBRs) or open ponds. There are several different kinds of photobioreactors, including airlift, tube, flat-plate, and vertical column designs.[52] A study presented by [53], explores different methods and systems for cultivating microalgae, which is a critical step in bioplastic production and CO₂ assimilation and conversion at a large scale. It also discusses the pros and cons of open systems and closed systems for microalgae cultivation. To maintain micro-algal dominance and prevent contamination by other microbes in the photobioreactor (PBR) setup prior to moving to larger-scale open ponds, a hybrid system integrates two or more culture systems.[54]

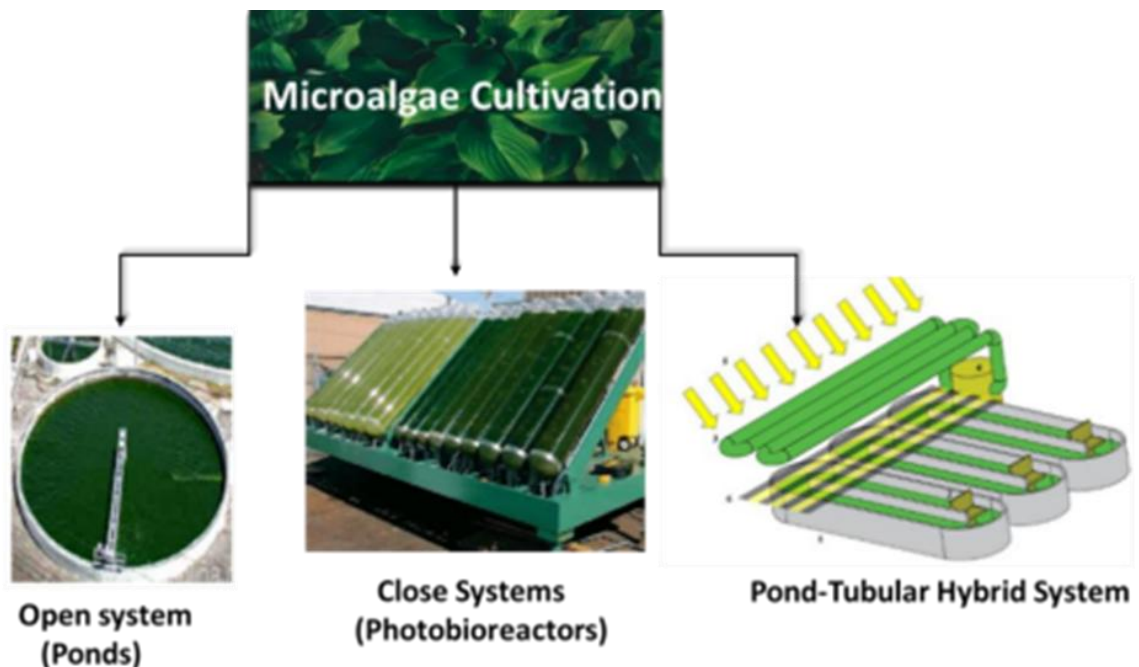


Figure 6: Cultivation Strategies used for microalgae

2.4 Microalgae as a Source of Bioplastic

Microalgae have great potential as a source of bioplastics. They are employed in the synthesis of Polyhydroxyalkanoates (PHAs) and Extracellular Polymeric Substances (EPSs) with a variety of industrial uses. Prized for their biodegradability, PHAs are essential ingredients in the production of bioplastics.[43] In order to increase strength and durability, PHA microalgae strains and the mixing of microalgae biomass with other plastics and synthetic polymers are the main subjects of current research on microalgae bioplastics. Microalgae also have the benefit of growing waste materials without interfering with food sources.[53] Bioplastics derived from microalgae provide a viable remedy for reducing plastic waste.[55] Scaled proof-of-concept initiatives for the manufacturing of microalgae plastic have focused mostly on using algae's lipids and proteins, not its sugars, for value-adding.[56] Sugars taken from microalgae can be converted into bioplastics, specifically polyhydroxyalkanoates (PHAs), according to recent research. These microalgal-derived PHAs have performance characteristics that are like those of polymers generated from fossil fuels, making them a viable substitute in the market.[57]

Promising results were obtained from an academic study on an algal biofuel-bioplastic refinery, with a sales price of about \$970 per tonne of bioplastic. In comparison to traditional plastic manufacture, it also showed greenhouse gas reductions ranging from 67.0 to 116.0%. Using microalgae in the production of bioplastics offers a comprehensive way to capture carbon emissions from industry.[44] The current annual output of bioplastics in the world is estimated to be 750,000 tons. One of the largest producers of bioplastics is NatureWorks, which produces 140,000 tons of polylactic acid (PLA)

annually, and Novamont, which produces 35,000 tons of starch-based bioplastics annually. The main substitute resource for traditional plastics in a variety of engineering application domains is polyhydroxyalkanoates, or PHAs.[58] Well-known microalgae like *Chlorella* (51–58%) and *Spirulina* (46–63%) are thought to be excellent for the development of bioplastics due to their high protein content.[55] Indeed, much like seaweed, microalgae and cyanobacteria are used as raw materials to make third-generation bioplastics. However, because of their poor economic feasibility, these third-generation bioplastics have drawn relatively less attention, which has slowed down development in this field.[59]

2.4.1 Polyhydroxyalkanoates (PHA) structure and properties

a. Overview of PHA's as biodegradable polymers

Due to high need of plastic and to reduce the plastic pollution in ecosystem, it is important to search for a source of degradable plastics as an alternative to conventional plastic.[80] Where the Polyhydroxyalkanoates called as “biopolymer”, can be a potential replacement to plastics made from petrochemical sources, also known as an energy and carbon source. PHA's can be synthesized by some microorganisms such as cyanobacteria or microalgae.[60]

In the field of microalgae-based products, polyhydroxyalkanoates (PHAs) that are isolated from microalgae are highly valued for their biodegradability, which makes them a perfect option for producing bioplastics.[43] It is widely recognized that microalgae can generate extracellular polymeric substances, which are extensively employed in commercial applications. They can also be a source of polyhydroxyalkanoates, which are valued for their biodegradable properties and can be used to produce bioplastics.[61] Currently, a majority of research on microalgae bioplastics focuses primarily on PHA microalgae

strains and the incorporation of microalgae biomass into synthetic polymers and other plastics to enhance their strength and durability.[55] By combining the carbon-capturing capabilities of microalgae with the production of bioplastics, it is possible to create a closed-loop system that actively reduces carbon emissions and plastic waste simultaneously.[62] Microalgal genetic engineering holds great potential for increasing CO₂ fixation and biomass accumulation, two processes that are essential to the synthesis of bioplastics.[63] Previous research on producing bioplastics from microalgae mostly focused on using proteins and lipids that were isolated from the algae. However, there is also an unrealized promise in using the sugars from microalgae to produce bioplastics.[56]

b. Types and Properties of PHA

PHA and PHB are biodegradable polymers, produced naturally by mainly two methods bacterial fermentation of sugars and lipids and biomass produced from microalgae.[44] In Polyhydroxyalkanoates (PHA), ester linkages bind Hydroxy Acid (HA) monomeric units together structure-wise. The number of carbons in the side chains of PHAs determines their classification. Short chain length (SCL) is defined as having fewer than five carbons; medium chain length (MCL) is defined as having five to fourteen carbon atoms; and long chain length (LCL) is defined as having more than fourteen carbon atoms.[64]

Polyhydroxyalkanoates, or PHAs, are polyesters that degrade naturally. The biodegradable quality of polymers made from microalgae is enhanced by the presence of PHB molecules in the cells of the algae.[65] In fact, it has been shown that certain cyanobacteria contain Polyhydroxyalkanoates (PHAs), which can make from 1% to 25% of their dry weight.[63] The majority Polyhydroxyalkanoates (PHAs) including PHB (polyhydroxybutyrate), PHBV (polyhydroxyvalerate), P(4HV) (poly (4-hydroxyvalerate) and P(3HB-co-3HV)

(poly (3-hydroxybutyrate-co-3-hydroxyvalerate) and P(3HB-co-4HB) (poly (3-hydroxybutyrate-co-4-hydroxybutyrate) are known to be synthesized by microalgae and cyanobacteria.[66] Indeed, PHAs have a variety of uses across a range of sectors. They are used as coatings, laminations, and biodegradable printing inks in addition to packaging components. Because the PHA frameworks contain a variety of hard thermoplastics and thermoplastic elastomers, they are also used in waxes, binders, and adhesives.[67] In fact, many microbes generate polyhydroxyalkanoates (PHA), a lipid-like molecule that is used as a storage medium.[64] In actuality, it appears that bacteria synthesize Polyhydroxyalkanoates (PHAs) in response to physiological stress brought on by nutritional limitation. This polymer is used by microorganisms as a molecule for storing energy that may be used when other energy sources are limited or nonexistent.[68] Without a doubt, in reaction to physiological stress brought on by dietary restrictions, bacteria do produce Polyhydroxyalkanoates (PHAs). When there are no or little alternative energy sources available, bacteria can use this polymer as a molecule for storing energy.[69]

One kind of polyhydroxyalkanoate is polyhydroxybutyrate (PHB)(PHAs).[67] Variations in the PHAs' molecular mass and crystallinity index point to the possibility that the polymer characteristics are modified by the extraction process.[70] PHA production usually varies

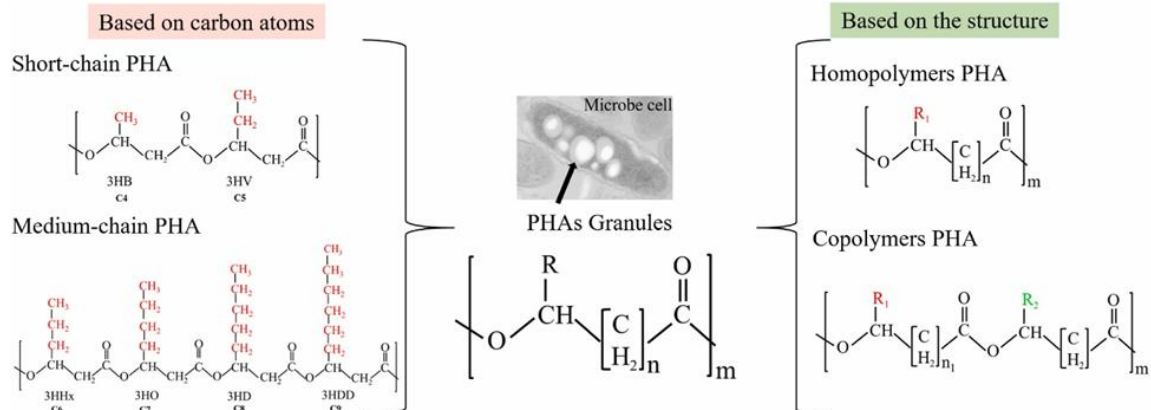


Figure 7: Structure of PHA and its classifications

with species and how it reacts to different nutritional circumstances. For example, in phototrophic circumstances, *Synechococcus elongatus* displays a PHA concentration of 17.15% when 1% sucrose is utilized as a carbon source. However, in chemotrophic settings, its concentration drops to 12.98%.[71]

c. PHA content and characterization of different microalgal species

Table 1: Microalgal species, and their PHA content and its characterization

<i>Species</i>	<i>PHA content</i>	<i>Thermal Stability</i>	<i>Mechanical Stability</i>	<i>PHA's</i>	<i>Ref.</i>
<i>Chlorella sp.</i>	Defatted (75.4%)	217 °C	81% Elongation at break [80]	P (3HB-co 3HV)	[72] [67] [73]
<i>Spirulina sp. LEB 18</i>	72.58%	269.76	5.5% Elongation at break and 51.65% of Crystallinity	PHB	[74] [75]
<i>Spirulina Plantesis</i>	7.4%, 30.7%	289.2	81% Elongation at break	P (3HB-co 3 HV)	[73]
<i>Synechococcus subsalsus</i>	51.78%	312.19	---	PHA	[76]
<i>Calothrix scytonemicola TISTR 8095</i>	25.2%	170.7°C	58% stronger than commercial PHB	PHA	[77] [78]
<i>Synechocystis sp.</i>	29%	----	5.5% Elongation at break	PHB	[78]

<i>Nostoc muscorum</i>	69%	223 °C	crystallinity of 62.4%. Young's modulus, tensile strength and elongation-to-break is 81%	P (3HB-co 3 HV)	[79] [80]
<i>Aulosira fertilissima</i>	85%	----	81% Elongation at break	P (3HB-co 3 HV)	[81] [82]
<i>Chlorella Pyrenoidosa</i>	27%	----	5.5% Elongation at break	PHB	[82]
<i>Anabaena sp.</i>	46%	----	5.5% Elongation at break	PHB [80]	[83]
<i>Scenedesmus.</i>	0.83–29.92%	----	5.5% Elongation at break	PHB [94]	[84]

d. Potential applications of PHA bioplastics in various industries

It is emphasized that the commercialization of PHA can be accomplished by broadening its application areas and exploring more high value uses, such as in medical applications.

Table 2: Microalgal species, and their application in bioplastic industries

Specie	Application	Characteristics application in Bioplastic Industry	References
<i>Chlorella pyrenoidosa</i>	Production of Bio-oil, Bioplastic, reduce the toxicity of heavy metals such as nickel and	Disposable products are used, such as packaging, agriculture, and even in medicine for drug delivery systems or for	[85]

	chromium, suggesting its potential use in treating industrial waste.	regenerative medicine applications as temporary implants, where the product can perform its function and then safely degrade.	
<i>Botryococcus braunii</i>	CO2 Sequestration, medical drug carriers	It can be used for preparing drug carriers for medical purposes	[86]
<i>Arthrospira;Spirulina</i>	Dietary supplements, Tissue Engineering	Bioplastic production, Food packaging, shoe soles	[82], [87]
<i>Chlorella Vulgaris</i>	Vegetable oil substitute, dietary supplements	Food Packaging and Agriculture	[82], [88]
<i>Synechococcus subsalsus</i>	Biopolymers	Industrial application in packaging area.	[74]
<i>Spirulina sp. LEB-18</i>	Biopolymers	Bio-Packaging area	[74]

A refinery using algae biofuel to create bioplastics was the focus of a recent scientific investigation. Positively, the trial showed encouraging results, producing bioplastics at a competitive \$970 per tonne sales price. In addition, the study showed significant decreases in greenhouse gas emissions, from 67.0% to 116.0%, as compared to traditional plastic production methods.[89]

2.4.2 Bioplastic extraction, production and characterization

Solvent extraction was used to extract PHA from several species of marine algae. This procedure involved centrifuging and incubating known dried biomass after treating it with a sodium hypochlorite solution. The biomass was then treated with heated chloroform, and cold methanol was used to precipitate the result. The material was centrifuged and then treated with hot chloroform one more time to further purify it.[67] It was made clear in a succinct analysis of PHA extraction that PHAs can be a financially and practically feasible option for large-scale commercial manufacturing. PHA's isolation and purity levels, however, vary according on the intended use; for example, lesser purity levels could be adequate for making trash bags, but greater purity levels are necessary for medicinal applications.[64] A 2L photobioreactor was used for the development of spirulina, and biopolymers were extracted using acetone and sodium hypochlorite as solvents.[60] The inclusion of sodium hypochlorite at the beginning of the extraction process promotes polymer buildup, whereas the addition of methanol at the conclusion yields PHAs with greater purity. The degree of crystallinity, monomeric composition, and molecular mass of PHAs are all influenced by these extraction techniques. This highlights how important the technique of choice is in producing polymers with the right properties that are appropriate for use in industry.[70] According to Das et al., the microalgae biomass that remains after lipid extraction may be used to produce bioplastics with a 27% PHB content.[90] It should be noted that the specific microalgae species and the intended kind of bioplastic may influence the choice of polymer extraction process.[53] Two methods have been used to extract PHB (a kind of PHA) from algal cells: the Wet Lipid Extraction Procedure (WETP) and the hydrolysis of dried material. When using WETP instead of hydrolyzing dry bulk,

total costs are lowered considerably.[53] PHA granules from microalgae can be extracted by cell disruption using a variety of techniques, such as chemical methods (using sodium hypochlorite), physical methods (using homogenizer mills and ultrasound), and biological methods (using specific enzymes like nucleases, lysozymes, and proteases).[61]

As *Chlorella pyrenoidosa* biomass was left behind after producing biofuel, Polyhydroxybutyrate was extracted using a chemical treatment technique in a study. UV photospectrometry was used for the characterisation.[85] The PHAs obtained from microalgae has a good plasticizing capacity and biodegradability.[61] *Leptolyngbya valderiana* and *Chlorella sp.* strains showed noteworthy growth and synthesis of polyhydroxyalkanoates. Both showed the fastest growth, with dry cell weight reaching around 2.75 g/L. As it investigated PHAs' thermal characteristics, *Leptolyngbya valderiana's* PHA was more stable than PHAs from other algae species (*Chlorella sp*, *Oscillatoria salina*, *Synchronous elongatus*). Its degradation start temperature was found to be 261 °C by Thermal Gravimetric Analysis, which is higher than the onset temperatures found in the other algae species under study.[67] When PHAs were subjected to thermal analysis, *Leptolyngbya valderiana* PHAs shown to be more stable than PHAs obtained from other algae species (*Chlorella sp*, *Oscillatoria salina*, *Synchronous elongatus*). Thermal Gravimetric Analysis revealed that it broke down at 261 °C, which was higher than the temperatures at which the other algae species under study showed their disintegration.[60]

2.5 Carbon footprint reduction; Balancing the ecosystem

A dual approach that addresses the issues of plastic waste and greenhouse gas emissions at the same time is the employment of microalgae to absorb CO₂ and produce bioplastics.

This not only reduces reliance on fossil fuels for plastic production but also helps to mitigate the environmental impact of plastic waste. Furthermore, microalgae-derived bioplastics offer potential economic benefits.[44] They are scalable in terms of production and easily incorporated into well-established industrial systems. Through integration, companies may capture carbon emissions from their processes and convert them into useful bioplastics, creating a closed-loop cycle that reduces carbon emissions worldwide.[91] Essentially, a complete carbon capture and utilization solution is provided by the combination of microalgae and bioplastic. It addresses the problems of plastic pollution and climate change while offering opportunities for the economy. Furthermore, by promoting a circular economy and reducing dependency on non-renewable resources, this combined strategy has the potential to further sustainable development goals.[92]

To sum up, the combination of microalgae and bioplastics offers a promising solution to sequester industrial carbon emissions and transform them into environmentally sustainable bioplastics. Because of these advantages and potential, microalgae can be a useful tool in the fight against climate change and energy-related issues.[93]

2.6 Life-cycle assessment of microalgae based PHA production

A thorough life cycle analysis of microalgae derived PHA synthesis requires assessing several important variables. The first things to look at are the energy sources that are used for microalgae cultivation (sunlight or artificial illumination), as well as the carbon emissions that are produced.[94] Using renewable energy sources when cultivating may significantly reduce carbon emissions. It's also critical to evaluate the amount of water used during the growing phase. Water volumes required for microalgae culture are frequently

high, which emphasizes the need to assess water consumption efficiency and putting in place water recycling devices to reduce waste.[95]

Moreover, it is crucial to closely examine the PHA extraction procedure from microalgae biomass. This involves determining the effects of energy inputs on the entire environmental footprint, such as the use of solvents or enzymes.[96] It is essential to consider the end-of-life stage of PHA products based on microalgae. This entails determining if the bioplastic can be recycled or composted and looking at the environmental effects of disposing of it.[97] According to recent evaluations of the life cycle and economic viability of microalgae-based production systems, increasing production may be achieved by the full use of biomass in an integrated biorefinery system. This entails taking every useful component that can be extracted from the biomass, processing it, and making it valuable.[43]

2.7 Techno-economic evaluation and cost considerations

The cost of CO₂ bio-fixation and reuse is dependent on the efficacy of algal biomass as well as the overall success of the culture or CO₂ collection process. The temperature, pond pH, and algal physiology all affect how well these systems capture CO₂. [98] It is advisable and shows potential to use biorefinery techniques to increase the technology's economic viability. This is accomplished by selling the secondary product together with the co-products, excess heat, and power produced.[5]

The primary cost-incorporating elements involve the initial fermentation processes and subsequent technologies employed for recovering PHA downstream.[64] Being a platform for carbon capture and utilization (CCU) from significant and sustainable CO₂ emission sources like power plant flue gas, the use of photosynthetic PHB production shows

promise. It also offers the possibility of additional cost savings.[99][100] The industrial exhaust gas is used as the only source of carbon to generate bioplastic material known as PHB, making up 21.1% of the content. By employing the *Synechococcus* 2973 strain, the biomass's volumetric productivity increased by a factor of 2.4. Furthermore, microalgae such as cyanobacteria are proposed as potential photosynthetic factories for producing bioplastics.[100] In a recent study, researchers proposed that algae biorefineries would need to rely on the sale of electricity and steam heat from their combined heat and power system, along with sales to the grid, to cover operational expenses and reduce material and energy costs. However, on larger scales of operation, the revenue generated from these sales was not enough to offset the total fixed capital investment and annual operating costs. Additionally, it was found that approximately 0.21% to 2.10% of the CHP plant's total CO₂ emissions could potentially be sequestered by algae biomass cultivation across different reactor scales.[101]

2.8 Challenges for Integrated approach and commercialization

The market for algal biorefineries is currently constrained by technological challenges, inefficient use of materials and energy, and intricate process management.[102] While it is possible to produce biodiesel, bio-oil, and bioplastics from algae, the associated costs are still significantly higher than in the conventional petroleum industries.[103] The methods adopted currently to manufacture bioplastics from microalgae primarily involve laboratory-scale processes that concentrate on improving strains that produce biopolymers through genetic engineering and fine-tuning blend compositions.[53] Consequently, difficulties include the high costs associated with large-scale farming as well as the ensuing procedures such as collecting and processing biomass.[43] A source of material with a

consistent level of quality, dryness, and no contamination is necessary for effective production in both biobased and conventional sectors.[104][105] So in this scenario, the industries mainly focused on utilizing or producing the biowastes and biomass on site have advantage.[106]

In order to satisfy industrial demands for bioplastics, microalgae culture must be scaled up. This will require further research and development to optimize growing methods, boost biomass yield, and enhance process efficiency.[68] One challenge is the scalability of microalgae cultivation and bioplastic production. To meet industrial demands, microalgae culture and bioplastic manufacture must be expanded. This will need ongoing research and development to improve growing techniques, increase biomass output, and improve process efficiency.[107] Moreover, a thorough analysis and optimization of the process's overall cost-effectiveness are required to guarantee its competitiveness with respect to existing technologies.[92] Therefore, integrating microalgae cultivation for bioplastic production with carbon capture and storage technologies and wastewater treatment can provide a more economically feasible and environmentally.[108] However, there are still challenges that need to be addressed for the widespread implementation of this integrated approach.

CHAPTER 3

RESEARCH OBJECTIVES

3.1 Research Gap

This study attempts to fill the gap of research by focusing on the production of PHA with cheap and new raw materials with algal specie not represented before. Previous study on *Algae (Chlorella Sorokiniana)* heavily focused Bio-oil, biodiesel and nutraceuticals, etc., and overshadowed the potential for biopolymer/bioplastic application. Much research has made on carbon capture and utilization in other domains lie bio-oil and biofertilizers but none of them deals with biodegradable plastic.

3.2 Overall objectives

The primary objective of this research is to enhance Polyhydroxyalkanoates (PHA) production using an integrated approach that utilizes industrial flue gases, predominantly composed of CO₂, and cultivates the microalgae species *Chlorella Sorokiniana* in wastewater media. This study aims to establish a cost-effective and sustainable alternative to synthetic plastics while contributing significantly to net-zero emissions. It will specifically focus on the effects of various cultivation parameters on PHA accumulation in *Chlorella Sorokiniana* grown in wastewater media.

3.2.1 Specific objectives

The following are the specific objectives that should be attained to achieve the overall objective.

1. Effect of Salinity on the accumulation of PHA(Bioplastic) with autotrophic cultivation of *Chlorella Sorokiniana*(Ck) in synthetic waste water media.
2. To study the effect of nitrogen to phosphorus(N/P) ratio on the accumulation of lipid and PHA in *Chlorella Sorokiniana* (Ck) autotrophically cultivated in synthetic wastewater media (BBM).
3. To see the synergetic effects of varied N:P with optimal salinity on the accumulation of PHA and lipid on *Chlorella Sorokiniana*(Ck) cultivated in synthetic wastewater media.
4. Effect of second stage cultivation with nitrogen deficient media on High N reactors with and without optimal salinity
5. To study the effect of CO₂ concentration, optimal N:P with salinity on Lipid and PHA accumulation in *Chlorella Sorokiniana* autotrophically cultivated in high saline synthetic modified wastewater media.

3.3 Task plan and mapping with objectives

Objective 1	
Effect of Salinity on the accumulation of PHA(Bioplastic) with autotrophic cultivation of <i>Chlorella Sorokiniana</i> (Ck) in synthetic wastewater media	
Task 1	Preparing 6 different concentration synthetic wastewater media with NaCl such as 0,1,2,4,6 and 8g in 1 liter of BBM.
Task 2	Adjusting the pH of the media around 6.8-6.9.
Task 3	Cultivation of the fresh algae culture in the prepared media.
Task 4	Checking optical density, Dry biomass, TN (total nitrogen),TP (total phosphorus) every alternate day
Task 5	Check the concentration of the Lipid by quantified protocol every 4 days.
Task 6	Check for the PHA accumulation by chemical extraction protocol at the end of the run (after 14 days)
Task 7	Characterization of the PHA and Lipid obtained through FTIR
Task 8	Arranging data and preparing the manuscript

Objective 2

To study the effect of nitrogen to phosphorus(N/P) ratio on the accumulation of lipid and PHA in *Chlorella Sorokiniana* (Ck) autotrophically cultivated in synthetic wastewater media (BBM)

Task 1	Preparing 5 different concentration synthetic wastewater media by changing N/P ratio such as (2:1, 1.5:1, 1:1, 0.8:1, 0.6:1, 0.4:1) in 1 liter of BBM.
Task 2	Adjusting the pH of the media around 6.8-6.9
Task 3	Cultivation of the fresh algae culture in the prepared media.
Task 4	Checking optical density, Dry biomass, TN (total nitrogen), TP (total phosphorus) every alternate day.
Task 5	Check the concentration of the Lipid by quantified protocol every 4 days.
Task 6	Check for the PHA accumulation by chemical extraction protocol at the end of the run (after 14 days)
Task 7	Characterization of the PHA and Lipid obtained through FTIR
Task 8	Arranging data and preparing the manuscript

Objective 3	
To see the synergetic effects of varied N:P with optimal salinity on the accumulation of PHA and lipid on <i>Chlorella Sorokiniana</i> (Ck) cultivated in synthetic wastewater media.	
Task 1	Preparing 5 of cultivation media with different N:P (2:1, 1.5:1, 1:1, 0.6:1, 0.4:1) with optimal saline condition of 2%.
Task 2	Adjusting the pH of the media around 6.8-6.9
Task 3	Cultivation of the fresh algae culture in the prepared media.
Task 4	Checking optical density, Dry biomass, TN (total nitrogen), TP (total phosphorus) every alternate day.
Task 5	Check the concentration of the Lipid by quantified protocol every 14 days at the end of cultivation
Task 6	Check for the PHA accumulation by chemical extraction protocol at the end of the run (after 14 days)
Task 7	Characterization of the PHA and Lipid obtained through FTIR and TEM for visualization
Task 8	Arranging data and preparing the manuscript

Objective 4	
Effect of second stage cultivation of high N reactors on accumulation of PHA and lipid on <i>Chlorella Sorokiniana</i> (Ck) cultivated in synthetic wastewater media.	
Task 1	Preparing 4 of cultivation media with different N:P (2:1-0, 1.5:1-0, 2:1-2, 1.5:1-2) with optimal saline condition of 2% and without saline conditions.
Task 2	Adjusting the pH of the media around 6.8-6.9
Task 3	Cultivation of the fresh algae culture in the prepared media.
Task 4	Checking optical density, Dry biomass, TN (total nitrogen), TP (total phosphorus) every alternate day.
Task 5	Check the concentration of the Lipid by quantified protocol every 14 days at the end of cultivation
Task 6	Check for the PHA accumulation by chemical extraction protocol at the end of the run (after 14 days)
Task 7	Characterization of the PHA and Lipid obtained through FTIR and TEM for visualization
Task 8	Arranging data and preparing the manuscript

Objective 5

To study the effect of CO₂ concentration, optimal N:P with salinity on lipid and PHA accumulation in *Chlorella Sorokiniana* autotrophically cultivated in high saline synthetic modified wastewater media.

Task 1	Preparing 5 sets of cultivation media and each set will get different CO ₂ concentration such as (0,2,4,6,8) percent.
Task 2	Adjusting the pH of the media around 6.8-6.9
Task 3	Cultivation of the fresh algae culture in the prepared media.
Task 4	Checking optical density, Dry biomass, TN (total nitrogen), TP (total phosphorus) every alternate day.
Task 5	Check the concentration of the Lipid by quantified protocol every 4 days.
Task 6	Check for the PHA accumulation by chemical extraction protocol at the end of the run (after 14 days)
Task 7	Characterization of the PHA and Lipid obtained through FTIR
Task 8	Arranging data and preparing the manuscript

CHAPTER 4

RESEARCH METHADODOLOGY

4.1 Materials and Methods

4.1.1 Microalgae strain

The University of Texas, Austin, USA, is the source of the microalgae strain *Chlorella Sorokiniana* (UTEX B 746), which is being used in the experiment.

4.1.2 Culture medium

Bold basal medium, or BBM, is the basic culture medium that will be utilized to grow microalgae. Its composition is directly sourced from the University of Texas website, which offers a list of solutions that have been created in advance and are then utilized to prepare the medium. In addition, for the study of the effects of N and P concentrations on lipid accumulation and PHA (bioplastic). The range of N:P ratio concentrations that were chosen and extrapolated. Artificial media is seen as a modified version of BBM. The sources of phosphorus and nitrogen are $\text{PO}_4^{3-}\text{-P}$ and $\text{NO}_3\text{-N}$, respectively.

Table 3: BBM components and solution concentrations/ Synthetic wastewater media

No.	Component	Amount	Stock soln. Concentration	Final Concentration
1	NaNO_3	10 mL	10.0 g/400 mL	2.94 mM
2	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	10 mL	1.0 g/400 mL	0.17 mM

3	MgSO ₄ •7H ₂ O	10 mL	3.0 g/400 mL	0.30 mM
4	K ₂ HPO ₄	10 mL	3.0 g/400 mL	0.43 mM
5	KH ₂ PO ₄	10 mL	7.0 g/400 mL	1.29 mM
6	NaCl	10 mL	1.0 g/400 mL	0.43 mM
7	EDTA Stock 1	1 mL		
8	Iron Stock 2	1 mL		
9	Boric acid Stock	1 mL		
10	Bold Trace Stock 3	1 mL		

Stock 1: (EDTA Stock solution)

1. Add the following ingredients to around 900 mL of deionized water in the given order, stirring constantly: • 50 grammes of acidic EDTA (final solubility ~ 8.5 µM) • KOH in the amount of 31 grammes
2. Reduce the total volume to one litre and store in the dark at room temperature.

Stock 2: (Acidic Iron stock solution)

1. Add the following ingredients to about 950 mL of deionized water in the given order, stirring constantly: • H₂SO₄ in a 10-mL volume; 5 grammes of FeSO₄• 7H₂O (final soln. ~ 0.9 µM FeSO₄)
2. Reduce the total volume to one litre and store in the dark at room temperature

Stock 3: (Boron Stock Solution)

1. Dissolve 11 grammes of H_3BO in about 900 mL of d. H_2O (final soln. $\sim 9 \mu\text{M}$ H_3BO_3).
2. Reduce the total volume to one liter and store in the dark at room temperature.

4.2 Experimental Procedure and Design

Batch photobioreactors (1L Erlenmeyer flask) are used to cultivate microalgae species, and each photobioreactor holds up to 850 working liquid material. To avoid studying the lag phase, the microalgae sp. is first pre-cultured before being added to the photobioreactors. Aluminum foil is placed over the flasks to shield them from outside contamination. To cover as much of the photobioreactor surfaces as possible, the photobioreactors are set up in a fume hood that has four vertically aligned Groulx fluorescent (Sylvania F18W/T8/GRO) 34 light tubes for continuous lighting.

The light intensity at the reactor surface was measured using a Fisher Scientifics TraceableTM Dual-Display Light Metre, which recorded light levels between 1600 and 1800 LUX. The schematic diagram of the experimental setup used in this investigation was shown in Fig. 8. Using an air- CO_2 mixture device FC-SH (Live Cell Instruments, South Korea), the air and air- CO_2 mixture are accurately delivered to the bottom of the photobioreactor. In order to keep conditions fully mixed, gas bubbles rise through the liquid medium and exit at the top of the reactor. This prevents cell sedimentation and supplies inorganic carbon (CO_2). Algae use carbon dioxide as their carbon source for cell development during photosynthesis. As photosynthetic microorganisms, *Chlorella Sorokiniana* can grow in aqueous suspension by ingesting nutrients from wastewater and

using solar energy to combine water and CO₂ to produce biomass that contains lipids, hydrocarbons, and PHA (bioplastic).

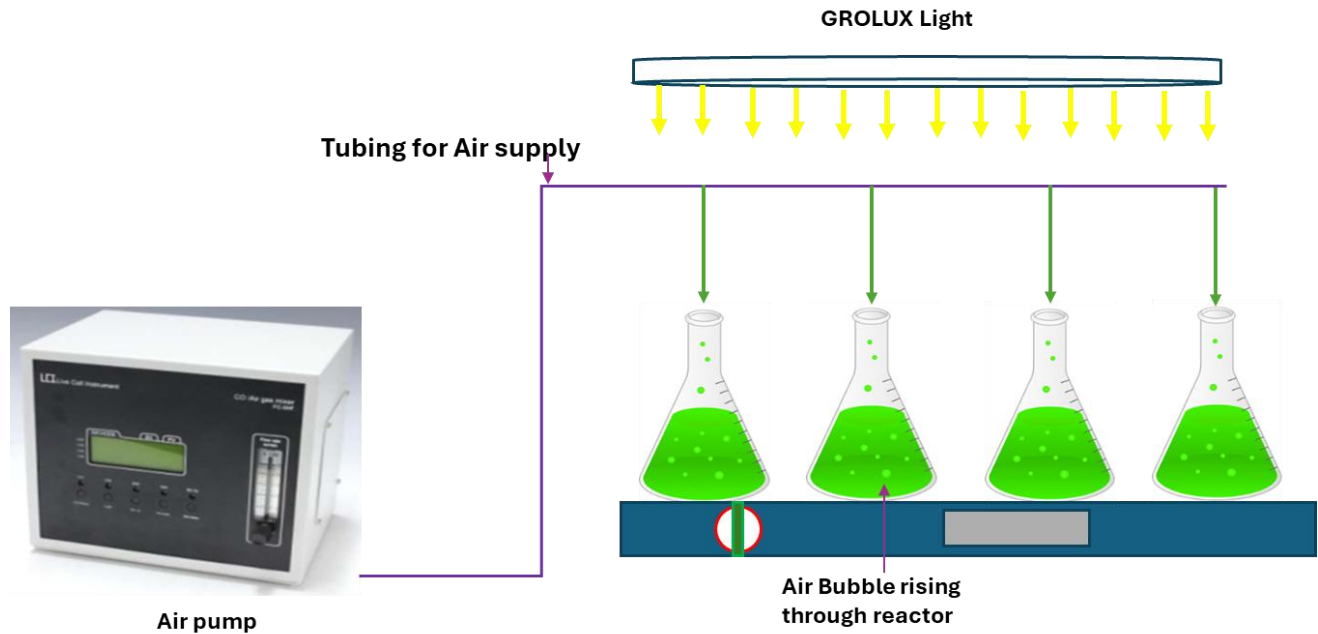


Figure 8: Experimental Setup design

4.3 Analytical Methods

4.3.1 Effect of salinity

Chlorella sorokinina taken from a fresh saline culture previously cultivated and added to six batch photobioreactors illuminated and provided with air contains 0.03% of CO₂ for each of them but a different range of sodium chloride is added as 0,1,2,4,6 and 8% to see the effects on the growth of microalgae and lipid and PHA accumulation. One of the photobioreactor would be exactly the in high saline concentrations, the microalgal culture is under stress and would behave differently in terms of CO₂ fixation and bioproduction. For example, the utilisation of high salinity conditions as a strategy to enhance the production of algal lipids has been the subject of numerous studies. All things considered,

salt stress encourages the creation of lipids and β -carotene, a phenomena that can be profitably marketed.[109] PHB accumulation is stimulated by increasing salinity in *Synechocystis* sp.[110]

4.3.2 Effect of nitrogen to phosphorus ratio (N:P)

Chlorella Sorokiniana was first cultivated in a medium that had been created with different N:P ratios to investigate the effects of N and P concentrations on microalgae growth, nutrient removal, lipid content, PHA accumulation, and CO₂ bio-fixation. The reported concentrations of domestic wastewater in weak to high strength are used to maintain the range of N and P concentrations. The MBBM medium is used for the batch tests, and several N:P ratios—2:1, 1.5:1, 1:1, 0.8:1, 0.6:1, and 0.4:1 is used. Additionally, other batch experiments have been conducted concurrently with modified BBM medium for comparison, with each experiment run maintaining a carbon dioxide concentration of 0.03%. The temperature, PH, and other cultivation parameters were all the same as those listed in the previous portion of the experimental process. More experiments will subsequently be conducted for both species in accordance with our experimental strategy. Total nitrogen (TN) in the form of N-NO₃⁻ and total phosphorus (PO₄³⁻) in culture media are measured daily using a spectrophotometer (DR 3900-HACH, USA) and digital reactor (DRB 200-HACH, USA) to analyze the concentration of nitrogen and phosphorus. The following formula is used to determine the proportion of TN and TP elimination rates in batch kinetics 39.

$$\%consumption = \frac{S_0 - S_t}{S_0} \times 100\% \dots \dots \dots (1)$$

Where S_0 and S_t represent the substrate concentrations at the initial time, t_0 and at the end of the cultivation period, t_t respectively.

4.3.3 Effect of nitrogen to phosphorus ratio (N:P) with optimal salinity

The influence of varying nitrogen-to-phosphorus (N:P) ratios-2:1, 1.5:1, 1:1, 0.6:1 and 0.4:1 was studied alongside optimal salinity levels (2% NaCl), known to promote PHA accumulation, to evaluate how nutritional balance affects microalgal efficiency. Different N:P conditions were monitored for alterations in growth rate, nutrient removal effectiveness, and polymer yield, particularly focusing on the shift from nitrogen-sufficient to nitrogen-limited environments. This integrated stress approach aimed to replicate real-life scenarios where salinity and nutrient levels fluctuate together, allowing for an assessment of how nutrient scarcity in saline conditions impacts metabolic pathways that enhance the production of biopolymers in *Chlorella sorokiniana*.

4.3.4 Effect of Second stage cultivation of High N reactors with and without salinity

Using cultures that had earlier been grown under high nitrogen (N) conditions, a second-stage cultivation method was employed to enhance PHA accumulation following a biomass-rich phase. These cultures were transferred to nitrogen-limited media after the initial growth phase to introduce metabolic stress that would benefit polymer synthesis. Two conditions were assessed simultaneously: one involving 2% NaCl, which had shown optimal results in PHA production, and the other using standard BBM media without added NaCl. This approach aimed to evaluate whether nitrogen deficiency and the osmotic stress caused by salinity could further stimulate PHA accumulation. Furthermore, this phase also

shed light on the resilience and adaptability of *Chlorella sorokiniana* when faced with sequential stress factors.

4.3.5 Effect of CO₂ concentration

By adjusting the CO₂ concentration, the impact of CO₂ levels on biomass production, concentration, carbon content, and CO₂ bio-fixation capability are examined. The culture will first be aerated at a 1500 cc/min air flow rate without adding CO₂, considering that the air contains roughly 0.03% of CO₂. The following batch is then run at the same flow rate with additions of 2%, 4%, 6%, and 8%, in that order. Through the examination of the impact of CO₂ concentration, the culture's N:P ratios are maintained at their ideal levels.

4.3.6 Biomass concentration, Specific growth rate and biomass productivity

a. Biomass concentration

Using a UV-VIS spectrophotometer (Evolution 260 Bio-Thermo Scientific), the optical density (OD) at a wavelength of 680 nm is measured every day to monitor the algal biomass. Simultaneously, a daily 15 ml microalgae sample was extracted from the photo-bioreactor to determine the biomass's dry weight (mg/l). Accordingly, the dry weight amount indicates the microalgae species' biomass concentration.

b. Specific growth

The formula below was used to determine the specific growth rate, or μ_g , which is the dry biomass weight increase each day.

$$\text{Specific Growth rate, } \mu_g = \frac{\ln\left(\frac{x_2}{x_1}\right)}{t_2 - t_1} \dots\dots\dots (2)$$

Where, x_1 and x_2 represent the dry biomass weight at time t_1 and t_2 respectively of the exponential growth phase.

c. Biomass Productivity

The following formula was used to calculate the biomass productivity (P_b) in mg/L/d, also known as the rate of biomass generation.

$$\text{Biomass Productivity, } P_b = \frac{X_t - X_0}{t_t - t_0} \dots \dots \dots (3)$$

Where X_0 and X_t indicate the dry biomass weight in mg/L at the initial time t_0 in days and at the end of the cultivation period t_t .

4.3.7 Determination of carbon content and carbon dioxide biofixation rate

The biomass's carbon content, evaluated through CHNS elemental analysis, was utilized to calculate the CO₂ fixation rate to evaluate the carbon capture abilities of *Chlorella sorokiniana*. To find the carbon percentage on a dry weight basis, samples of dried algal biomass collected at the end of the growth phase were analyzed using CHNS (Elementar Vario EL or similar). The CO₂ fixation rate (g CO₂/L/day) was calculated with the assumption that all fixed carbon originates from the CO₂ absorbed during the photosynthesis process.

Where:

$$\text{CO}_2 \text{ Fixation rate} = P \times \left(\frac{C}{100} \right) \times \left(\frac{44}{12} \right) \dots \dots \dots (4)$$

P represents the productivity of biomass (g/L/day), which is determined from dry weight measurements over a specified time period. C indicates the carbon content of the biomass

(%_{DWB}), as analyzed through CHNS methods. The ratio of CO₂ to carbon molar mass is expressed as $\frac{44}{12}$ (where CO₂ = 44 g/mol and C = 12 g/mol). Using this approach, the quantity of atmospheric CO₂ that is physiologically incorporated into microalgal biomass under different cultivation conditions can be quantitatively assessed. The resulting data provides insight into the effectiveness of the system in terms of bioconversion and carbon sequestration.

4.3.8 Determination of lipid content in microalgae biomass

The Sulfo technique methodology is followed when analyzing lipid content.[111] A calibration curve process that the SPV method describes. To quantify the amount of lipid within the cell, 15 milliliters of microalgae were taken from each photobioreactor. To get rid of salt from the cell surface, samples are further diluted if needed, centrifuged for ten minutes at 4500 rpm, and then washed three times with distilled water. To the biomass, one milliliter of concentrated sulfuric acid (98%) is added. Afterwards, an ice bath is used to cool it for five minutes. Five milliliters of recently made SPV reagent are added to the sample once it has cooled. Samples were then placed in an incubator shaker at 200 rpm and 37°C. Lastly, using a spectrophotometer to measure the absorbance at 530 nm, the lipid content of the sample was determined. illustrated the lipid colorimetric assay's schematic form. Lipid contents within the samples were determined to be $y_{OD_{530}} = 0.0333X_{\text{lipid}} + 0.1316$ with an R² of 0.9878 (Fig. S1 in Supplementary information).[112] Finally, total lipid contents were expressed as percent of dry biomass weight using the following equation (5) [113].

$$\text{Lipid Content}(\%) = \frac{X_L}{X_B} * 100 \dots\dots\dots (5)$$

where X_L is the amount of lipid in the biomass dry weight (X_B) of the sample. Moreover, lipid productivity was determined according to the equation described by a study [114].

$$\text{Lipid Productivity}(\text{mgL}^{-1}\text{d}^{-1}) = \text{Biomass Productivity}(\text{mgL}^{-1}\text{d}^{-1}) * \frac{\text{Lipid Content}(\%)}{100} \dots\dots\dots (6)$$

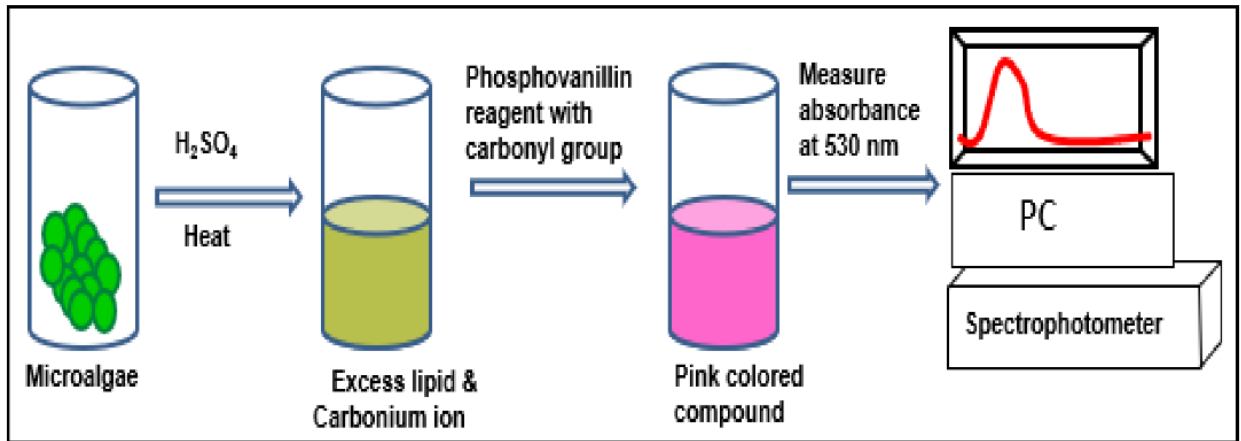


Figure 9: Lipid quantification technique(SPV-method)Adapted from[115]

4.3.9 Determination of PHA accumulation in microalgae biomass

Each photobioreactor run was centrifuged to precipitate the biopolymers after the cultivation period of fourteen days. Then, 10% to 12% v/v and 4% sodium hypochlorite solution (20 min 45 degree) were added to the mixture, and the mixture was again centrifuged. After discarding the supernatant, the precipitate was cleaned with distilled water, centrifuged once more, and acetone was added to precipitate the biopolymer (2 hours at 45 degrees). After that, it was dried for 48 hours at 35°C in an oven.[116][117]

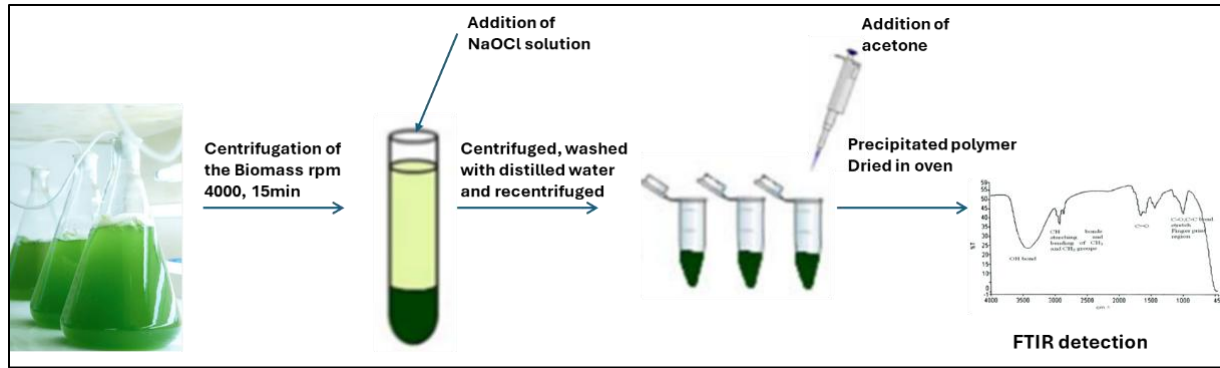


Figure 10: PHA quantification and detection method

According to equation (4), which has been applied in earlier research, the dry weight of a 1L culture after 14 d of cultivation was utilized to calculate the percentage of PHA buildup inside the microalgal cell [118].

$$\% \text{ PHA accumulation} = \frac{\text{Weight of extracted polymer}}{\text{Weight of dry biomass}} \times 100 \dots \dots \dots (7)$$

4.3.10 Proposed Model for PHA productivity with Nitrogen deficiency

PHA productivity varies over time and is primarily influenced by the nitrogen concentration present in the cultivation media. It has been noted that under conditions where nitrogen is limited, the accumulation of PHA increases rapidly [119]. With this in mind, we have developed a model that aims to forecast PHA growth over time, allowing for quick predictions of its behavior in specific conditions. Microalgae experience three distinct phases, with each phase exhibiting different characteristics in terms of PHA accumulation. The phases include the lag phase: where the cells adapt to their new environment, and no PHA accumulation takes place. The growth rate constant (k) is approximately zero. Next is the exponential phase (growth-driven accumulation): In this phase, the cells are dividing, leading to a gradual increase in initial PHA accumulation. The k value is primarily determined by growth in this phase and is associated with the growth

rate. Finally, there is the stationary phase (storage-driven accumulation): here, a higher k value is employed to represent the rate of PHA accumulation as growth begins to decelerate [120]. To capture this, we can employ a modified logistic function that exhibits a more pronounced rise in PHA production following nitrogen depletion. A sigmoidal (S-curve) model featuring an inflection point once nitrogen is exhausted is superior to a basic logistic model [121]. The proposed function:

$$PHA(t) = \frac{P_{max}}{1+e^{-k_t(t-t_c)}} \dots \dots \dots (8)$$

t denotes the time (typically measured in days) at which the concentration of PHA is being evaluated. P_{max} signifies the peak concentration of PHA attained. k_t refers to the constant for the growth/accumulation rate of PHA. t_c indicates the critical time (or inflection point) at which the rate of PHA accumulation reaches its peak. Thus, t is the time variable that varies as PHA is monitored over several days. By inputting a specific day into t , the equation provides the predicted PHA concentration for that moment. Additionally, the value of k alters during various growth stages, such as the lag phase, exponential phase, and stationary phase [122]. When nitrogen levels are high, k remains low. As nitrogen levels diminish, k rises towards k_{max} . Currently, k is a function that is increasing [122].

$$k_t = k_{min} + (k_{max} - k_{min}) * \left(1 - \frac{N(t)}{N_0}\right)$$

Table 4: Variable function in PHA productivity model and their dependence

Variable	Formula	Depends on
$N(t)$	Interpolated from Data	Experimental Values
k_t	$k_{min} + (k_{max} - k_{min}) * \left(1 - \frac{N(t)}{N_0}\right)$	Nitrogen Concentration
PHA_t	$PHA(t) = \frac{P_{max}}{1 + e^{-k(t-t_c)}}$	Growth Rate k_t

4.4 PHA characterizations and visualizations

4.4.1 Fourier Transform infrared spectroscopy (FTIR)

FTIR spectroscopy was conducted to verify the functional groups found in the extracted PHA. The spectra were obtained using a (Model: Thermo Scientific Nicolet Is5) over the wavenumber range of 4000–400 cm^{-1} . Peaks associated with ester bonds (C=O stretch) and additional functional groups typical of PHA were recognized.

4.4.2 Thermogravimetric Analysis (TGA)

The thermogravimetric analysis of microalgae PHA provides essential insights into the thermal behaviour of the PHA sample. TGA measurements were conducted using a TGA instrument model STDG 600 from TA Instruments, USA. Samples weighing 1 mg were placed in the sample holder and heated from room temperature to 800 °C in an inert nitrogen atmosphere at a heating rate of 10 °C/min. The proximate analysis of moisture content (MC), volatile matter (VM), fixed carbon (FC), and ash content of the PHA sample was directly determined using the TG curve.

4.4.3 Transmission Electron Microscopy (TEM)

TEM investigation was conducted to examine the morphology of PHA granules located within the microalgae cells. The samples were dispersed in absolute ethanol and sonicated for 30 min and analyzed with a [Jeol JEM-F200] at an accelerating voltage of [200 kV]. The resulting micrographs illustrated the arrangement and dimensions of intracellular PHA granules.

4.4.4 CHNS Analysis

Elemental analysis (CHNS) was performed to determine the carbon content in the dried microalgal biomass, thereby quantifying the amount of carbon fixed during cultivation. A known amount of lyophilized biomass was subjected to CHNS analysis using a CHNS elemental analyzer (2400 Series II CHNS Elemental Analyzer). The sample was combusted in an oxygen-rich environment, and the resulting gases were analyzed to determine the percentage composition of carbon, hydrogen, nitrogen, and sulfur. The carbon content obtained was used to estimate the extent of carbon assimilation by the microalgae during the growth period.

4.5 Flow chart of Methodology

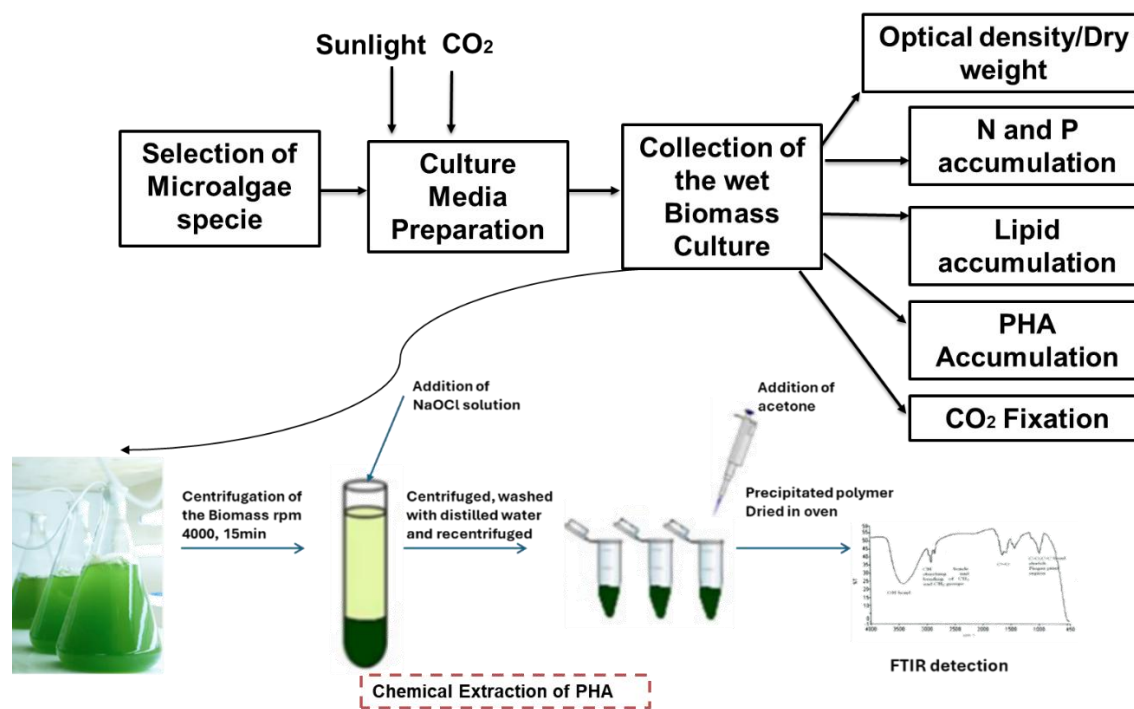


Figure 11: Flow Chart of Methodology

CHAPTER 5 RESULTS AND DISCUSSION

5.1 Effect of Salinity on microalgae growth

5.1.1 Biomass Concentration

The adaptability of *Chlorella sorokiniana* to saline stress was assessed to determine its growth characteristics as a freshwater microalga. This species generally flourishes in wastewater environments or synthetic wastewater media [123]. Growth was tracked in six photobioreactors (0% salinity, 1% salinity, 2% salinity, 4% salinity, 6% salinity, and 8% salinity) by measuring dry weight on alternate days and evaluating live cell counts using OD₆₈₀. Biomass concentration (mg/L) served as a primary indicator of growth. The results indicated that the highest biomass production occurred in 2% salinity at 1133 mg/L, followed by 8% salinity at 1066 mg/L (Figure 11). The control media with 0% salinity showed reduced growth at 866 mg/L, suggesting that *Chlorella sorokiniana* can withstand and potentially thrive in moderate saline environments. These results are consistent with earlier research on salt-tolerant microalgae, such as *Chlorella sp.* MACC-360, which showed robust growth up to 600 mM NaCl (35 g/L) [124]. Other species, such as *E. marina*, demonstrate increased cell growth at ideal salinity conditions [125]. However, high salinity levels can interfere with metabolic functions, resulting in lower biomass production or cell mortality [126]. Likewise, the growth of *Scenedesmus obliquus* was hindered by elevated NaCl concentrations, which caused oxidative stress and damage to photosynthesis [127]. A maximum biomass concentration of 1.08 g/L was recorded at 85.56 mM (5 g/L NaCl), but higher concentrations (10–20 g/L NaCl) resulted in ion toxicity, diminishing biomass by as much as 9.29%. These findings emphasize the necessity of adjusting salinity levels

to optimize microalgal growth while reducing stress-induced limitations in commercial production systems [128].

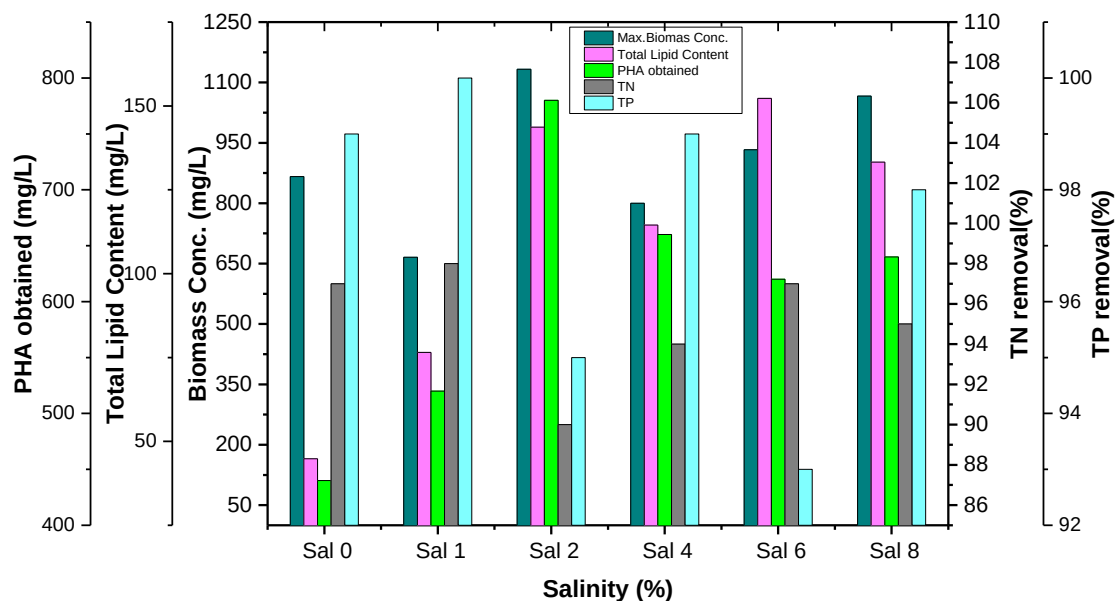


Figure 11: Effect of varying levels of salinity (0,2,4,6,8 percent i.e., 2% is 2g of NaCl) on maximum biomass concentration X_{max} in mg/L, Total lipid Content (mg/L), Quantity of PHA obtained(mg/L) and percent removal of total nitrogen(TN) and total phosphorus(TP) at the end of 14 day cultivation for *Chlorella Sorokiniana*.

5.1.2 Biomass productivity and specific growth rate

In this experiment, other growth parameters of *C. Sorokiniana* were also evaluated which were essentially helpful to see a clear behaviour of microalgae in saline environment. A salinity level of 2% resulted in the highest biomass productivity at 57.14 mg/L/d, accompanied by a specific growth rate (μ_{max}) of 0.26 d⁻¹. In contrast, a salinity level of 8% yielded a biomass productivity of 52.35 mg/L/d with a μ_{max} of 0.12 d⁻¹ (Figure 12a). The optimal salinity level was identified as 2%, as this concentration supports favourable outcomes regarding maximum PHA accumulation and biomass concentration. The impact

of salt stress on growth and pigment synthesis was examined in research involving the halotolerant microalga *Dunaliella salina*. The findings indicated that while *D. salina* thrives in high salinity environments, its growth rates decline when salinity exceeds a specific range that maximizes growth and pigment formation, Research on *Nannochloropsis* sp. examined how different levels of light intensity and varying salinity (13, 27, and 40 g/L NaCl) influenced lipid productivity and growth parameters. It was found that changes in salinity significantly affected both biomass productivity and the specific growth rate [129].

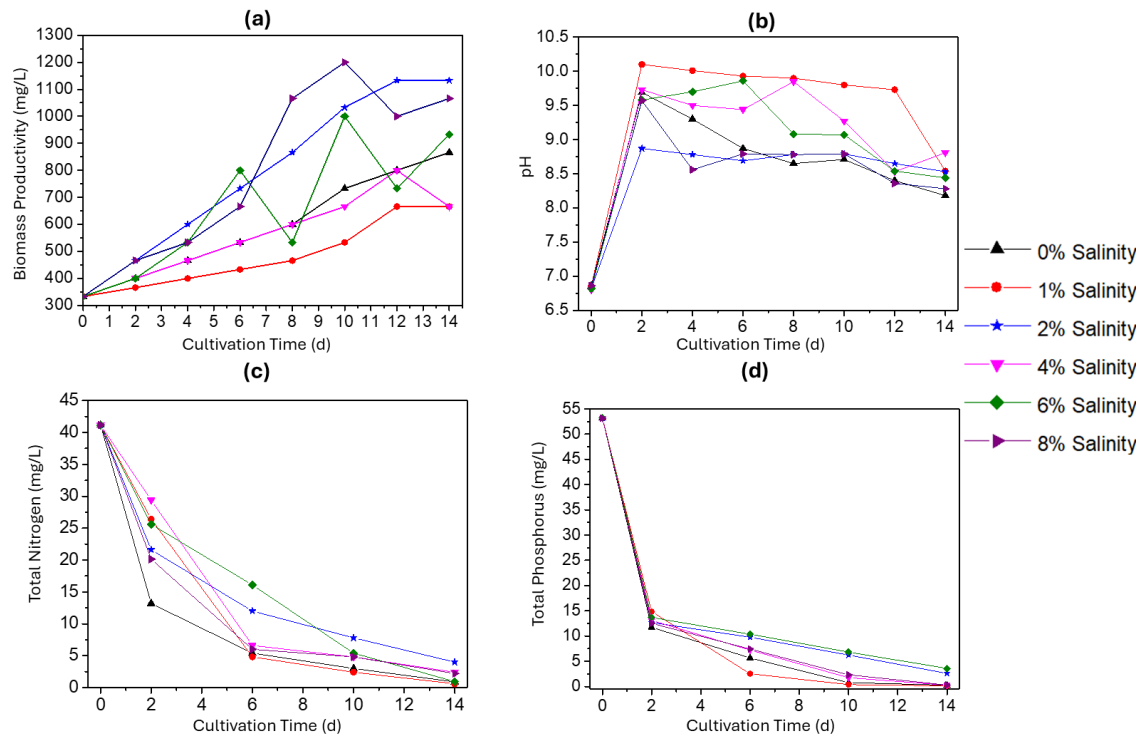


Figure 12: Effect of varying level of salinity (0,1,2,4,6,8 percent i.e., 2% is 2g/L of NaCl) (a) biomass growth over time(biomass productivity P_B (mg/L/d), (b) variation in pH during 14-days cultivation,(c) total nitrogen in media with time (mg/L) and (d) total phosphorus in media with time (mg/L)

5.1.3 Nutrient removal analysis (NO_3^- And PO_4^{3-})

Nitrate (NO_3^- -N) removal efficiency varied with salinity. The highest removal efficiency was recorded at 0% salinity (97.69%), with a slight increase noted at 1% salinity (98.54%). However, as salinity rose to higher levels, the efficiency declined, such as at 2% salinity (90.28%), then showed an increase at 4% salinity (94.17%), followed by an elevation at 6% salinity (97.71%), and finally a decrease again at 8% salinity (94.65%) (Figure 12c). While *Chlorella Sorokiniana* is halotolerant, it requires an adaptation period, evident in the delayed nitrogen uptake in saline conditions [130]. The most significant nitrogen removal in BBM was observed during the initial two days (67.93%), while uptake was more gradual under saline conditions. By the tenth day, nitrogen removal at 1% salinity achieved 94%, exceeding the removal rates of 0% salinity (92%) and 6% salinity (87%). Higher nitrate removal in 6% salinity may be attributed to bioadsorption by both live and dead algal cells, as salinity can enhance nitrate adsorption capacity. Nitrate uptake mechanisms include bioadsorption, bioaccumulation, and metabolic consumption. Literature suggests that dead algae exhibit increased bioadsorption capacity in saline environments, contributing to higher nitrogen removal [131]. Overall, lower salinity levels promoted faster nitrogen uptake, but *C. sorokiniana* required additional time to fully acclimate to high salinity conditions. The 90.28% nitrogen removal observed in a 2% saline environment, which also showed the peak biomass of 1133 mg/L, suggests that moderate stress boosts both growth and nutrient absorption [131].

Phosphate concentration directly influences microalgal growth kinetics and nutrient removal efficiency [132]. Studies indicate that nitrogen uptake is hindered when phosphorus availability is insufficient. Phosphates act as a limiting substrate, and

maintaining an optimal nitrogen-to-phosphorus (N:P) ratio is crucial [131]. Research shows that reducing total phosphorus concentration from 2.00 to 0.10 g/L decreases nitrogen removal efficiency from >99% to 39%, highlighting the interdependence of these nutrients [133]. In this study, the BBM and modified BBM (with NaCl) provided an N:P ratio of 0.8:1. The efficiency of phosphorus removal was significant in conditions of 0% salinity (99.5%), 15% salinity (99.73%), 4% salinity (99.47%), and 8% salinity (99.5%), indicating that saline environments facilitate phosphate removal (Figure 3d). However, in 2% and 6% saline conditions, phosphorus removal was lower (77% and 74%, respectively) after two days, indicating that early phosphate depletion may have restricted nutrient uptake and nitrogen removal. Previous studies confirm that phosphorus limitation can significantly reduce nitrogen removal efficiency and nutrient assimilation in microalgae [134]. These findings suggest that while salinity enhances nutrient removal, an optimal balance is required to maximize both phosphorus and nitrogen uptake efficiency in microalgal-based wastewater treatment.

5.1.4 pH Profile

pH is essential for the growth of microalgae as it influences the solubility and availability of nutrients [135]. In this research, initial pH was established between 6.8 and 6.9, which is the optimal range for *Chlorella sorokiniana*, allowing for maximum biomass production [136]. In the first two days, pH increased rapidly, reaching around 10, which corresponds with the ideal growth range (6–10). In 0% salinity, the peak pH recorded was 9.69, attributed to nutrient availability, a pattern noted in all reactors except 2% salinity. By the end of day 14, pH declined to a range of 8.18 to 8.80 across all reactors, reflecting nutrient depletion and peak algal growth. Interestingly, in 2% salinity, pH remained below 8.87,

indicating favorable conditions for swift biomass growth even within the initial two-day period (Figure 12b). This underscores the significance of moderate salinity in improving the efficiency of microalgal growth. A research study examined the impact of different salinity levels on the proximate composition and growth performance of microalgae. The findings indicated that fluctuations in salinity could influence metabolic activities, which may subsequently alter the pH of the culture medium [137].

5.2 Effect of N:P ratio on microalgae growth

5.2.1 Biomass concentration

This research assessed the impact of different initial total nitrogen levels on the growth and growth rate of the microalga *Chlorella sorokiniana*. Nitrogen, supplied as sodium nitrate, is the second most important inorganic nutrient for the growth of microalgae [138]. Since the availability of nitrogen plays a crucial role in cell growth, changing its concentration has a direct effect on biomass productivity and growth rate. The highest biomass concentration ($X_{\max}$) recorded for *Chlorella sorokiniana* was 1000 mg/L on day-14 with a nitrogen to phosphorus (N:P) ratio of 2:1 (Figure 13). Comparable growth trends were noted in nutrient-dense media (1.5:1 and 1:1), with biomass still increasing on day-14, indicating that the elevated nitrogen levels facilitated a prolonged exponential growth phase, as other research has also shown [139] (Figure 14a). The elevated nitrogen content also resulted in a delay of nutrient depletion during this prolonged exponential growth. Optical density (OD_{680}) measurements corroborated this vigorous growth, measuring 2.410 (2:1-N:P), 2.633 (1.5:1-N:P), and 2.487 (1:1-N:P) after 14 days. However, nitrogen depletion results in a superior accumulation rate of PHA per dry weight biomass, as N:P-0.4:1 created 40% PHA per dry weight biomass. For a low N:P ratio, we found a poor

biomass production because nitrogen was depleted early. These findings indicate that enhanced nitrogen availability encourages continued exponential growth, thereby prolonging the biomass production phase [140].

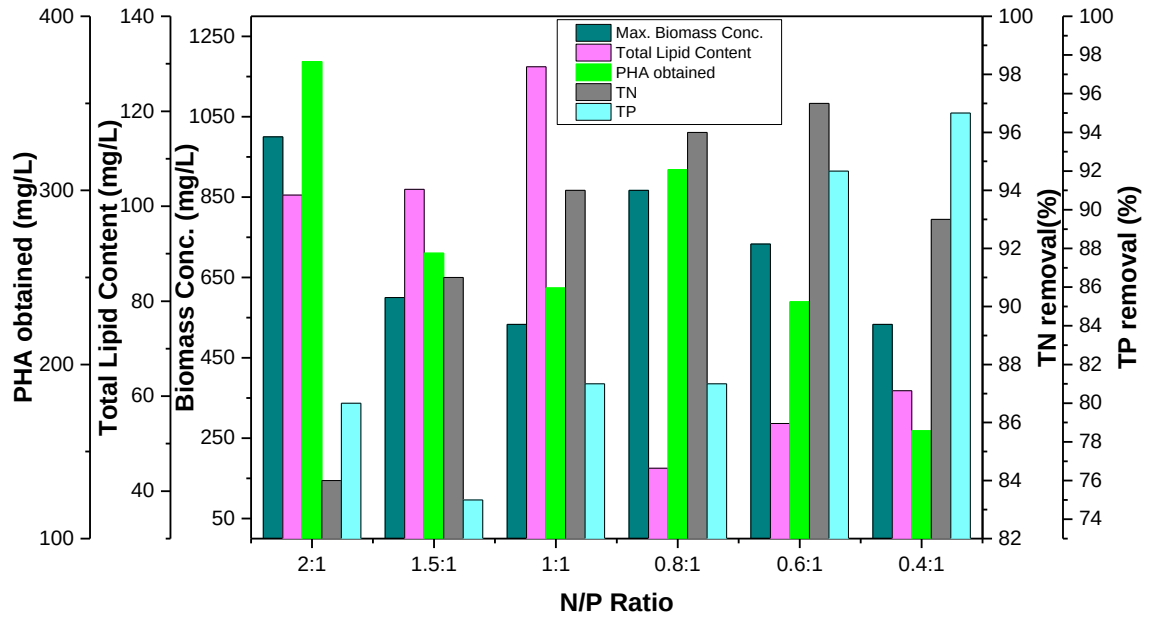


Figure 13: Effect of varied N:P(2:1, 1.5:1, 1:1, 0.8:1, 0.6:1, 0.4:1) ratios on maximum biomass concentration X_{max} (mg/L), Total lipid content (mg/L), Quantity of PHA obtained(mg/L) and percent removal of total nitrogen(TN) and total phosphorus(TP) at the end of 14-days cultivation for *Chlorella Sorokiniana*.

5.2.2 Biomass productivity and specific growth rate

In this study, the highest biomass productivity (52.42 mg/L/d) and the most significant specific growth rate (0.1339 d^{-1}) were recorded in the 0.8:1-N:P photobioreactor (using standard BBM) among all N:P ratios, likely attributed to minimal adaptation time and the lack of a lag phase. Generally, the exponential growth phase in BBM concludes around day-10 (Figure 14a). Reduced nitrogen levels (0.6:1-N:P and 0.4:1-N:P) resulted in a shorter exponential phase and a diminished maximum biomass yield, even though the

initial growth rate was satisfactory. For example, for N:P of 0.6:1, the exponential phase was briefer, and for 0.4:1, the biomass only reached 533 mg/L (Figure 13). This finding supports earlier research suggesting that nitrogen scarcity drives microalgal metabolism towards the accumulation of energy storage compounds, like biopolymers, instead of continuous biomass production. A study demonstrated that there was a 20% decrease in the maximum productivity of the *Chlorella minutissima* culture, decreasing from $0.025 \text{ g L}^{-1} \text{ d}^{-1}$ with the standard medium to $0.020 \text{ g L}^{-1} \text{ d}^{-1}$ using the limited medium. A comparable trend was noted in the specific growth rate (μ_{max}), which experienced a 23% decline when nitrogen availability was restricted [74]. A different study showed that raising nitrogen levels from 72 mg/L to 144 mg/L enhanced biomass production in *Isochrysis galbana*. Additionally, the same research found that biomass yield under nitrogen-limited conditions—given that phosphorus was adequate—was similar to biomass production under ideal nutrient conditions (control) [141].

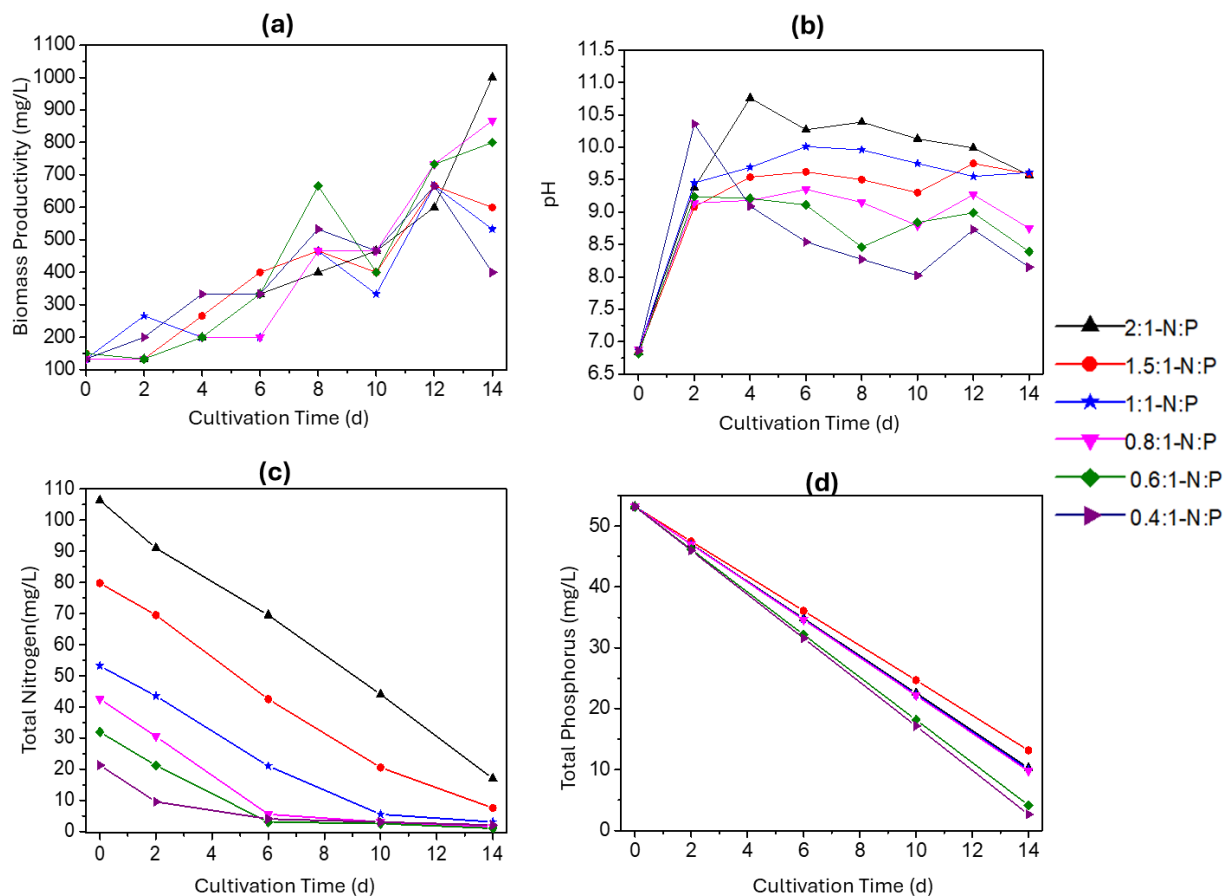


Figure 14: Effect of varying N:P (2:1, 1.5:1, 1:1, 0.8:1, 0.6:1, 0.4:1) on (a) biomass growth over time biomass productivity P_B (mg/L/d), (b) variation in pH during 14-days cultivation, (c) Total nitrogen in media with time (mg/L) and (d) total phosphorus in media with time (mg/L)

5.2.3 Nutrient removal analysis (NO_3^- And PO_4^{3-}) under varied nutrient loading

The initial concentration of nitrogen and phosphorus available in the synthetic wastewater media affects how it microalgal growth and the accumulation of lipid, protein and carbohydrates [142]. Different microalgal species demonstrate unique reactions to variations in nitrogen levels. In this study, total phosphorus (TP) was kept constant across all conditions, while only nitrogen concentrations varied from the standard BBM. The results indicated that increased nitrogen levels prolonged the exponential growth phase, leading to ongoing biomass production even after 14 days of cultivation. For N:P ratios of

2:1 (106.34 mg/L of N), 1.5:1 (79.75 mg/L of N), and 1:1 (53.17 mg/L of N), a consistent rise suggests that microalgae require additional time to fully utilize all the nitrogen in the media. This finding aligns with other research indicating that, at the maximum nitrogen loading rate of $63 \text{ mg N L}^{-1} \text{ d}^{-1}$, microalgae were unable to fully assimilate all the nitrogen that was added [140]. The residual nitrogen levels for N:P ratios of 2:1, 1.5:1, and 1:1 were recorded at 17 mg/L, 7.5 mg/L, and 3 mg/L, respectively (Figure 14c). Confirmations from OD680 indicate that the microalgae remain in the exponential growth phase and have not yet begun to decline. However, under nitrogen-deficient conditions (N:P ratios of 0.6:1 and 0.4:1), the total nitrogen removal rate is higher, achieving peak efficiency over a 14-day period.

Nitrogen and phosphorus are both essential macronutrients for the growth of microalgae, and they are typically found in high concentrations in wastewater. However, the proportion of these nutrients in the media influences the behaviour of microalgae. Numerous studies have indicated that there is an ideal level of phosphorus that must be present in the media to enhance nitrogen removal and subsequently impact growth and biochemical composition [143]. In our research, we discovered that a nitrogen to phosphorus ratio of 2:1 results in a significant reduction in nitrogen levels by 84% (Figure 13), whereas the efficiency of phosphorus removal is comparatively lower. This could be due to microalgae focusing primarily on nitrogen absorption. Additionally, it was observed that the rate of nitrogen removal declines as the initial nitrogen concentration decreases, likely because biomass flourishes in nitrogen-rich conditions, while phosphorus levels remain optimal and adequate throughout the cultivation period, making the process primarily reliant on nitrogen. In nitrogen-deficient media (N:P-0.4:1), total nitrogen removal reaches 97%

(Figure 4), yet the daily removal rate is 1.41 mg, which is the lowest of all, while phosphorus shows a notable removal rate. It has been determined that *Chlorella Sorokiniana* performs well even in nutrient-dense environments and modifies its biochemical pathways to enhance growth and improve nitrogen uptake. However, in nitrogen-poor conditions, it exhibits a slow growth rate while effectively removing nitrogen along with phosphorus, as nitrogen is utilized first and resources are redirected towards phosphorus for survival during nitrogen stress [144].

The rate of phosphorus removal increases as the initial nitrogen concentration decreases. Media that is deficient in nitrogen shows the highest efficiency for phosphorus absorption by microalgal cells, as demonstrated by the N:P ratio of 0.4:1, which achieves 85% phosphorus removal with a residual total phosphorus of 8.12 mg/L, and at the N:P ratio of 0.6:1, it reaches 76% with a residual total phosphorus of 12.78 mg/L (Figure 14c, 14d). However, in nutrient-rich photobioreactors, there is minimal variation in phosphorus removal efficiency. These findings align with a study that indicates microalgae under stress conditions tend to utilize all available phosphorus found in the media, including that stored within the cells [145].

5.2.4 pH profile behaviour with N:P

In various N:P cultured conditions, the pH of *C. sorokiniana* was measured every other day. The pH levels across all culture conditions varied from 6.8 to 10.8 (Figure 14b). Overall, the control BBM culture with a N:P ratio of 0.8:1 generally maintained a pH range of 8-9, while the nitrogen-limited culture conditions of 0.6:1 and 0.4:1 N:P ratios showed comparable levels by the end of the 14-days cultivation period. The highest pH was recorded on the 6th day for all nutrient-rich cultures and for control at N:P-0.8:1. However,

the maximum pH for the nutrient-deprived conditions peaked on day-4 for the N:P ratio of 0.6:1 and on day-2 for 0.4:1 ratio.

5.3 Investigation of N:P+2% salinity effect on microalgae growth

5.3.1 Biomass concentration

Figure 15 illustrates the total biomass concentration achieved after a 14-days cultivation period of *C. sorokiniana*, with varying N:P ratios (both high and low) alongside the optimal salinity previously examined for PHA and biomass. In Figure 11, the 2% saline media produce 1133 mg/L of biomass; however, optimizing it with N:P ratios do not yield a higher biomass concentration. The peak biomass was observed at a 1:1-2 N:P ratio with 2% salinity, likely because it closely resembles the BBM media (0.8:1-N:P), and the microalgal cells had already adapted to this environment from the earlier experiment. In contrast, for all other N:P ratios combined with 2% salinity (2:1-2, 1.5:1-2, 0.6:1-2, 0.4:1-2), the microalgal cells required some time to adjust to the higher or lower nutrient conditions, resulting in a stress environment, which was exacerbated by the saline media, as noted in another study [146]. Based on our earlier findings, a N:P ratio of 2:1 without 2% salinity proved more effective for biomass growth, yielding around 1000 mg/L (Figure 15). In contrast, the 2:1-2 (N:P with the ideal 2% salinity) achieved only 600 mg/L since the algae is still in the exponential growth phase. Nonetheless, this treatment influenced the microalgae cell size, leading to larger cells due to the high nutrient loading [147] (Figure 14c). A further study on *C. sorokiniana* demonstrated that it can adjust to different salinity levels, which affects its ability to absorb phosphate and nitrogen. Because of its adaptability, it could be efficiently employed in settings with changing salinity and nutrient concentrations. [148].

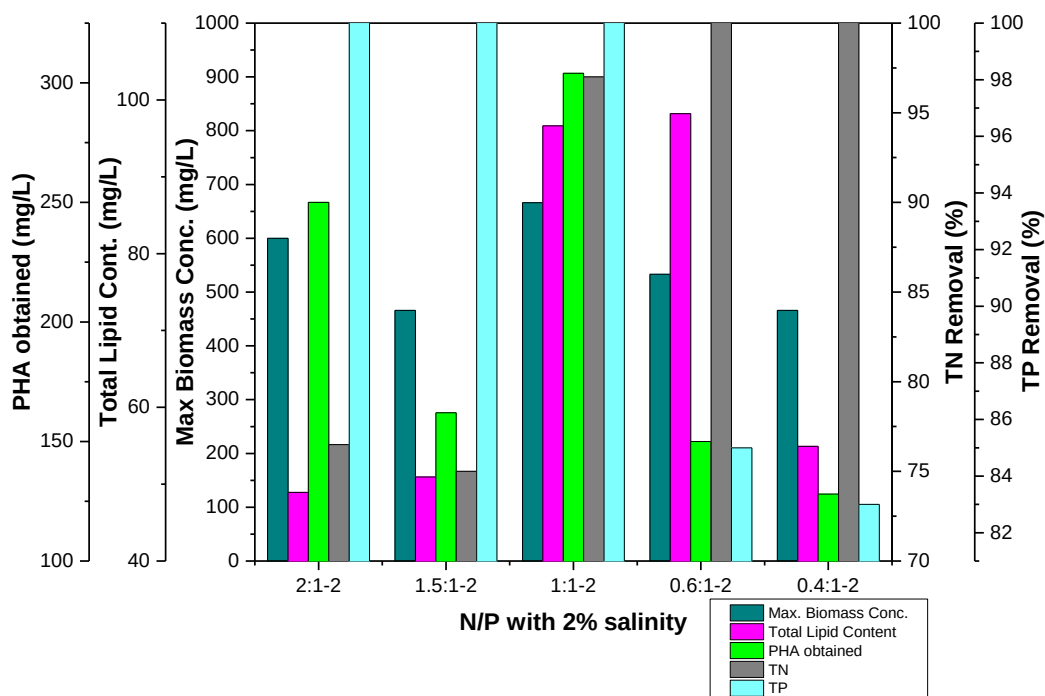


Figure 15: Effect of varying N:P ratio (2:1, 1.5:1, 1:1, 0.6:1, 0.4:1) with optimal salinity obtained 2% (2g NaCl) on maximum biomass concentration X_{max} (mg/L), Total lipid content (mg/L), Quantity of PHA obtained (mg/L) and percent removal of total Nitrogen (TN) and total Phosphorus (TP) at the end of 14-days cultivation

5.3.2 Biomass productivity and specific growth rate

Increased nitrogen levels typically lead to higher peak biomass productivity and maximum specific growth rates; for a 2:1-2(N:P with 2% Salinity), the values are 38.14 mg/L/d (Figure 16a) and 0.157 (d^{-1}), respectively. However, an imbalance between nitrogen and phosphorus in the medium can hinder long-term productivity, causing growth to plateau due to secondary limitations like CO_2 availability or light [149]. In the case of 1:1-2 (N:P with 2% Salinity), the maximum specific growth rate is lower than all others; however, the maximum biomass productivity is greater at 48.85 mg/L/d (Figure 16a) due to the media composition being like standard or moderate levels.

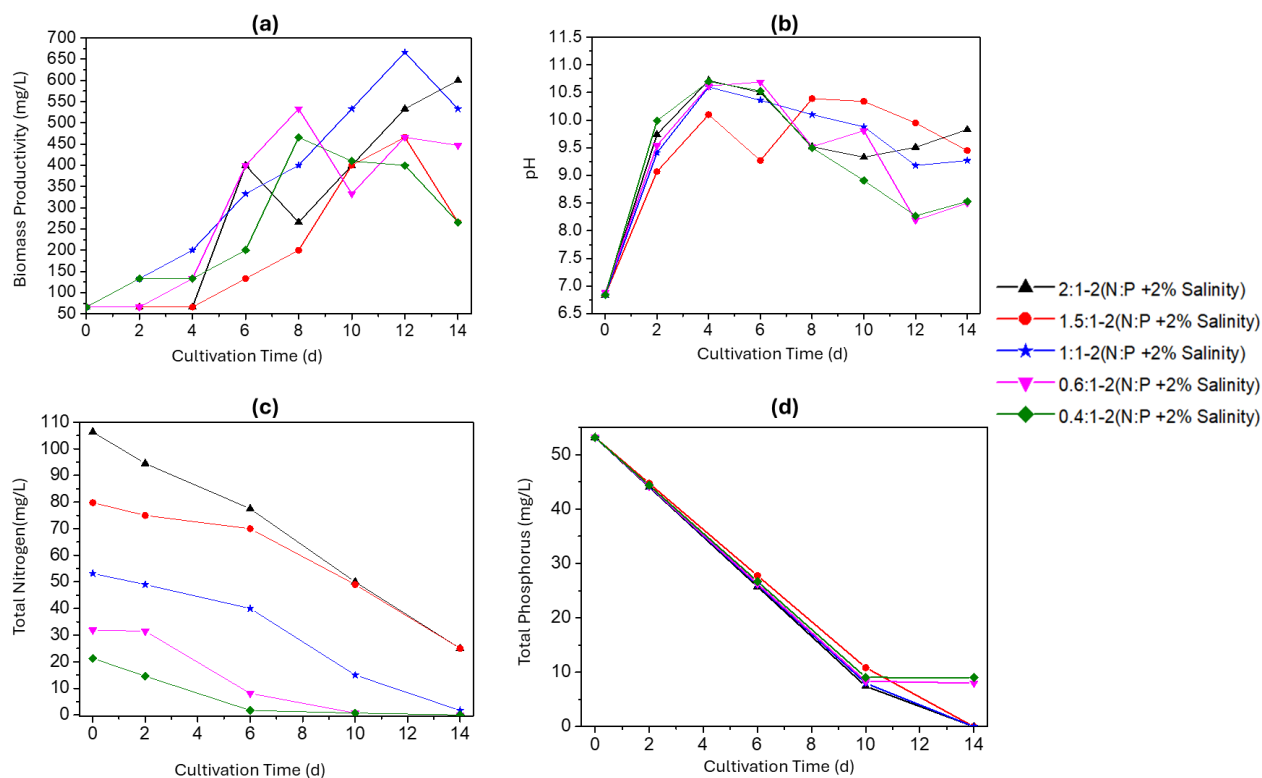


Figure 16: Effect of varying N:P ratio (2:1, 1.5:1, 1:1, 0.6:1, 0.4:1) with optimal salinity obtained 2% (2g NaCl) on (a) Biomass growth over time (Biomass productivity P_B (mg/L/d)), (b) variation in pH during 14-days cultivation, (c) total Nitrogen in media with time (mg/L) and (d) total Phosphorus in media with time(mg/L).

5.3.3 Nutrient removal analysis

The interaction between the N:P ratio and salinity may result in both synergetic and antagonistic impacts on nutrient removal, specifically total nitrogen and total phosphorus (Figler et al., 2019). Within saline environments of 2% salinity, an elevated N:P ratio of 2:1-2, 1.5:1-2, and 1:1-2 appears to achieve complete phosphorus removal, acting as a limiting factor for the effective removal of nitrogen itself (Figure 15). Nevertheless, the nitrogen removal rate is notably high for the N:P ratios in a 2% saline environment, as microalgal cells absorb more nitrogen in nitrogen-rich media (Figure 16c). However, an unbalanced nitrogen to phosphorus ratio results in suboptimal nutrient removal efficiency, as emphasized in another study examining the green alga *Coelastrum morus* [150]. An opposite trend was noted with N:P ratios that were less than the actual BBM, as nitrogen

became the limiting factor and a decline in the nitrogen removal rate was observed at 2.27 mg/L/d and 1.51 mg/L/d for N:P ratios of 0.6:1-2 and 0.4:1-2, respectively, at 2% optimal salinity. Research has shown that phosphorus removal is affected by insufficient nitrogen levels. Moreover, salinity adds another level of stress, causing osmotic pressure and changes in metabolic processes, which ultimately leads to a decrease in the efficiency of phosphorus removal in *Chlamydomonas reinhardtii* [151]. In our case, *C. sorokiniana* successfully manages these conditions, and the only limiting factor influencing the removal rate in both high and low N:P ratios is this nutrient input. In a separate study, *C. sorokiniana* demonstrated the capability to eliminate nearly 99% of nitrogen and phosphorus within just seven days. The removal rate is dictated by the limiting nutrient, as evidenced by the significant influence of the initial concentrations of these nutrients on the performance of removal [152].

5.3.4 pH profile

We noticed a rapid increase in pH as nitrogen was converted into nitrate (NO_3^-) and there was a higher generation of hydroxide at elevated N:P ratios under 2% salinity (2:1-2, 1.5:1-2, 1:1-2), resulting in a sharp rise in pH [153]. This rise stabilizes under saline stress, leading to a drop in pH by day four across all scenarios (Figure 16b). However, in scenarios with higher N:P ratios combined with salinity, the decrease in pH is attributed to nitrogen assimilation. In the cases with lower N:P ratios and salinity (0.6:1-2 and 0.4:1-2), pH will also decline because nitrogen is depleted more quickly, while NaCl (salinity) encourages the formation of organic acids, which further reduces pH over the following days. Furthermore, a gradual rise in pH was observed during the initial four days due to the restricted availability of NaNO_3 , resulting in a reduced presence of OH (Figure 16b) [154].

5.4 Investigation of second stage cultivation in nitrogen deficient media on microalgae growth

5.4.1 Biomass concentration

High nutrient reactors (2:1-2, 2:1-0, 1.5:1-2, 1.5:1-0) with or without salinity resulted in a very high biomass concentration among all optimizations. The first and second stages took about 28 days, which could have contributed to the prolonged cultivation period. Additionally, the high level of nutrient loading likely caused the microalgae to take longer to adapt to the environment and absorb more nitrogen. However, due to insufficient available phosphorus, their growth rate wasn't very high [139]. When the nutrient-rich microalgae were then subjected to a second shift in available media, they continued to grow and achieve a higher biomass concentration [155]. In Figure 17 we can see that 1.5:1-0 (N:P-without salinity) resulted in 1713mg/L, 2:1-0 (N:P-without salinity) resulted in 1533mg/L, 2:1-2 (N:P-2% Salinity) resulted in 1253mg/L and for 1.5:1-2 it is 1240mg/L biomass concentration obtained. However, the first stage resulted in 600 mg/L for 1.5:1-0 (Figure 13), 1000mg/L for 2:1-0 (Figure 13), 600mg/L for 2:1-2 (Figure 15) and 466mg/L for 1.5:1-2 (Figure 15) in maximum biomass concentration achieved.

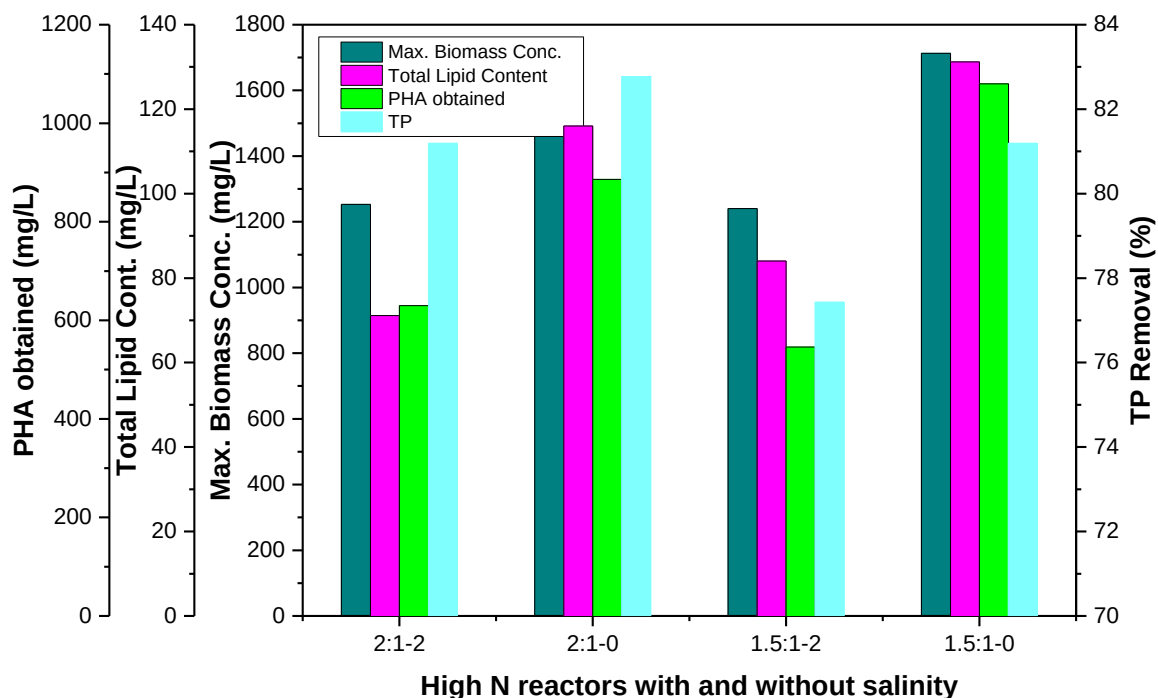


Figure 17: Second stage cultivation of high N:P photobioreactors with optimal salinity 2% (2g NaCl) and without optimal salinity on maximum biomass concentration X_{max} in (mg/L), Total lipid content (mg/L), Quantity of PHA obtained (mg/L) and percent removal of Total nitrogen (TN) and Total phosphorus (TP) at the end of 14-days cultivation

5.4.2 Biomass productivity and specific growth rate

C. sorokiniana showed a significant increase in biomass production in the second stage of cultivation under the 1.5:1–0 N:P ratio without salinity condition, in which no more nitrogen was added but the medium remained rich in phosphorus and other nutrients. While biomass production increased significantly from 28.67 mg/L/d in the first stage to 83.35 mg/L/d, the specific growth rate was measured at 0.081 d⁻¹ (Figure 18a). This reaction suggests that the microalgal cells were able to sustain growth and metabolic activity in the nitrogen-depleted second stage after assimilating enough nitrogen in the preceding stage,

most likely with the help of internal nitrogen reserves and high phosphorus availability. During the second-stage cultivation, biomass productivity ($P_{\max}$) increased by 37% under the 2:1-0 N:P ratio compared to the previous stage. The specific growth rate decreased in tandem with this productivity boost, indicating that although biomass accumulation persisted, the rate of cell division may have reduced, maybe because of physiological adaptation or shifts in resources from the initial stage as this behaviour was discussed in previous studies either [156]. Other conditions with salinity also show improvement in biomass productivity due to prolonged cultivation period but their specific growth rate was also lower. Improvements in biomass productivity were also seen in other culture circumstances that included 2% salinity along with the corresponding N:P ratios; this was mainly because the cultivation period was longer. However, the specific growth rates were somewhat lower under these saline circumstances, indicating that although salinity may promote biomass buildup and sustained cellular activity, it also imposes a mild osmotic stress that slows down cell division [157].

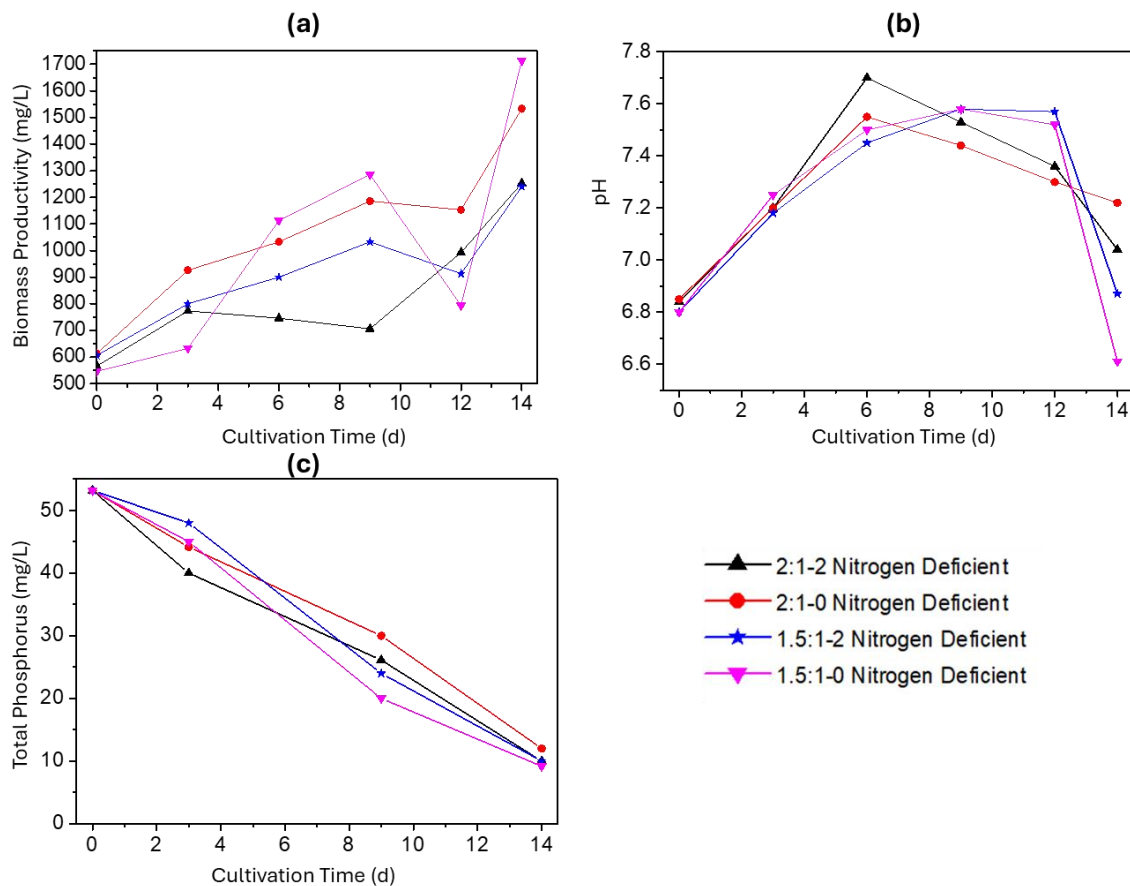


Figure 18: Effect of second stage cultivation of high N:P photobioreactors on (a) biomass growth over time (Biomass productivity P_B (mg/L/d)), (b) variation in pH during 14-day cultivation and (c) total Phosphorus in media with time.

5.4.3 Nutrient removal analysis

During the second stage cultivation (Nitrogen deficient) after initial cultivation for high nutrient reactors (2:1-0, 1.5:1-0, 2:1-2, 1.5:1-2) the media became phosphorus sufficient contained 53.17 mg/L of phosphorus in the form phosphate as K_2HPO_4 and KH_2PO_4 . We can still observe efficient removal of phosphorus in culture media as 2:1-2 PBR (N:P with 2% Salinity) as 81.19% and even higher for 2:1-0 PBR (N:P without salinity) as 82.77% removal of phosphorus.(Figure 18) In many research studies, phosphorus is crucial for growth, but it appears to have little impact on growth when there is sufficient nitrogen available in the medium. Nevertheless, limited phosphorus availability has occasionally

been shown to yield positive outcomes regarding lipid production, although it adversely affects biomass productivity [158]. In this case, it was clear that phosphorus supported the growth of microalgae while the cells had been subjected to high nitrogen media. The cells became so concentrated with nitrogen that they could effectively accumulate phosphorus in conditions of scarcity and continue their growth. The excess phosphorus was stored in the cell as polyphosphate, which could be utilized by microalgae in times of complete phosphorus and nitrogen deficiency to aid survival. Additionally, one study indicates that under stressful conditions, *I. galbana* tends to mobilize all the available phosphorus within their cells for survival [159].

5.4.4 pH profile

The second phase was found to achieve the maximum pH across all reactors in all scenarios that were previously examined. The pH continued to rise until the sixth day of the second stage of cultivation, with the reactors in saline environments and high N:P ratio (2:1-2 and 1.5:1-2) showing higher pH levels compared to the reactor that was not influenced by saline conditions. Nevertheless, the increase in pH could possibly be attributed to several factors in this situation (Figure 18b). Research indicates that a lack of nitrogen may lead to decreased generation of acidic metabolites, resulting in a more alkaline surrounding; this nitrogen assimilation process is particularly observed in *Chlorella* species [160]. The increase in pH might also be associated with the buildup of lipids and polymers in conditions of nitrogen deficiency and adequate phosphorus. Since phosphorus is crucial for vital metabolic processes like energy transfer and nucleic acid synthesis, it helps to stabilize variations in pH [161].

5.5 Effect of CO₂ with optimal salinity and balanced N:P on microalgae growth

5.5.1 Biomass Concentration, Biomass Productivity and Specific Growth Rate

To evaluate the influence of carbon availability on microalgal performance, *Chlorella sorokiniana* was cultivated under varying CO₂ concentrations (2%, 4%, 6%, and 8% v/v) using previously optimized conditions of 2% salinity and a 1:1 nitrogen-to-phosphorus (N:P) ratio with 0% CO₂ (only air). These conditions were selected based on prior experiments that demonstrated enhanced biopolymer accumulation and stress-adapted growth. The introduction of CO₂ aimed to stimulate photosynthetic activity, promote carbon assimilation, and assess its impact on biomass concentration, biomass productivity, and specific growth rate.

Across all treatments, a notable increase in biomass production was observed with elevated CO₂ levels up to a threshold, beyond which growth either plateaued or declined. Specifically, the 2% and 6% CO₂ treatments exhibited the highest biomass concentration ($X_{\max}$) as 746mg/L and 770mg/L respectively (Figure 19), which is higher than gained with 0% CO₂ integrated 2% saline conditions and N:P ratio of 1:1 and biomass productivity noticed to be really high for 6% CO₂ as 52.14 (mg/L/d) (Figure 20a) among all suggesting an optimal carbon enrichment range for maximizing growth. However, at 8% CO₂, a slight inhibitory effect was observed than 6% CO₂, likely due to excessive CO₂-induced acidification or cellular stress. Specific growth rate trends mirrored biomass productivity, further validating the growth response to CO₂ availability. A similar study observed the impact of CO₂ concentration on the biomass productivity of *Chlorella sorokiniana* IPPAS C-1. They found that at an optimal CO₂ flow rate corresponding to 1.5% CO₂ in the gas-air mixture, the specific productivity reached approximately 1 g dry weight L⁻¹ day⁻¹ in

flat-panel photobioreactors. This suggests that elevated CO₂ levels can enhance biomass productivity under controlled conditions [162]. Although direct studies on *Chlorella sorokiniana* under the combined conditions of elevated CO₂, a 1:1 N:P ratio, and 2% salinity are limited, existing research indicates that, elevated CO₂ concentrations can enhance biomass productivity, optimal N:P ratios are crucial for maximizing growth, with higher N:P ratios generally favoring biomass accumulation and salinity levels can affect growth and lipid accumulation, with effects varying by species and salinity concentration. These findings highlight the importance of balancing inorganic carbon supplementation with other cultivation parameters to optimize microalgal growth and downstream bioproduct generation.

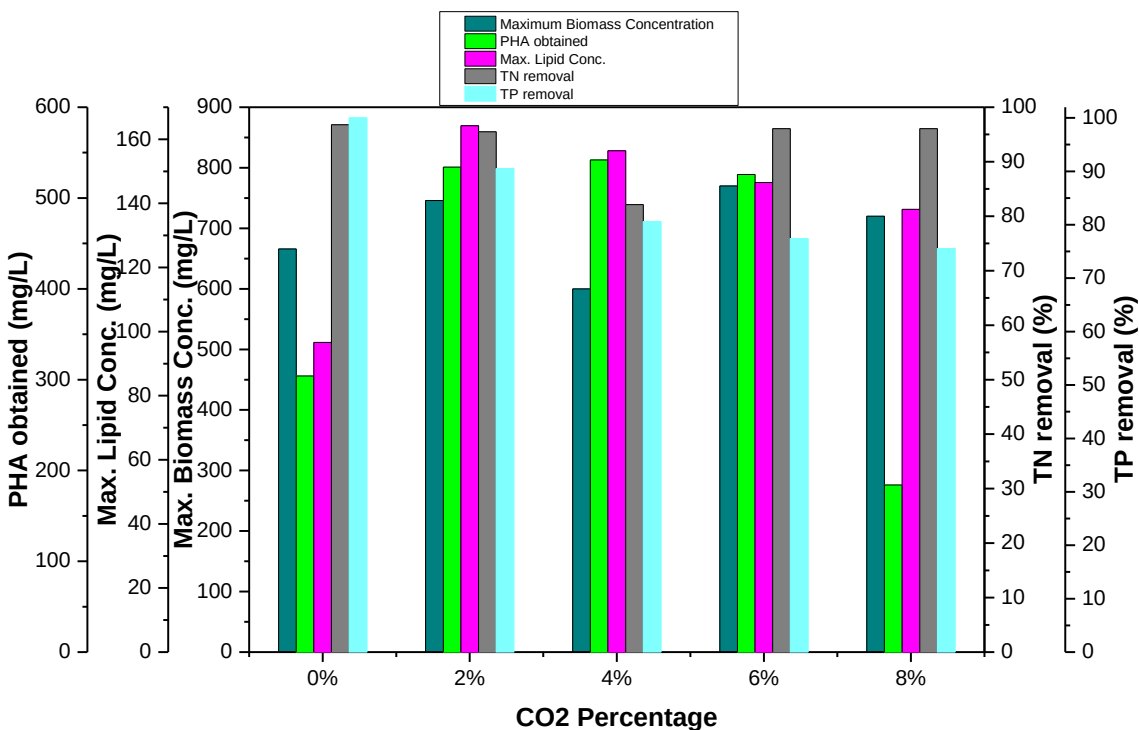


Figure 19: Biomass Concentration, PHA obtained, Total Lipid Content, TN removal and TP removal under different CO₂ concentration

5.5.2 Effect of CO₂ on Nutrient Removal (NO₃⁻ And PO₄³⁻)

The ability of *C. sorokiniana* to remove nutrients from wastewater media was assessed under different CO₂ concentrations (0%(control), 2%, 4%, 6%, and 8% v/v). Nutrient removal, particularly nitrate and phosphate, plays a critical role in wastewater treatment and reflects the physiological uptake and assimilation capabilities of the microalgae. The integration of CO₂ enrichment was hypothesized to influence nutrient uptake by boosting photosynthetic metabolism. Results indicated that moderate CO₂ levels 6% and 8% CO₂ significantly enhanced both nitrogen removal rate as % removal of 96% is achieved for both cases but percent phosphorus removal is decreasing as 77% for 6% CO₂ and 75% for 8% CO₂ with increase CO₂ percentage as it was recorded as 100% and 90% TP (Total phosphorus) removal for 0% CO₂ and 2% CO₂ respectively (Figure 19). It might happen because at higher CO₂ percentage microalgae undergo enhanced carbon fixation and growth but without sufficient nitrogen that growth can't be sustained and phosphorus demand remain low [163]. P-uptake in *Chlorella sorokiniana* is not purely passive and often linked with metabolic demand. As residual phosphorus for 6% and 8% CO₂ is quite higher than other conditions as 12 (mg/L) and 13 (mg/L) respectively (Figure 20). The media with saline stress and unbalanced N:P with high CO₂ concentration leads to medium acidification (carbonic acid formation) further stressing cells in already saline conditions. A decline in nutrient removal was observed potentially due to stress-induced inhibition of enzymatic pathways involved in nutrient assimilation.[164]

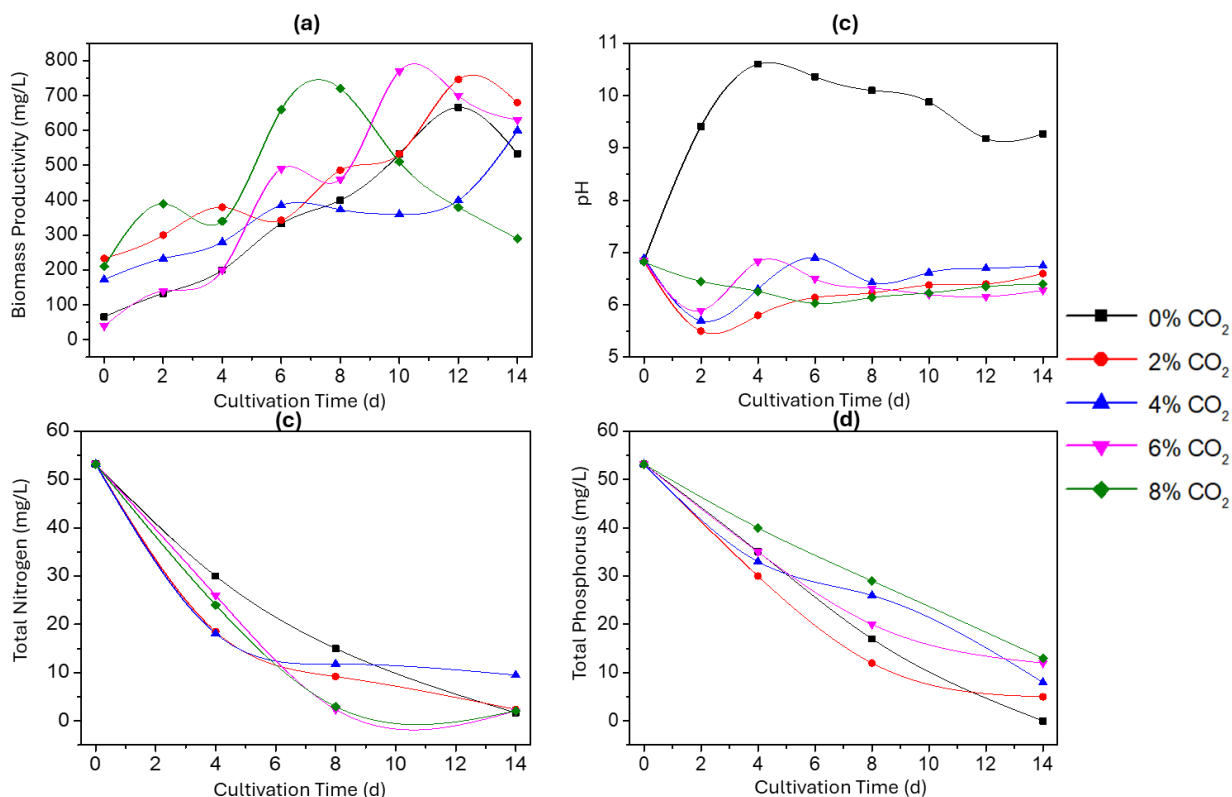


Figure 20: Biomass productivity, pH profile, TN and TP under 14-days cultivation with varied CO₂ concentrations

5.5.3 Effect of CO₂ on pH profile

Monitoring pH variation during cultivation provides insight into the physiological status of the culture and the buffering behavior of the media. Under all CO₂-enriched conditions, the injected carbon dioxide initially reduced the pH of the culture medium due to the formation of carbonic acid as the 0% CO₂ conditions shows complete opposite behavior as it increases to its maximum (pH 10.7) and then started getting down after day-4 due to the accumulation of CO₂ for photosynthesis most likely belongs to exponential phase (Figure 20). Over the cultivation period after day-2 a gradual pH increase was observed, particularly in 2–6% CO₂ treatments, suggesting active CO₂ uptake and photosynthetic activity but initial drop is because of acidification of media probably belongs to a lag phase as microalgae is trying to adapt the CO₂ conditions. The 8% CO₂ condition maintained a

relatively lower pH throughout, likely due to CO₂ oversaturation and limited fixation, which may have contributed to the observed inhibition in growth and nutrient removal efficiency. While specific studies on *Chlorella sorokiniana* under 2% salinity are limited, research on other microalgae indicates that salinity can influence pH stability and nutrient availability. High salinity levels may exacerbate the acidifying effects of CO₂ supplementation, making pH control even more critical to ensure optimal growth conditions.

CHAPTER 6

PHA Accumulation, Lipid Production and CO₂ Fixation

6.1 PHA Accumulation and Productivity

6.1.1 Effect of Salinity

This research examines how saline stress affects PHA production in *C. sorokiniana* at different sodium chloride concentrations. PHA accumulation occurs in response to stress, especially after the exponential growth phase when nutrient availability decreases. Although stress can increase polymer synthesis, high salinity may impede cell growth, indicating the need for an optimal balance to maximize both biomass and PHA yield [165]. Experimental findings reveal that raising salinity to 2% (2g NaCl/L of culture media) enhances PHA production, achieving a maximum of 780 mg/L (Figure 11). However, as salinity increases further, PHA yield slightly decreases, falling to 660 mg/L at 4% salinity, 620 mg/L at 6% salinity, and 640 mg/L at 8% salinity. In comparison to the control group (440 mg/L at 0% salinity), even at 1% salinity (520 mg/L), there was a significant increase (Figure 11). These results are consistent with earlier studies on *Synechocystis* and *Spirulina subsalsa*, which reported higher PHB accumulation under moderate NaCl stress [110].

A Study discovered that by incorporating 1 g L⁻¹ of NaCl into *Nostoc muscorum* NCCU-442, the average PHB content (based on triplicates) increased from 7.6% (dry cell weight) in the control group to 8.15% (dry cell weight) [166]. Similarly, it was noted that with

Spirulina subsalsa, there was an increase from 5.9%_{DCW} to 7.45%_{DCW} when 50 g NaCl L⁻¹ was introduced [69]. All studies regarding salinity indicate that the optimal stress level for maximizing PHA production varies depending on the specific cyanobacteria or microalgal species [167]. For *Chlorella sorokinina*, it has been determined that the ideal salinity is 2 g NaCl L⁻¹ of BBM, as it can achieve maximum polymer production without requiring a very high saline concentration. The rapid polymer accumulation at moderate saline levels is attributed to its nature as a freshwater microalga [168], which can quickly adapt its metabolic pathways when subjected to saline stress. Numerous microalgae, including species from *Chlorella* and *Dunaliella*, have demonstrated impressive resilience to fluctuating salinities. Optimal growth is frequently noted at low to moderate salinity levels (such as 1–3% NaCl), where osmotic stress is balanced by cellular adaptations without incurring excessive energy costs to maintain cell integrity [169]

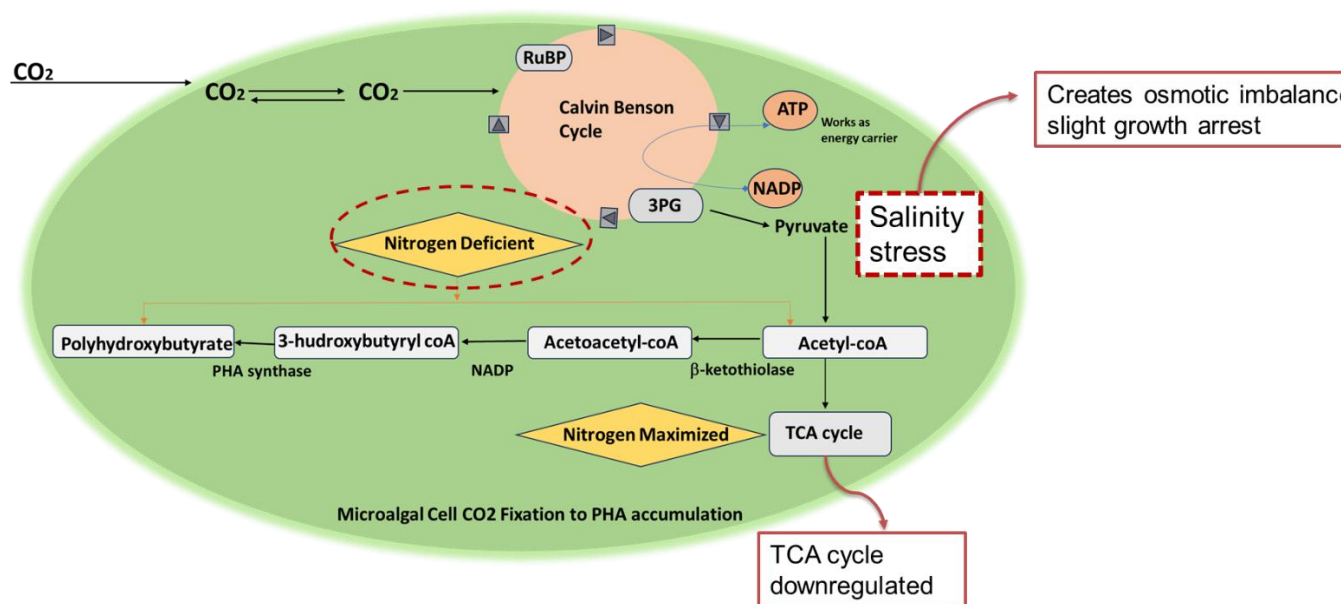


Figure 21: Mechanism of PHA accumulation in microalgae cell in saline condition

6.1.2 Effect of N:P ratio

In this research, the effect of nitrogen and phosphorus levels on the accumulation of PHA by *C. sorokiniana* was examined using a modified version of Bold's Basal Medium (BBM). Numerous studies have emphasized substantial PHA production when nitrogen is limited. [84]; however, restricting nitrogen generally diminishes overall biomass growth, which constrains its practical use [170]. Consequently, different nitrogen-to-phosphorus (N:P) ratios were assessed to find a balance between biomass growth and polymer accumulation. Six photobioreactors (PBR) were set up with distinct N:P ratios: PBR1 (2:1, highest nutrient levels), PBR2 (1.5:1), PBR3 (1:1), PBR4 (0.8:1, standard BBM), PBR5 (0.6:1), and PBR6 (0.4:1, maximum deficiency). After 14 days, the results showed that the maximum total PHA accumulation (mg/L) occurred at higher nutrient levels (374 mg/L at 2:1), while the highest percentage of PHA per dry biomass was achieved under conditions of significant nitrogen deficiency (0.4:1) (Figure 13). This inversely proportional relationship arises because limiting nutrients reduces biomass growth but enhances the PHA content per cell.[84] Even though higher nutrient ratios led to increased biomass and total PHA yield, nitrogen utilization was still not fully complete after 14 days [167]. Therefore, an economically viable approach must strike a balance between high biomass productivity and effective nutrient consumption. The 1:1 N:P ratio emerged as the most favourable condition, yielding elevated PHA accumulation rates alongside satisfactory nutrient utilization, underscoring the adaptability of *Chlorella sorokiniana* in both nutrient-rich and nutrient-deficient environments [171].

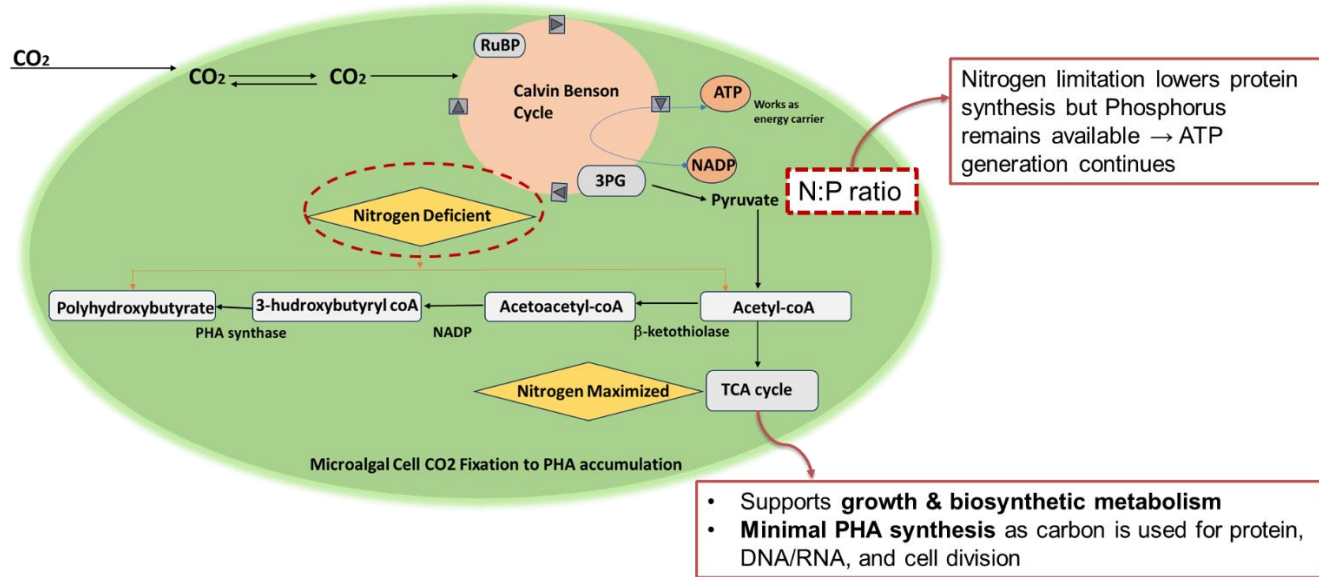


Figure 22: Mechanism of PHA accumulation in microalgae cells with varied N:P conditions

6.1.3 Effect of N:P ratio + 2% Salinity

The interaction between N:P ratio and salinity can have synergistic or antagonistic effects on PHA accumulation and productivity [172]. We can see that the PHA accumulation is triggered by the presence of saline environment as an additional stress over low N:P ratio as the percent accumulation of PHA obtained is even higher as 48%_{DCW} than we get from low N:P ratio which is 0.4:1-0 without salinity as discussed in earlier but the PHA obtained overall is lower (128mg/L) (Figure 15) as compared to N:P ratio without optimal salinity as shown in Figure 13. This occurs because increased stress alters the growth mechanism, focusing on the survival and storage of materials such as PHA and lipids. In terms of higher N:P ratio with optimal salinity, PBR 2:1-2 and 1:1-2 demonstrates improved performance regarding PHA accumulation as the total biomass produced is greater, resulting in higher PHA yields [173]. Among all the best synergetic effects obtained from 1/1-2 (N/P with 2% salinity) which shows a higher polymer obtained around (304 mg/L). Hence, it is proved that the stress from both nitrogen limitation and salinity forces microalgae to store more

carbon as PHA. The results obtained are like some other studies reported that combining nitrogen limitation with moderate salinity stress can enhance PHA accumulation. Research conducted on *Synechocystis* sp. has indicated that a reduced N:P ratio alongside moderate salinity levels i.e. 2% salinity considerably enhances PHA content and productivity [76].

PHA productivity (mg/L/day) depends on both PHA content and biomass productivity. While nitrogen limitation increases PHA content, it often reduces biomass productivity, leading to a trade-off [74]. In Figure 26c PHA productivity is plotted for the highest amount of PHA obtained for 1/1-2 depending on availability of nitrogen in the media showed that PHA accumulation start rising in media after 8th day of cultivation and increase sharply as there is deficiency of nitrogen increases in media.

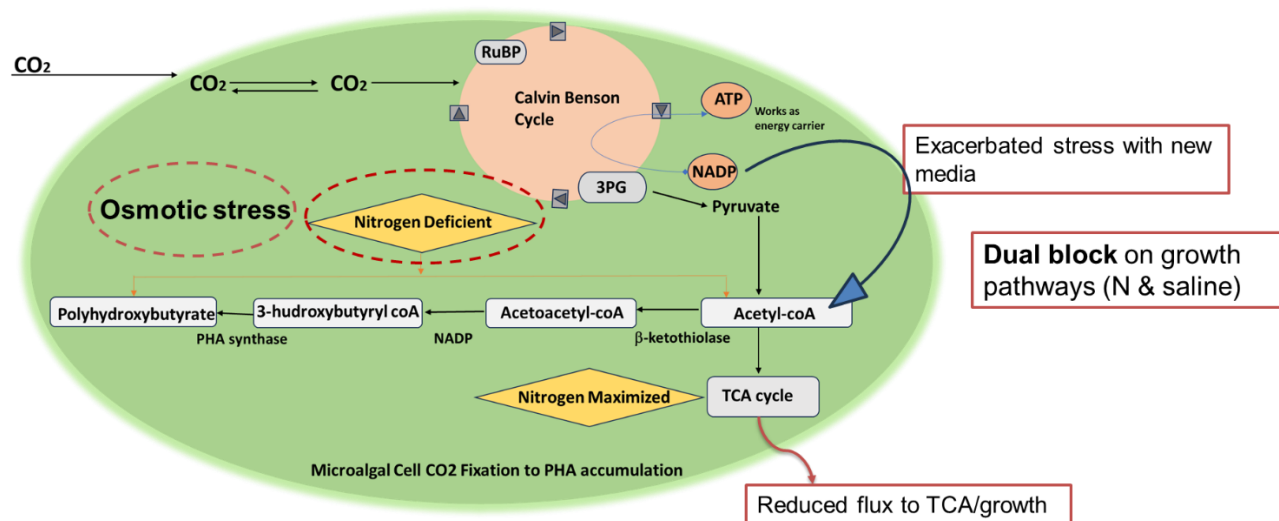


Figure 23: Mechanism of PHA accumulation in microalgae cell with synergetic stress of salt and N:P

6.1.4 Effect of High N reactors with or without salinity (Second stage cultivation)

We have seen in previous sections that a lower N:P with or without the optimal salinity is the cause of decrease in the biomass productivity which is directly influencing the PHA

content obtained as the highest amount of 304 mg/L from 1:1-2(N:P with 2% salinity) and 374 mg/L for 2:1(N:P) presented in Figure 15 and Figure 13 respectively which is quite less than obtained only for 2% salinity around 780mg/L for the same specie *C. sorokiniana* (Figure 11). In general, starting with elevated nitrogen levels (high N:P ratios) tends to enhance biomass production. The increased biomass can lead to a higher accumulation of PHA when subjected to both nitrogen depletion and saline stress. Therefore, relying solely on a low nitrogen strategy might not be commercially viable [174]. Eventually the high N photobioreactors such as 2:1-0, 1.5:1-0, 2:1-2 and 1.5:1-2 in previous experiments is not fully able to utilize all the nitrogen in media for 14 days and still in the exponential phase. Consequently, the production of PHA remains low since the process has not yet transitioned towards carbon storage and continues to prioritize the growth of microalgae [175]. High nitrogen reactors are once more cultivated in nitrogen-deficient or nitrogen-free media while maintaining all other conditions as outlined in earlier sections. The PHA produced from this second stage cultivation in the high nitrogen reactors is extremely high, even surpassing the levels we achieved in the saline environment alone. The highest amount of PHA obtained for 1.5:1-0 and 2:1-0 (N:P without salinity) around 1080 mg/L and 886mg/L respectively (Figure 17). Also, PHA obtained in high N reactors together with saline stress contained even better % accumulation per dry weight biomass as 50% and 44% for 2:1-2 and 1.5:1-2 (N:P with 2% salinity) respectively. Due to the enhanced biomass productivity resulting from the higher nitrogen levels in the media during the initial cultivation (as discussed earlier), the microalgae continue to thrive at the same rate into the second stage after being harvested from the first. Once the microalgae adapt to their new environment and deplete their growth reserves, they begin to accumulate PHA

and lipids for survival, as our predicted model shows a significant increase in PHA production per day (PHA productivity) just after the second day of the second run (Figure 26d).

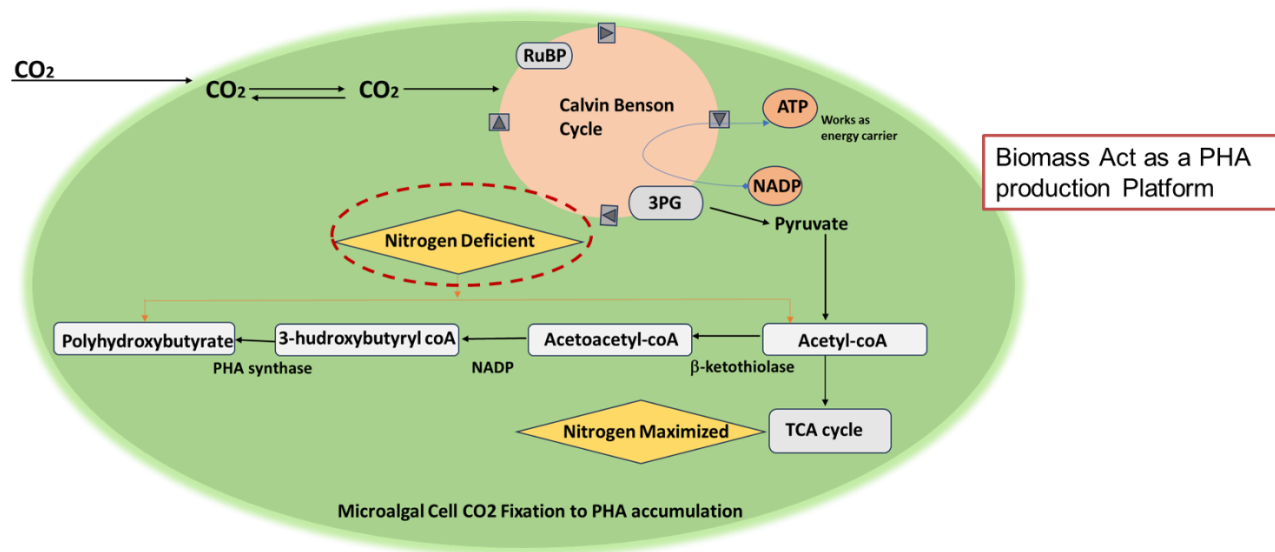


Figure 24: Mechanism of PHA accumulation in microalgae cell with Nitrogen deficient conditions

6.1.5 Effect of CO₂ on PHA accumulation and productivity

One of the primary goals of this study was to enhance polyhydroxyalkanoate (PHA) production under CO₂-supplemented, stress-optimized conditions. PHA accumulation is known to increase under nutrient-limiting yet carbon-rich environments. By applying previously determined optimal conditions (2% salinity and 1:1 N:P), the influence of CO₂ levels on PHA productivity was evaluated. In the current experiment, varying concentrations of CO₂ (2%, 4%, 6%, and 8%) were supplied to the culture system to assess their effect under these integrated conditions.

The results showed a synergistic effect of the optimized salinity (2%-2g NaCl) and nutrient ratio (1:1-N:P) with elevated CO₂ levels. Among all tested conditions, 4% CO₂ yielded the

highest PHA content % dry weight cell (Figure 13), indicating an enhanced carbon assimilation rate and redirection of metabolic pathways towards polymer synthesis. Both lower (2%) and higher (6%) CO₂ concentrations led to enhanced PHA accumulation as 78.52% and 83.49% dry weight cell than without any CO₂ supplementation (57% dry weight cell), but 8% CO₂ resulted in reduced PHA accumulation (48.42% dry weight cell) possibly due to CO₂-induced saline stress with limited nutrient availability. This integrated approach highlights the importance of coupling multiple optimized environmental factors—salinity, nutrient balance, and CO₂ availability—to maximize the bioplastic production potential of microalgae. The enhanced PHA productivity under 4% CO₂ demonstrates the role of carbon enrichment in further pushing the metabolic limits of the strain, when foundational conditions (salt and nutrients) are already favorable. However, the PHA concentration (mg/L) obtained with 4% CO₂ conditions together with N:P ratio 1:1 and salinity concentration of 2% was the most favourable and produced 542 (mg/L) of PHA obtained (Figure 11). The results showed that moderate CO₂ levels (2–6%) significantly promoted PHA accumulation, likely due to improved carbon flux toward polymer synthesis pathways. At 8% CO₂, however, a drop in PHA yield was recorded, possibly due to metabolic stress or imbalanced intracellular redox conditions [176]. While direct studies on polyhydroxyalkanoate (PHA) production by *Chlorella sorokiniana* under elevated CO₂ concentrations are limited, research on other microalgae species provides valuable insights into how CO₂ levels influence PHA accumulation. A study demonstrated that under nitrogen-depleted conditions, up to 87% of the carbon in polyhydroxybutyrate (PHB) was derived from intracellular carbon reserves rather than directly from CO₂ fixation. This suggests that while CO₂ contributes to overall biomass, PHA accumulation

is more closely tied to internal carbon metabolism under nutrient stress [84]. Considering the outcomes of this study alongside previous findings, it becomes evident that an integrated cultivation strategy—combining controlled salinity stress, nutrient limitation, and elevated CO₂ supply—not only enhances CO₂ fixation but also significantly promotes PHA accumulation. The findings support the notion that while CO₂ drives overall biomass productivity, PHA synthesis under nutrient stress relies heavily on intracellular carbon metabolism. This integrated approach, therefore, proves highly effective for boosting both carbon capture and biopolymer production in microalgal systems

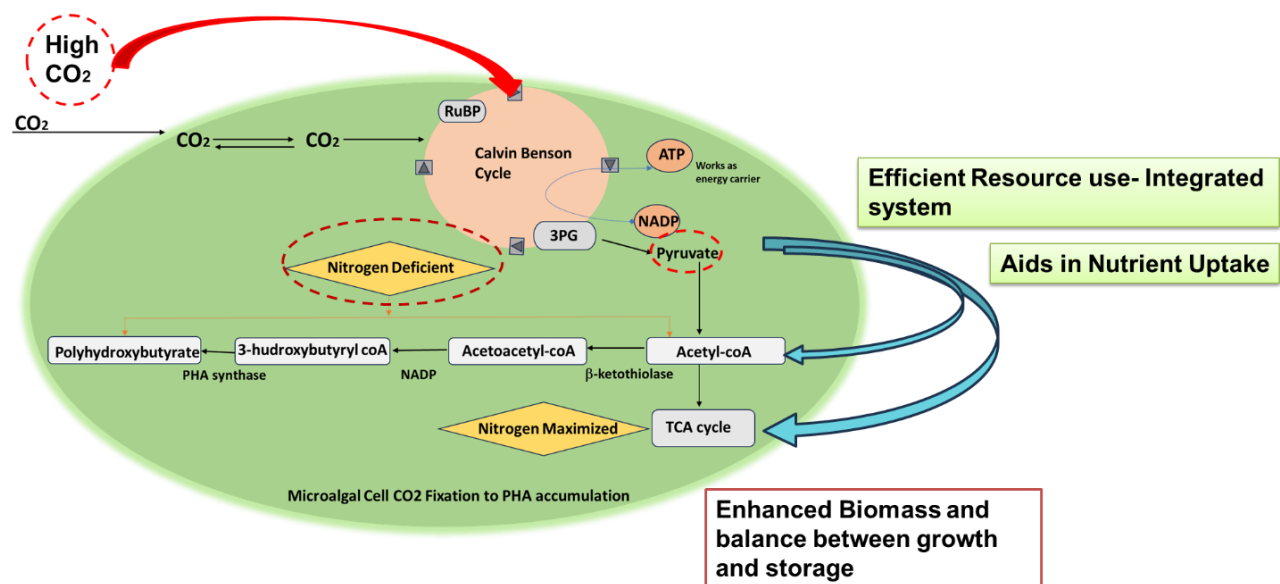


Figure 25: Mechanism of PHA accumulation in microalgae cells with induced CO₂ saline stress with balanced N:P ratio

6.2 Modelling of PHA productivity with nitrogen depletion

6.2.1 Optimal conditions for salinity, N:P, integrated salinity with N:P and

Second stage

Since PHA productivity and nitrogen depletion are closely related, the sigmoid model was utilized to track PHA productivity over time for various culture conditions. In some situations, nitrogen depletion is not noticeable until the end of the growing season, thus we are unable to verify whether PHA accumulated satisfactorily or whether this scenario had a favorable outcome. Additionally, the PHA quantification method is a lengthy process that requires time if we wish to calculate it frequently; therefore, this method makes the PHA computation easier over time. k which is a PHA accumulation or growth constant was increases as nitrogen concentration in media decreases and when nitrogen is very low called an inflection point is when k is at higher rate. PHA accumulation was noted to be higher in 2% saline (a), 2/1-N/P (b), 1/1-2 (N/P+2% Salinity) (c) and 1.5/1-0 (second stage nitrogen deficient) (d) conditions and they were plotted against their nitrogen removal data (calculated experimentally). We noted a higher PHA production in saline condition(a) as compared to high nutrient N:P (2:1) (b) or integrated N:P with 2% salinity (1:1-2) (c) because nitrogen removal rate is faster in saline condition observing an inflection point near 7th day but for it was past 11 days for other conditions resulting in a lower PHA at the end. Interestingly, second stage cultivation with completely nitrogen deficient conditions (d) microalgae utilizes all the nitrogen salt reserves from previous cultivation and suddenly reaching inflection point in just 2 days and PHA accumulation was boosted even before because the reserves for salt from previous cultivation was very less in media around 7mg/L of nitrogen. Therefore, the model predicts that PHA production significantly

increases at an inflection point, which corresponds to when nitrogen depletion occurs in the media. Graphs illustrate that cultures with higher initial nitrogen concentrations reach the inflection point later, while those with lower nitrogen levels or enhanced removal reach it sooner. The results extend the model by demonstrating how environmental factors (salinity, N:P ratio, nitrogen starvation) influence the timing of this transition, making it more adaptable to real wastewater treatment scenarios.

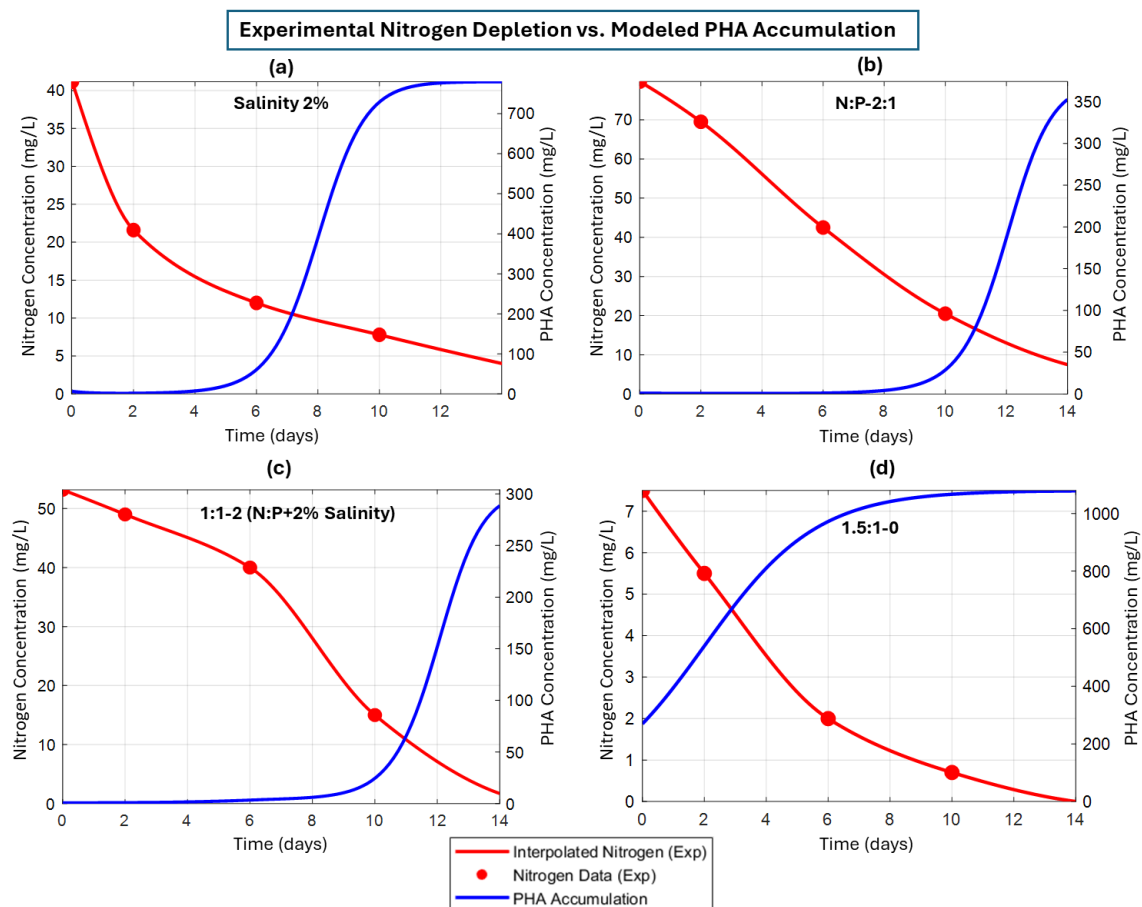


Figure 26: Model validated with nitrogen removal calculated experimentally for high PHA accumulating conditions as (a) for 2% salinity (2g NaCl) with a decrease in nitrogen in media increase in PHA (b) Modeled PHA for nitrogen depletion in 2:1-N:P (c) Modeled PHA for nitrogen depletion in integrated media of 1:1-2 (N/P with 2% Salinity) (d) Modeled PHA for 1.5:1-0 nitrogen depleted media in second stage.

6.2.2 Varied CO₂ concentrations with Optimal salinity and N:P ratio

To better understand the dynamics of PHA accumulation under nutrient stress, a sigmoidal model was applied to simulate PHA productivity as a function of time (14 days cultivation period), with nitrogen availability as a key parameter. The model assumes that PHA accumulation accelerates as nitrogen depletes, with the constant rate increasing inversely with nitrogen concentration. Experimentally measured nitrogen values were interpolated using a shape-preserving cubic spline (pchip), and used to dynamically update k , thus influencing the PHA productivity curve. The modeled PHA productivity (mg/L/d) followed the classic sigmoidal trajectory, with a lag phase in the early days, rapid increase around the critical time point ($t_a = 6 \text{ days}$), and saturation near the final maximum yield (542 mg/L) for 4% CO₂ with 2% saline stress and nutrient limited condition as N:P ratio of 1:1 (Figure 27c).

The modeled trends were further supported by experimental measurements of PHA concentration, which followed a similar accumulation pattern under nitrogen-limited conditions. The close agreement between simulated and observed PHA values validates the reliability of the sigmoidal model in capturing nutrient-driven metabolic shifts. Moreover, the timing of the highest modeled PHA productivity coincided with the period of maximum nitrogen limitation, reinforcing the metabolic linkage. This modeling approach not only complements the experimental findings but also provides a predictive framework for optimizing cultivation conditions in future scale-up strategies.

Although experimental productivity was not directly measured on a daily basis, the final PHA concentration and percent accumulation were determined for each condition. These end-point measurements aligned well with the trends predicted by the model. Specifically,

conditions with faster nitrogen depletion led to higher final PHA concentrations and accumulation percentages, reinforcing the accuracy and relevance of the sigmoidal model.

Furthermore, to visualize and compare the influence of nutrient availability, the modeled PHA productivity over time was plotted for all five cultivation conditions (0%, 2%, 4%, 6%, 8% CO₂) from day 0 to day 14. The comparison clearly demonstrates how differences in nitrogen consumption rate affect the timing and extent of PHA accumulation, providing valuable guidance for optimizing nutrient regimes in future bioplastic production setups.

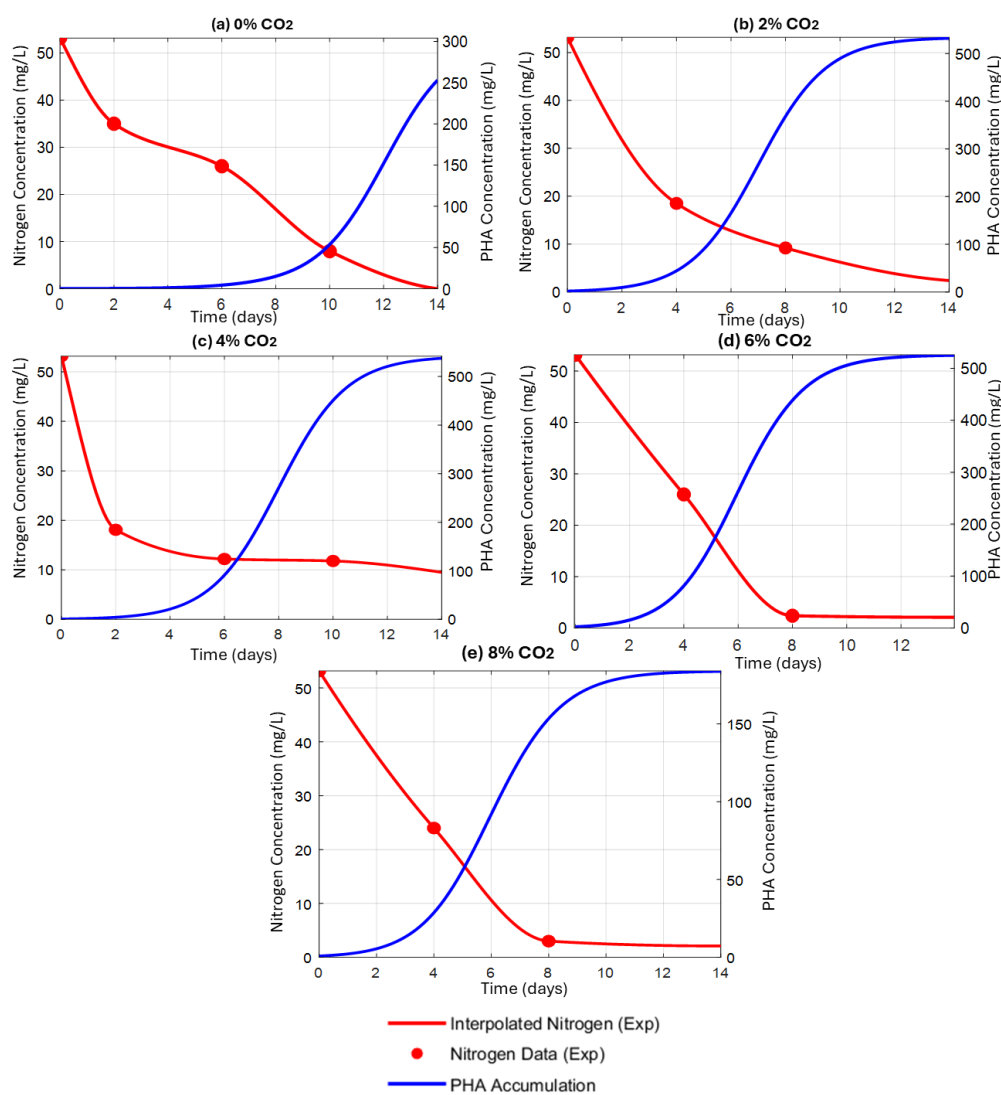


Figure 27: Modeled PHA productivity with Nitrogen deficiency under different CO₂ conditions

6.3 Lipid Content and Productivity

6.3.1 Optimal salinity, N:P ratio, synergetic effect of N:P with salinity and Nitrogen deficient condition

Biodiesel represents a promising substitute for conventional diesel, and microalgal biomass is regarded as a suitable feedstock due to its rapid growth and substantial lipid accumulation [177]. This research investigated how salinity affects lipid production in *Chlorella sorokiniana*, utilizing the sulfo-phospho-vanillin (SPV) colorimetric technique for measurement [178]. Since 6% salinity (6g NaCl) yields the greatest lipid concentration, higher salinity levels led to larger lipid accumulation, as demonstrated by the increased lipid productivity under more salinised conditions (Figure 11). Lipid productivity was very modest at 0% salinity, but it increased as NaCl concentrations rose, reaching 5.46 mg/day at 1% salinity and 10.26 mg/day at 2% salinity (Figure 28). The highly stressed environment, 6% salinity, had the highest lipid productivity (10.87 mg/day). 4% salinity had the highest lipid content as a proportion of dry biomass, whereas 6% salinity was superior in terms of productivity and total lipid content. A high lipid content in biomass indicates effective nutrient conversion and resilience to saline stress, encouraging lipid accumulation rather than growth [179]. These results are consistent with earlier studies, where microalgae accumulated between 20% and 60% lipid per dry cell weight (DCW) (Figure 28), depending on the species and environmental conditions [180]. Additional research suggests that lipid content rises under elevated NaCl concentrations, with one study showing a 2.52-fold increase at 0.20 M NaCl (32.26%) compared to non-saline environments (12.82%) [127]. These findings underscore the potential of regulating salinity to bolster lipid production for biodiesel purposes.

Lipid production by microalgal cells is triggered by many factors and it mainly depends on the concentration of nutrients in media. Although the initial concentration of nitrogen and phosphorus and the stress condition mainly define the production of biochemical compounds by it is also dependent on the type of microalgal specie [181]. In this study with constant optimum phosphorus concentration the initial nitrogen concentration was changes and we investigated the effect on lipid production. We can demonstrate from the results that *Chlorella Sorokiniana* shows significant change in lipid production by just changing the initial nitrogen concentration in bold bassel media (BBM). The two interesting factors showed by this specie that it behaves well in term of growth and lipid production even in nutrient rich media as we can see that 2:1, 1.5:1 and 1:1 N:P ratios showed a better result in terms of lipid concentration achieved as 102.36, 103.56 and 129.36 mg/L than control which is 0.8:1 BBM media (Figure 13). In terms of lipid content per dry cell weight and lipid productivity nutrient rich condition shows importance. The reason behind the enhance lipid production in nitrogen rich media is that besides nitrogen removal the phosphate uptake is not significant, making them a phosphate sufficient media which has effect on the microalgal lipid production as reported by many other studies. As in one study, *Chlorella vulgaris* showed highest lipid productivity (58.39 mg/L/day) was obtained under nitrogen deprived with sufficient phosphorus supply (35 mg/L) [141]. For the cultures with nitrogen deprived conditions, then control 0.6:1 and 0.4:1 shows somewhat better performance than 0.8:1 because nitrogen starvation with limited phosphate which is now utilized for survival causing stress to alter the biochemical pathway and induce the lipid production. But still overall the nitrogen enriched cultures show better performance and the best lipid production in terms of productivity and content

were found for 1:1 (53.17 mg/L N and 53.14mg/L P) showing the optimum ratio for the bio-oil production by *C. sorokiniana*. Therefore, according to the high uptake rate of phosphorus in a study concluded, the cultivation conditions in other studies were more likely to be “phosphorus limited” with nitrogen starvation. In other words, insufficient phosphorus will be a suppression factor under nitrogen starvation stage for the increase of lipid productivity [141]. Another study highlights that nitrogen sufficient medium was more suitable for bio-oil production. *Isochrysis galbana* grew in varying nitrogen levels to stimulate stress for lipid synthesis. The findings indicated that lipid synthesis in *I. galbana* diminished when nitrogen concentrations fell below 288 mg/L [132]. Research has shown that *Chlorella vulgaris* can be grown for biodiesel production when there is a sufficient supply of phosphorus. The findings indicated that the lipid yield of *C. vulgaris* for biodiesel was 58.39 mg/L/day in phosphate-rich conditions, which was notably higher compared to a phosphate-limited environment. Therefore, it can be inferred that phosphorus is a crucial macronutrient for the production of lipids in microalgae [141].

Higher N:P ratio (nitrogen is increased and phosphorus is constant) resulted in a better biomass productivity and favorable for biomass growth but for lipid accumulation high N:P is not very favorable together with saline stress microalgae exacerbate growth inhibition leading to reduced biomass productivity which results even less lipid content(%_{DWB}) [182] as it is 8.15(%_{DWB}) and 10.93(%_{DWB}) , lipid productivity as 3.10 (mg/L/d) and 3.12(mg/L/d) and lipid concentration 48.93mg/L and 50.94 mg/L for 2:1-2 and 1.5:1-2 (N/P with 2% salinity) respectively (Figure 28). The results were aligned with but for moderate levels of N:P ratio with saline stress (1:1-2 and 0.6:1-2 and 0.8:1-2-BBM) results in better lipid productivity (7.76 mg/L/d and 7.32 mg/L/d) (Figure 28) and lipid

concentration 96.64mg/L and 96.21mg/L (Figure 15). The lowest concentrations of nitrogen and phosphorus combined with 2% salinity, which showed a significant deficiency (80%) (0.4:1-2 PBR) resulted in the highest lipid content of 20.5% on a dry weight basis. However, due to decreased biomass productivity, the overall lipid concentration was lower, but it remained preferable compared to a high nitrogen and phosphorus ratio, as demonstrated in another study conducted with *Chlorella Vulgaris* [183].

Lipid content was also enhanced by nitrogen deficient conditions as also reported by other literature. Nitrogen deficient conditions promote the lipid accumulation, but it strongly depends on the time during your cultivation period [156]. As the nitrogen deficiency was created after 14-days of cultivation period when microalgal cells already goes to decline phase and starts dyeing possibly. So, there were less cells left who can actively accumulate the lipid in nitrogen deficient conditions. For 2:1-0 and 1.5:1-0 (High Nutrient photobioreactors without salinity) the total lipid content obtained was slightly increased during second stage cultivation with zero nitrogen in media as 116mg/L and 131.21 mg/L of lipid (Figure 17) as compared to first stage (Figure 13) for N:P-1:1 contained lipid concentration of 129mg/L with 24.27% of lipid content (mg of lipid percent dry weight of biomass), which is the highest among all scenarios as it had a balanced nitrogen and phosphorus approach for microalgal growth in initial days of cultivation and lipid accumulation is higher after nitrogen levels go down. The lipid content for 2:1-2 and 1.5:1-2 (N/P with 2% salinity) also enhanced 71.15 mg/L and 84.06mg/L respectively (Figure 17), then for the first stage it was 48.93mg/L and 50.94mg/L respectively (Figure 15). But in terms of lipid content %_{DCW} overall content is lower, as it was 8.15%_{DCW} for 2:1-2(N:P

with 2% salinity) in first stage and drop to 5.67%_{DCW} in second stage of deficient N cultivation. Similar drop was found for other PBRs also as for 1.5/1-2 it drops from 10.93%_{DCW} (first stage) to 6.77%_{DCW} (second stage) (Figure 28).

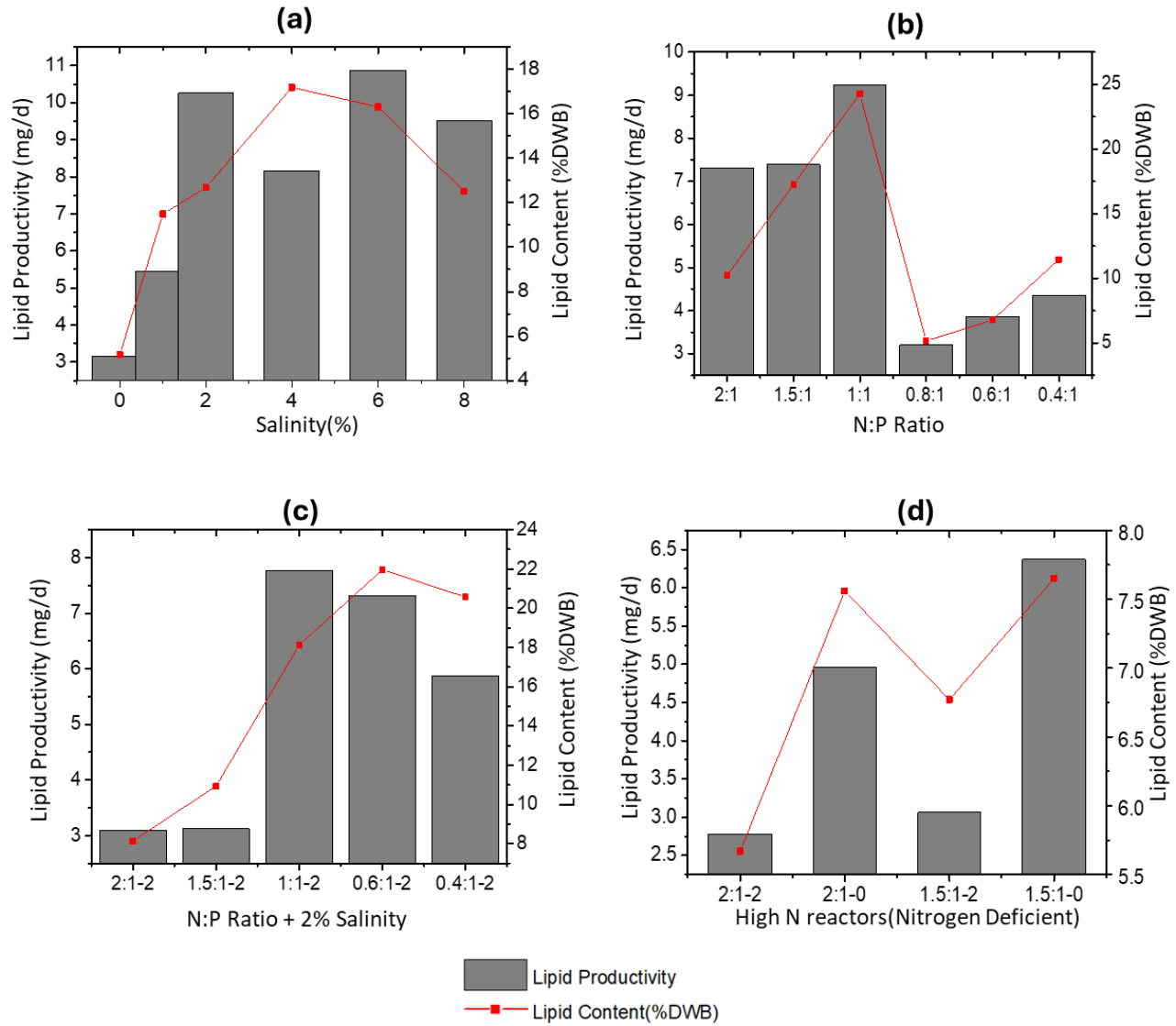


Figure 28: (a)Effect of varied saline levels (0,1,2,4,6,8 percent), (b)Effect of varied N:P (2:1, 1.5:1, 1:1, 0.8:1, 0.6:1, 0.4:1), (c)Effect of varying N:P ratio(2:1, 1.5:1, 1:1, 0.6:1, 0.4:1) with optimal salinity obtained 2%(2g NaCl), (b)Effect of second stage cultivation of High N/P photobioreactors, on lipid production per day basis and lipid content %_{DWB} in microalgae cell *Chlorella sorokiniana*

6.3.2 Varied CO₂ concentrations with Optimal salinity and N:P ratio

Lipid accumulation in *C. sorokiniana* is another key metabolic response to environmental and nutrient stress, with potential applications in biofuel and bioplastic co-production. Under the selected baseline conditions, the introduction of CO₂ at different concentrations affected the balance between growth and lipid biosynthesis. Similar to the trend observed with PHA, lipid content and productivity peaked at 4% , 6% and 8% CO₂ as 26%, 23% and 36% dry weight biomass of lipid content obtained and lipid productivity of 7.94, 12.12 and 13.23 (mg/L/d) (**Figure 15**), suggesting that carbon enrichment facilitated lipid pathway activation without severely compromising growth. Conversely, lipid accumulation declined at 8% CO₂ (138.12 mg/L) (**Figure 11**), likely due to carbon overloading and oxidative stress, reinforcing the need for optimal CO₂ dosing in integrated wastewater–bioproduct systems. Elevated CO₂ levels can enhance lipid accumulation in *Chlorella sorokiniana*. A study investigated the effects of elevated CO₂ on lipid production in *Chlorella sorokiniana* LS-2. The researchers found that supplying extra CO₂ improved lipid accumulation by increasing carbon metabolism. Transcriptomic analysis revealed upregulation of genes involved in carbon fixation and lipid biosynthesis pathways under high CO₂ conditions, indicating that elevated CO₂ levels can enhance lipid production in this species [184]. Another study explored the cultivation of high CO₂ tolerant *Chlorella* strains using flue gas containing 40% CO₂. The study confirmed that *Chlorella sorokiniana* promoted lipid accumulation while fixing CO₂ from flue gas, demonstrating its potential for biofuel processing under high CO₂ conditions [185].

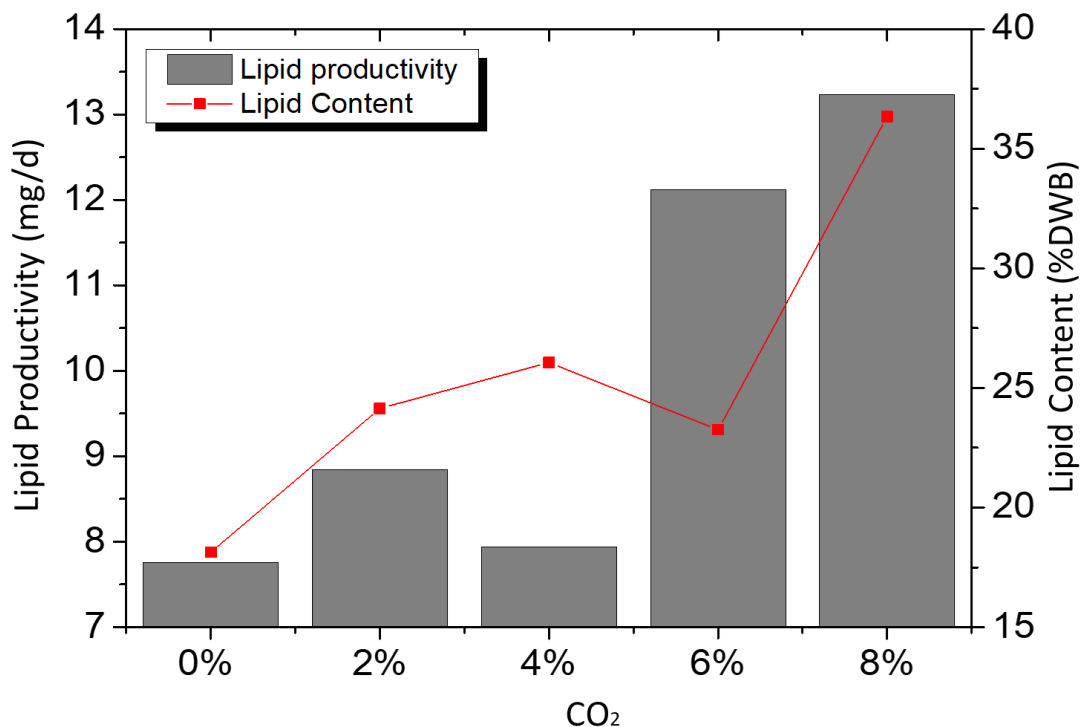


Figure 29: Lipid content and productivity under varying CO₂ conditions

6.4 CO₂ bio fixation rate

The effectiveness of *Chlorella sorokiniana* in removing CO₂ from the provided gas mixtures was evaluated by measuring its carbon biofixation performance at different CO₂ concentrations (0%, 2%, 4%, 6%, and 8%). The CO₂ fixation rate first rose with CO₂ enrichment, as the bar chart illustrates, going from about 60 mg/L/day at 0% CO₂ (ambient air only) to about 70 mg/L/day at 2% CO₂. This pattern suggests that photosynthetic activity and biomass growth are enhanced by moderate CO₂ availability. Interestingly, at 4% CO₂, the fixation rate decreased somewhat to about 55 mg/L/day. our shows that carbon may have been diverted from biomass accumulation to biopolymer synthesis, even though it supports the highest PHA accumulation in our study. The best carbon uptake for biomass production was indicated by the maximum fixation rate, which reached about 95 mg/L/day at 6% CO₂. Nevertheless, the fixation rate decreased once again to about 65 mg/L/day at

8% CO₂, most likely because of CO₂ oversaturation or pH-induced stress that prevented algal metabolism. These findings show a trade-off between PHA formation and carbon fixation, with 4% CO₂ being the most advantageous for biopolymer accumulation and 6% CO₂ being the most successful for CO₂ biofixation. Therefore, the particular application focus—whether it be higher PHA productivity or maximum CO₂ capture—determines the right CO₂ concentration.

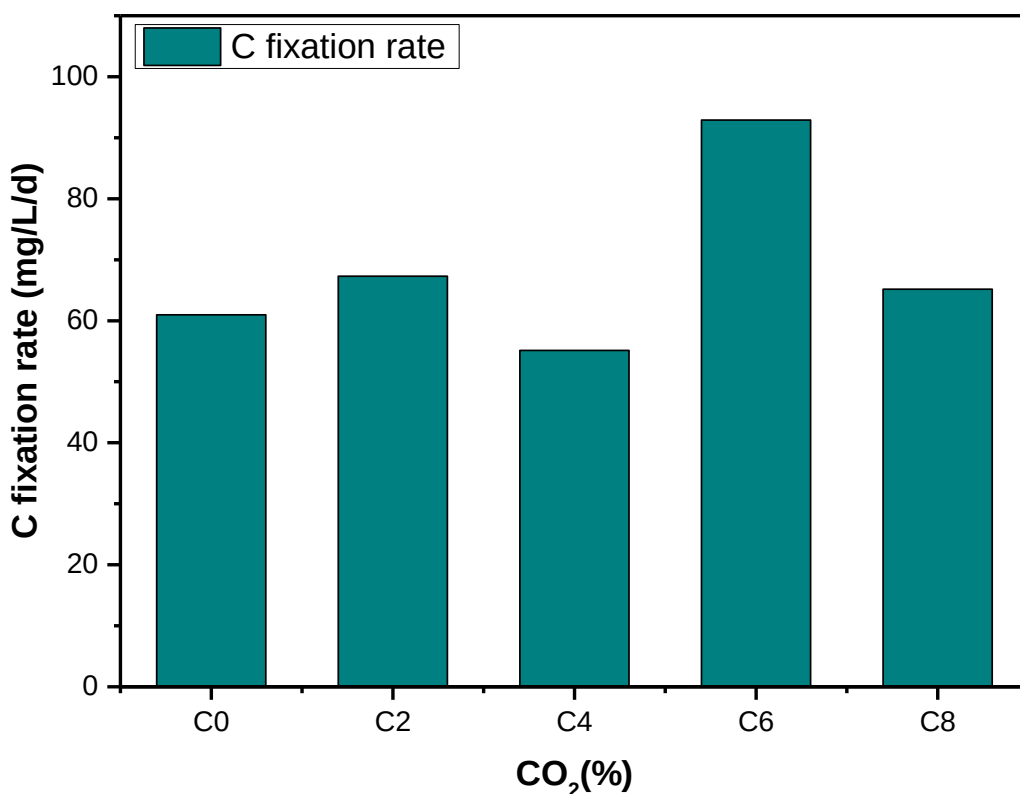


Figure 30: C biofixation rate under different CO₂ percentages

With CO₂ enrichment, the algal biomass's elemental carbon content (%C) significantly rose, suggesting improved carbon assimilation in conditions of elevated CO₂. The carbon content was 38.76% at ambient circumstances (0% CO₂), indicating a limited amount of CO₂ available for photosynthesis. The carbon content increased dramatically to 50.14% after enrichment to 2%, indicating a notable improvement in the incorporation of carbon

into algal biomass. This pattern implies that cellular biosynthesis and carbon absorption efficiency are increased by even modest CO₂ input. Remarkably, the carbon content stayed high at 49.25% at 4% CO₂, which is consistent with the previously noted rise in PHA buildup, which is naturally carbon-rich. The carbon content was somewhat lower than the peak at 2% and remained reasonably consistent at 6% and 8% CO₂ (48.58% and 48.79%, respectively). This could suggest that the cells prioritise rapid biomass synthesis over dense carbon storage after a specific CO₂ threshold. Stress-induced constraints or carbon absorption pathway saturation may possibly be the cause of the stabilisation or minor decrease in carbon content at higher doses. According to these findings, the ideal range for increasing the amount of carbon in biomass is between 2 to 4% CO₂, whereas greater concentrations may speed up development but not always increase the carbon density per biomass unit.

Table 5: CHNS Analysis for total Carbon fixed with Nitrogen, Hydrogen and Sulphur

CO₂ percent (%)	C (%)	N (%)	H (%)	S (%)
0	38.76	7.6	6.226	0.505
2	50.14	6.86	6.261	0.598
4	49.25	5.73	6.316	0.431
6	48.58	5.68	6.33	0.347
8	48.79	5.54	6.29	0.415

CHAPTER 7

PHA Characterization and Identification

7.1 FTIR (Fourier Transform Infrared Spectrometry) of obtained polymer

First spectrum (Figure 31a) had a clear PHB profile, with a sharp peak at 1720 cm^{-1} corresponding to the ester carbonyl stretch, typical asymmetric CH_2/CH_3 bending vibrations (1450 and 1375 cm^{-1}) and ester linkage is situated at 1077 cm^{-1} . The second spectrum (Figure 31b) appears to have slight shift in ester carbonyl stretch at 1723 cm^{-1} reflecting the copolymer composition, methylene and methyl groups vary depending on the HV content. However, the peak at 1621 cm^{-1} is more common in PHBV due to influence of HV units and typically not observe in pure PHB strongly suggesting the presence of PHB-co-PHV or PHBV(Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)) [186] Because of its reduced crystallinity, PHBV has larger peaks, while PHB often has a more defined structure [187]. A higher 3HV content makes a material more malleable and processable by lowering its melting point and crystallinity. Thus, in addition to confirming the existence of PHBV, the FTIR results demonstrate how even minute compositional variations can have a big impact on the structural and thermal properties of the polymer [188].

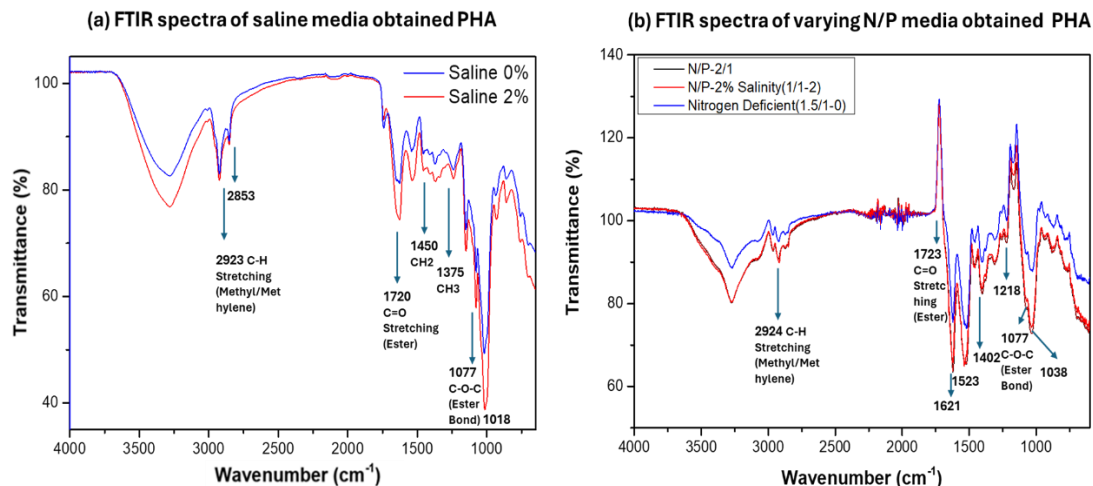


Figure 31: FTIR analysis of extracted PHA of optimal conditions

The FTIR spectrum of the extracted polymer from *Chlorella sorokiniana* exhibited several characteristic peaks confirming the presence of polyhydroxyalkanoates (PHAs), most likely polyhydroxybutyrate (PHB). A prominent absorption band observed around 1720 cm^{-1} corresponds to the stretching vibration of the ester carbonyl (C=O) group, which is a key functional group in PHAs. Additional peaks at approximately 2920 cm^{-1} and 2850 cm^{-1} are attributed to asymmetric and symmetric stretching of methylene (CH₂) groups, respectively, indicating the presence of saturated aliphatic chains. The absorption band near 1450 cm^{-1} corresponds to CH₃ bending vibrations, while peaks in the region of 1270–1050 cm^{-1} are associated with C–O–C stretching vibrations of ester linkages. A broad peak around 3300–3500 cm^{-1} suggests residual moisture or hydroxyl-containing compounds. Collectively, these spectral features are consistent with the structure of PHB, suggesting successful intracellular accumulation of this polymer under the applied stress conditions. In summary, while elevated CO₂ levels can enhance PHA production in microalgae, current research does not provide substantial evidence of significant alterations in the polymer's chemical structure due to increased CO₂ concentrations.

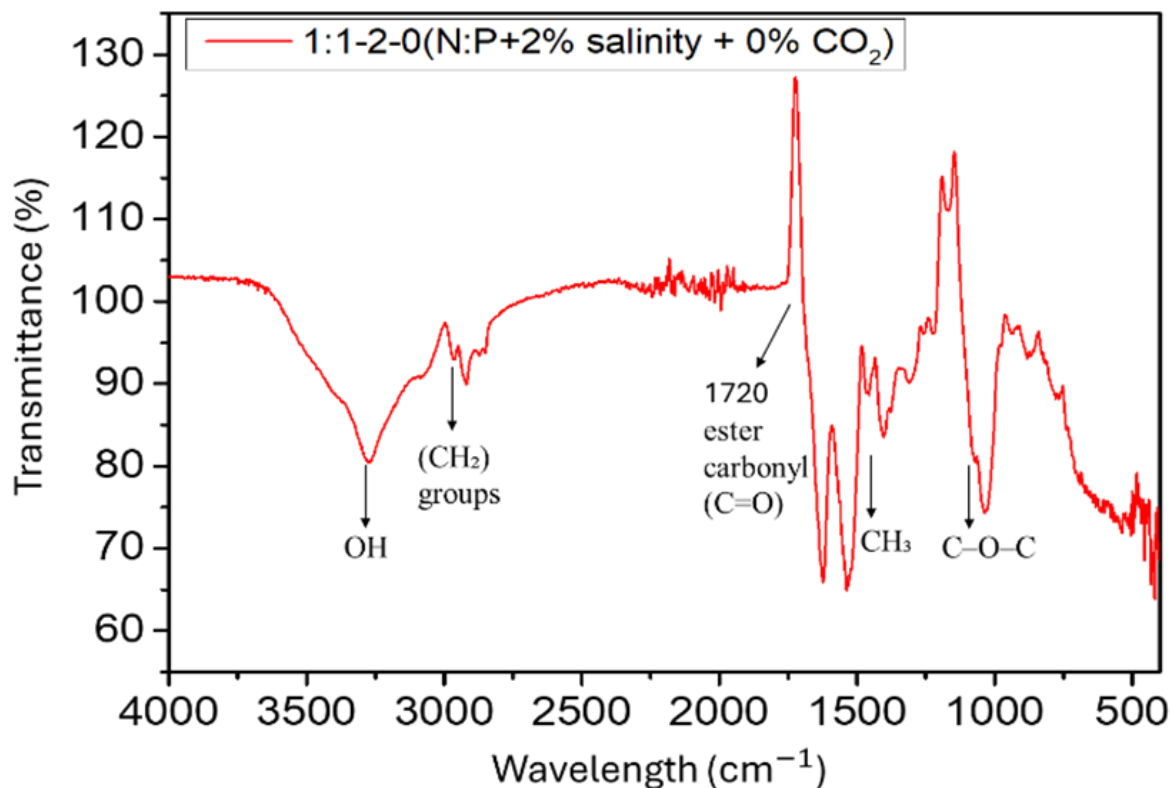


Figure 32: FTIR analysis for CO₂ variants

7.2 Thermogravimetric Analysis of obtained polymer

Thermal Analysis (TGA) – PHB has a sharp melting point ($\sim 175^{\circ}\text{C}$), while PHBV melts at a slightly lower range ($140\text{--}170^{\circ}\text{C}$) [189]. Thermal properties of the obtained PHA were observed through thermogravimetric analysis. Experiments resulting in higher quantity of PHA among all 5 sections were degraded to the maximum of 800°C . The degradation started between $250\text{--}300^{\circ}\text{C}$ (T_{onset}) for all curves and the maximum weight loss occurred between $300\text{--}400^{\circ}\text{C}$ (T_{max}). For all the samples tested complete degradation doesn't occur as there were some residues left (20-30% at 550°C). For the samples consisting of salinity seems to have least residue and indicate a more crystalline structure which is generally a characteristic of PHB, or it contains low value of HV (hydroxy velarate units) (Saline 2%-red curve) (Figure 33a). Similarly, for 1:1-2 (N:P-2% Salinity) the residue is less as

compared to other conditions, it could be considered that saline conditions pushed the PHB polymerization and N:P conditions pushed PHBV polymers inside microalgae cells. A lower melting temperature results from the disruption of PHB's crystalline structure caused by the addition of HV units, which also reduces crystalline. PHBV is more processable than pure PHB because of its lower melting point. Melting temperatures for HBV specimens with different HV contents ranged from 170 to 180°C, while thermal decomposition temperatures exceeded 220°C. This suggests that by expanding the processing window in comparison to PHB, adding HV to PHBV polymers can greatly increase their workability [190]. In summary, the melting temperature of PHBV decreases as the HV content increases, making it more suitable for applications requiring lower processing temperatures.

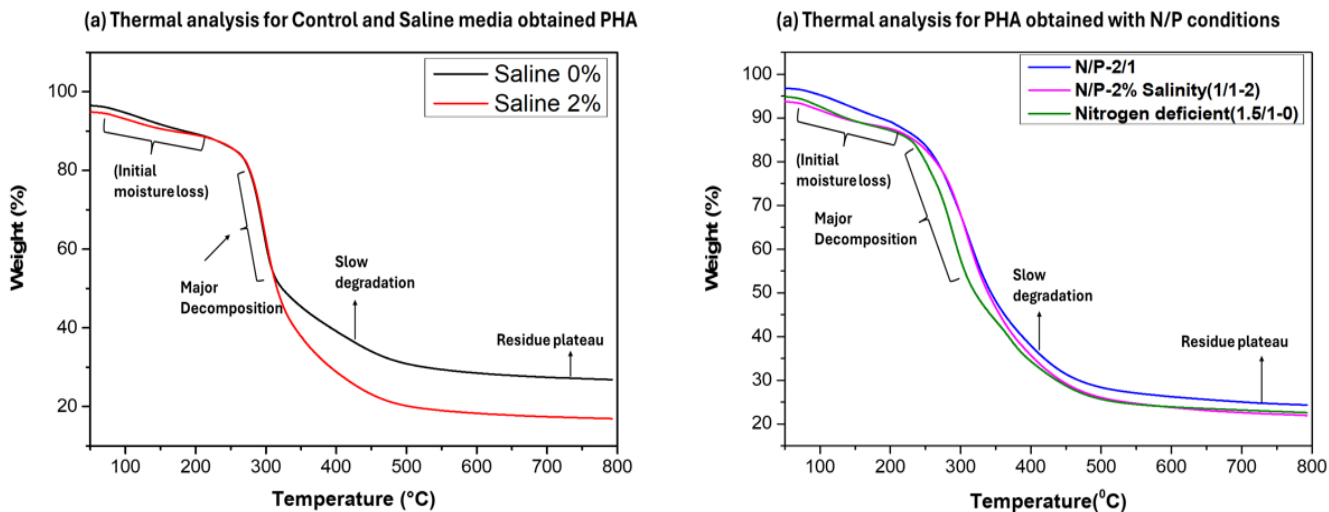


Figure 33: Thermal Analysis obtained from extracted PHA with optimal conditions

Thermal degradation range: 250–350°C, aligning with or exceeding commercial PHB/PHBV (240–290°C). Good thermal stability, suitable for real-world bioplastic applications. Slight variations with nutrient levels show potential for tuning polymer

properties for industrial use. FTIR & TGA confirmed structural and thermal behavior comparable to industrial-grade PHAs.

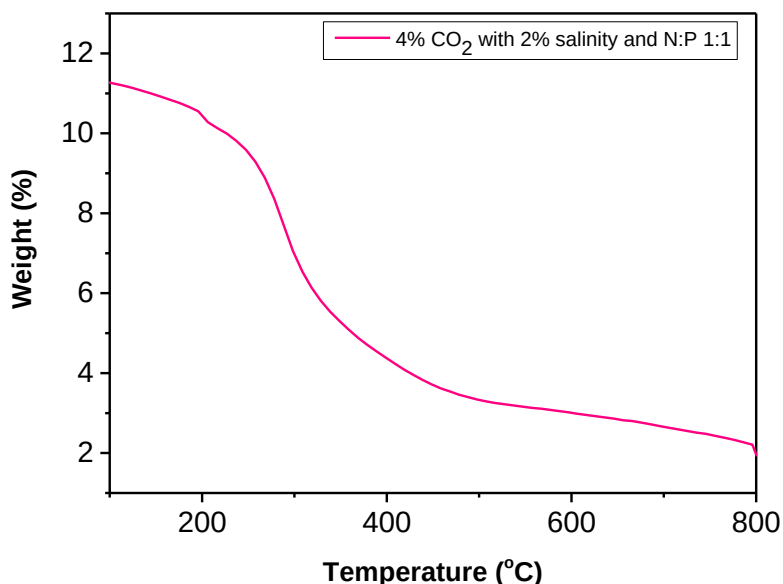


Figure 34: Thermal analysis of extracted PHA with CO₂

7.3 Transmission Electron Microscopy of microalgae cell

TEM analysis tells us about the cell size and accumulation of PHA granules inside the cells. Most studies have reported the presence of white or grey white coloured PHA granules inside the microalgal cell but the range and size of accumulated PHA is largely depends on the specie and the conditions in which microalgae grown [191]. In our study, we dealt with *Chlorella Sorokiniana* and observed the PHA accumulation under 19 different experiments and one as control (BBM). For TEM we presented the highest accumulating conditions among all of them and how it has been changed while we shift from one condition to another. We can see that *Chlorella sorokiniana* cell size is smaller (around 280nm) in control media with very small diameter of PHA granules visualized (Figure 35a). But for a condition with 2% salinity cell size and quantity of PHA granules

increased highly as clusters of grey-white granules appeared (**Figure 35b**). For condition (N:P-2:1) microalgae cell size is relatively very large (around 1 μ m) [147], and dense that PHA granules are very hard to visualize as % accumulation of PHA within the cell is less (**Figure 35c**). But if we see the synergetic effect of both optimum saline (2%) and optimum N:P (1:1) on cell size and PHA accumulation it could be visualized that cell size is relatively smaller (less than 200nm) and less dense but there is better accumulation of PHA granules inside the cell than N:P ratio 2:1 (**Figure 35d**). For fifth analyzation (second stage cultivation in nitrogen deficient media) we attained the best results among all as very large cell size (bigger than 1 μ m) along with very bright white granules fully occupied inside the cell were found (**Figure 35e**). Researchers discovered the existence of PHA granules within the microalgal cells of *Scenedesmus* sp., *Coelastrella* sp., and *Phormidium* sp. when nitrogen availability was limited [119].

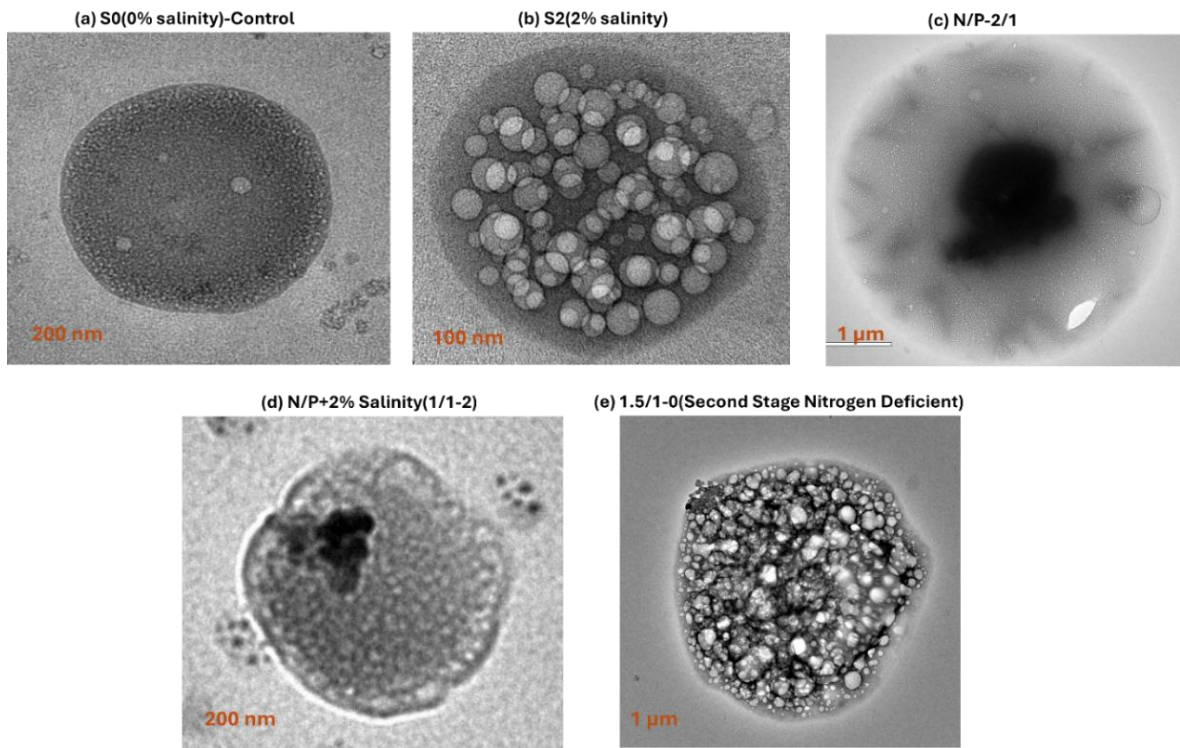


Figure 35: Transmission electron microscopy for visualization of PHA inside microalgal cells

To further confirm the intracellular accumulation of polyhydroxyalkanoates (PHAs) in *Chlorella sorokiniana*, Transmission Electron Microscopy (TEM) was employed. This technique enables high-resolution visualization of cellular ultrastructure, allowing direct observation of PHA granules within the cytoplasm. Cells cultivated under elevated CO₂ and stress conditions were carefully fixed and sectioned for imaging. The presence of electron-dense, spherical inclusions within the microalgal cells provided morphological evidence of PHA accumulation [192]. These granules were distributed throughout the cytoplasmic matrix, often occupying a significant portion of the intracellular space, which aligns with previously reported microalgal responses under nutrient-limited or carbon-enriched environments. It is evident that at 0% CO₂ with a 1:1 nitrogen to phosphorus ratio, saline stress leads to the accumulation of material within the cell; however, the amount and size of PHA are minimal, with only tiny white droplets visible at the cell's perimeter, making the cell appear delicate. In contrast, at CO₂ levels of 2% and 4%, PHA was distributed throughout the entire cell, occupying more space, yet the cells still exhibited fragility (Figure 36a and 36b). As the CO₂ concentration increased to a moderate 4%, we observed robust cells with strong walls and significant accumulation of material (Figure 36c and 36d). This highlights the significance of elevated CO₂ levels for enhancing microalgal cell growth, enabling them to withstand greater stress and produce higher quantities of bioproducts when faced with increasing salinity and nitrogen limitations.

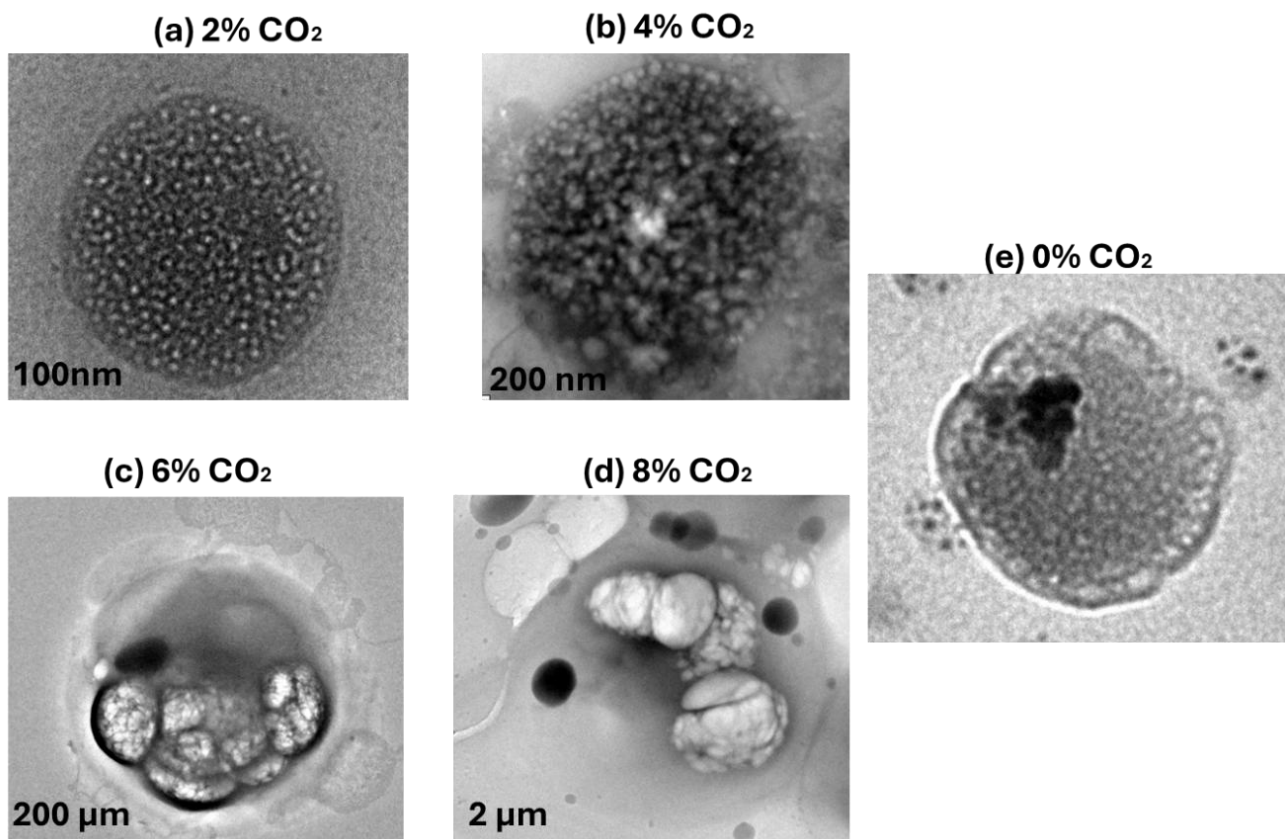


Figure 36: Effect of varied CO₂ concentration on accumulation of PHA granules inside microalgae smell

CHAPTER 8

Conclusions and Recommendation

8.1 Conclusions

This research explores the lipid and polyhydroxyalkanoate (PHA) production of *Chlorella sorokiniana* while treating synthetic wastewater, focusing on the influence of salinity, nitrogen-to-phosphorus (N/P) ratio, and sequential cultivation. The results demonstrate how various environmental conditions impact microalgal metabolism, biomass generation, polymer production, and nutrient removal efficiency.

The levels of salinity had a significant effect on productivity. The highest PHA (780 mg/L) and biomass productivity (1133 mg/L) were achieved at a salinity of 2%. At 4% salinity, the efficiency of PHA accumulation peaked at 99%, which represents a 27% increase compared to 2%. Lipid production reached its highest point at 6% salinity (152 mg/L), while the maximum lipid concentration relative to dry biomass was observed at 4% salinity. The importance of optimizing salinity for wastewater treatment is highlighted by the fact that nitrogen removal was most successful at 6% salinity (98%), while phosphorus removal was most effective at 1% salinity.

The highest yield of PHA and optimal biomass production (1000 mg/L) were attained with a 2/1 nitrogen to phosphorus (N/P) ratio. In comparison to nitrogen-rich settings, a 15-20% rise in PHA accumulation was noted at a 1/1 N/P ratio, which also resulted in the highest lipid concentration (129.36 mg/L) and PHA accumulation (46%). When nitrogen

availability was reduced (0.6/1 and 0.4/1 N/P), nitrogen removal increased by as much as 20%, underscoring the importance of nutrient limitation in wastewater treatment.

A biomass productivity of 666 mg/L was achieved, along with a 97% elimination of nitrogen and 100% elimination of phosphorus, resulting in a PHA accumulation of 57% by combining 2% salinity with a 1/1 N/P ratio. In the second phase of cultivation under nitrogen limitation (1.5/1-0 N/P), PHA production reached 1080 mg/L, corresponding to a 63% accumulation. Saline conditions promote the production of PHB, whereas the 1/1 N/P ratio enhances the production of PHBV. The nitrogen-dependent growth rate constant was validated through sigmoid modelling, which provided insights for optimizing scalable bioprocesses.

A 4% CO₂ air mix significantly enhanced PHA production in *Chlorella sorokiniana*, based on the combination of ideal cultivation parameters, which included 2% salinity, a nitrogen to phosphorus ratio of 1:1, and various CO₂ levels. The optimal balance between cell growth and the accumulation of biopolymers due to stress was achieved under these conditions. Although higher CO₂ concentrations (6% and 8%) may have slightly boosted biomass, they did not lead to greater PHA yields. This could be attributed to an overload of carbon in the system or a shift in metabolic priorities. These findings indicate that 4% CO₂ supplementation is the most effective condition for enhancing the production of biodegradable polymers when facing combined salinity and nutrient stress, offering a promising strategy for sustainable bioplastic production.

8.2 Recommendations

The following recommendations are proposed for further investigation and potential expansion based on the outcomes of the five tests conducted to enhance PHA production using *Chlorella sorokiniana*:

1. Salinity Variability

Since it yielded the highest PHA production, it is recommended to maintain salinity levels at 2% NaCl. Elevated salinity levels (4–8%) created stress that hindered growth and production.

Future Direction: Explore long-term cultivation in varying salinity conditions to simulate wastewater environments found in various industries or natural settings.

2. N:P Ratio Variability

Recommendation: To achieve the optimal balance between growth and PHA accumulation, a 1:1 N:P ratio should be utilized. Lower ratios led to premature nutritional limitation, while a 2:1 N:P ratio enhanced growth but delayed PHA formation.

Future Direction: Investigate dynamic nutrient feeding strategies to more effectively transition from the growth phase to the polymer accumulation phase.

3. Stress from Salinity with Optimal N:P Ratio Suggestion

Unlike individual optimization efforts, applying 2% NaCl salt together with a 1:1 N:P ratio resulted in increased PHA productivity. This indicates that a proper nutritional balance combined with mild osmotic stress can have synergistic benefits.

Future Considerations: To better understand the mechanisms that govern PHA production, further research should focus on the metabolic and biochemical pathways that are engaged under this specific combination of stress.

4. High N:P Second-Stage Cultivation Recommendation:

Inducing PHA accumulation was effectively achieved by transitioning high-nitrogen cultures (2:1 N:P) to nitrogen-limited conditions during a second-stage cultivation. It is recommended to implement this two-phase strategy to enhance the production of polymers and biomass.

Future Considerations: Investigate the optimal timings and nutrient transition intervals to achieve cost-effective production and successful scaling.

5. Variations in CO₂ Levels under Integrated Conditions

Suggestion: The highest accumulation of PHA was achieved with 4% CO₂ supplementation at optimal salinity (2% NaCl) and a nitrogen to phosphorus ratio (1:1), indicating an ideal balance between stress and carbon availability.

Future consideration: Further studies should focus on quantifying carbon absorption, practical applications of actual flue gas, and systems for continuous CO₂ supply to advance integrated CO₂ capture and bioplastic production.

References

- [1] W. Yi *et al.*, “Environmental Science and Ecotechnology Nature ’ s fi ght against plastic pollution: Algae for plastic biodegradation and bioplastics production,” *Environ. Sci. Ecotechnology*, vol. 4, p. 100065, 2020, doi: 10.1016/j.esec.2020.100065.
- [2] D. Kweku *et al.*, “Greenhouse Effect: Greenhouse Gases and Their Impact on Global Warming,” *J. Sci. Res. Reports*, vol. 17, no. 6, pp. 1–9, 2018, doi: 10.9734/jsrr/2017/39630.
- [3] A. Aslam, S. R. Thomas-Hall, T. A. Mughal, and P. M. Schenk, “Selection and adaptation of microalgae to growth in 100% unfiltered coal-fired flue gas,” *Bioresour. Technol.*, vol. 233, pp. 271–283, Jun. 2017, doi: 10.1016/j.biortech.2017.02.111.
- [4] J. G. Speight, “Unconventional gas,” in *Natural Gas*, Elsevier, 2019, pp. 59–98. doi: 10.1016/B978-0-12-809570-6.00003-5.
- [5] S. R. Chia, K. W. Chew, H. Y. Leong, S.-H. Ho, H. S. H. Munawaroh, and P. L. Show, “CO₂ mitigation and phycoremediation of industrial flue gas and wastewater via microalgae-bacteria consortium: Possibilities and challenges,” *Chem. Eng. J.*, vol. 425, p. 131436, Dec. 2021, doi: 10.1016/j.cej.2021.131436.
- [6] R. Morales-Ospino, R. G. Santiago, R. M. Siqueira, D. C. S. de Azevedo, and M. Bastos-Neto, “Assessment of CO₂ desorption from 13X zeolite for a prospective TSA process,” *Adsorption*, vol. 26, no. 5, pp. 813–824, Jul. 2020, doi:

10.1007/s10450-019-00192-5.

- [7] L. Wang *et al.*, “Experimental evaluation of adsorption technology for CO₂ capture from flue gas in an existing coal-fired power plant,” *Chem. Eng. Sci.*, vol. 101, pp. 615–619, Sep. 2013, doi: 10.1016/j.ces.2013.07.028.
- [8] J. de Riva, J. Suarez-Reyes, D. Moreno, I. Díaz, V. Ferro, and J. Palomar, “Ionic liquids for post-combustion CO₂ capture by physical absorption: Thermodynamic, kinetic and process analysis,” *Int. J. Greenh. Gas Control*, vol. 61, pp. 61–70, Jun. 2017, doi: 10.1016/j.ijggc.2017.03.019.
- [9] C. A. Scholes, M. T. Ho, A. A. Aguiar, D. E. Wiley, G. W. Stevens, and S. E. Kentish, “Membrane gas separation processes for CO₂ capture from cement kiln flue gas,” *Int. J. Greenh. Gas Control*, vol. 24, pp. 78–86, May 2014, doi: 10.1016/j.ijggc.2014.02.020.
- [10] A. K. Vuppaladadiyam *et al.*, “Impact of Flue Gas Compounds on Microalgae and Mechanisms for Carbon Assimilation and Utilization,” *ChemSusChem*, vol. 11, no. 2, pp. 334–355, Jan. 2018, doi: 10.1002/cssc.201701611.
- [11] G. Li, W. Xiao, T. Yang, and T. Lyu, “Optimization and Process Effect for Microalgae Carbon Dioxide Fixation Technology Applications Based on Carbon Capture: A Comprehensive Review,” *C*, vol. 9, no. 1, p. 35, Mar. 2023, doi: 10.3390/c9010035.
- [12] C.-Y. Kao, S.-Y. Chiu, T.-T. Huang, L. Dai, L.-K. Hsu, and C.-S. Lin, “Ability of a mutant strain of the microalga *Chlorella* sp. to capture carbon dioxide for biogas

- upgrading,” *Appl. Energy*, vol. 93, pp. 176–183, May 2012, doi: 10.1016/j.apenergy.2011.12.082.
- [13] D. Cheng *et al.*, “Adaptive evolution and carbon dioxide fixation of *Chlorella* sp. in simulated flue gas,” *Sci. Total Environ.*, vol. 650, pp. 2931–2938, Feb. 2019, doi: 10.1016/j.scitotenv.2018.10.070.
- [14] F. Perera, “Pollution from Fossil-Fuel Combustion is the Leading Environmental Threat to Global Pediatric Health and Equity: Solutions Exist,” *Int. J. Environ. Res. Public Health*, vol. 15, no. 1, p. 16, Dec. 2017, doi: 10.3390/ijerph15010016.
- [15] S. V. Zakharov, V. P. Lushpey, L. R. Abbasova, and S. Zhongkai, “Analysis of the impact of the energy industry on the environment,” *IOP Conf. Ser. Earth Environ. Sci.*, vol. 1070, no. 1, p. 012044, Jul. 2022, doi: 10.1088/1755-1315/1070/1/012044.
- [16] P. Gupta, “Environmental and ecotoxicology,” in *Illustrated Toxicology*, Elsevier, 2018, pp. 373–425. doi: 10.1016/B978-0-12-813213-5.00014-6.
- [17] I. Manisalidis, E. Stavropoulou, A. Stavropoulos, and E. Bezirtzoglou, “Environmental and Health Impacts of Air Pollution: A Review,” *Front. Public Heal.*, vol. 8, Feb. 2020, doi: 10.3389/fpubh.2020.00014.
- [18] A. Krishnan, A. Nighojkar, and B. Kandasubramanian, “Emerging towards zero carbon footprint via carbon dioxide capturing and sequestration,” *Carbon Capture Sci. Technol.*, vol. 9, p. 100137, Dec. 2023, doi: 10.1016/j.ccst.2023.100137.
- [19] K. W. Bandilla, “Carbon Capture and Storage,” in *Future Energy*, Elsevier, 2020, pp. 669–692. doi: 10.1016/B978-0-08-102886-5.00031-1.

- [20] L. M. Alsarhan, A. S. Alayyar, N. B. Alqahtani, and N. H. Khdary, “Circular Carbon Economy (CCE): A Way to Invest CO₂ and Protect the Environment, a Review,” *Sustainability*, vol. 13, no. 21, p. 11625, Oct. 2021, doi: 10.3390/su132111625.
- [21] W. Klinthong, Y.-H. Yang, C.-H. Huang, and C.-S. Tan, “A Review: Microalgae and Their Applications in CO₂ Capture and Renewable Energy,” *Aerosol Air Qual. Res.*, vol. 15, no. 2, pp. 712–742, 2015, doi: 10.4209/aaqr.2014.11.0299.
- [22] Y. Gao, C. Gregor, Y. Liang, D. Tang, and C. Tweed, “Algae biodiesel - a feasibility report,” *Chem. Cent. J.*, vol. 6, no. S1, p. S1, Dec. 2012, doi: 10.1186/1752-153X-6-S1-S1.
- [23] S. Nagappan, P.-C. Tsai, S. Devendran, V. Alagarsamy, and V. K. Ponnusamy, “Enhancement of biofuel production by microalgae using cement flue gas as substrate,” *Environ. Sci. Pollut. Res.*, vol. 27, no. 15, pp. 17571–17586, May 2020, doi: 10.1007/s11356-019-06425-y.
- [24] G. P. D. De Silva, P. G. Ranjith, and M. S. A. Perera, “Geochemical aspects of CO₂ sequestration in deep saline aquifers: A review,” *Fuel*, vol. 155, pp. 128–143, Sep. 2015, doi: 10.1016/j.fuel.2015.03.045.
- [25] S. C. Doney, V. J. Fabry, R. A. Feely, and J. A. Kleypas, “Ocean Acidification: The Other CO₂ Problem,” *Ann. Rev. Mar. Sci.*, vol. 1, no. 1, pp. 169–192, Jan. 2009, doi: 10.1146/annurev.marine.010908.163834.
- [26] J. C. M. Pires, “COP21: The algae opportunity?,” *Renew. Sustain. Energy Rev.*, vol. 79, pp. 867–877, Nov. 2017, doi: 10.1016/j.rser.2017.05.197.

- [27] J. Podder, B. R. Patra, F. Pattnaik, S. Nanda, and A. K. Dalai, “A Review of Carbon Capture and Valorization Technologies,” *Energies*, vol. 16, no. 6, p. 2589, Mar. 2023, doi: 10.3390/en16062589.
- [28] S. N. A. Shafie *et al.*, “[EMIM][Tf2N]-Modified Silica as Filler in Mixed Matrix Membrane for Carbon Dioxide Separation,” *Membranes (Basel)*, vol. 11, no. 5, p. 371, May 2021, doi: 10.3390/membranes11050371.
- [29] T. Tomioka, T. Sakai, and H. Mano, “Carbon Dioxide Separation Technology from Biogas by ‘Membrane/Absorption Hybrid Method,’” *Energy Procedia*, vol. 37, pp. 1209–1217, 2013, doi: 10.1016/j.egypro.2013.05.219.
- [30] N. R. H. Rao, B. Tamburic, Y. T. T. Doan, B. D. Nguyen, and R. K. Henderson, “Algal biotechnology in Australia and Vietnam: Opportunities and challenges,” *Algal Res.*, vol. 56, p. 102335, Jun. 2021, doi: 10.1016/j.algal.2021.102335.
- [31] J. C. M. Pires, F. G. Martins, M. C. M. Alvim-Ferraz, and M. Simões, “Recent developments on carbon capture and storage: An overview,” *Chem. Eng. Res. Des.*, vol. 89, no. 9, pp. 1446–1460, Sep. 2011, doi: 10.1016/j.cherd.2011.01.028.
- [32] S. Suliestyah and I. P. Sari, “Effect of temperature and time of carbonization on coal-based activated carbon adsorption,” *IOP Conf. Ser. Mater. Sci. Eng.*, vol. 1098, no. 6, p. 062020, Mar. 2021, doi: 10.1088/1757-899X/1098/6/062020.
- [33] E. Smith, J. Morris, H. Kheshgi, G. Teletzke, H. Herzog, and S. Paltsev, “The cost of CO₂ transport and storage in global integrated assessment modeling,” *Int. J. Greenh. Gas Control*, vol. 109, p. 103367, Jul. 2021, doi:

10.1016/j.ijggc.2021.103367.

- [34] D. Y. C. Leung, G. Caramanna, and M. M. Maroto-Valer, “An overview of current status of carbon dioxide capture and storage technologies,” *Renew. Sustain. Energy Rev.*, vol. 39, pp. 426–443, Nov. 2014, doi: 10.1016/j.rser.2014.07.093.
- [35] H. T. Schaef, B. P. McGrail, and A. T. Owen, “Carbonate mineralization of volcanic province basalts,” *Int. J. Greenh. Gas Control*, vol. 4, no. 2, pp. 249–261, Mar. 2010, doi: 10.1016/j.ijggc.2009.10.009.
- [36] E. M. Valdovinos-García, J. Barajas-Fernández, M. de los Á. Olán-Acosta, M. A. Petriz-Prieto, A. Guzmán-López, and M. G. Bravo-Sánchez, “Techno-Economic Study of CO₂ Capture of a Thermoelectric Plant Using Microalgae (*Chlorella vulgaris*) for Production of Feedstock for Bioenergy,” *Energies*, vol. 13, no. 2, p. 413, Jan. 2020, doi: 10.3390/en13020413.
- [37] Y. Ni, G. S. Eskeland, J. Giske, and J.-P. Hansen, “The global potential for carbon capture and storage from forestry,” *Carbon Balance Manag.*, vol. 11, no. 1, p. 3, Dec. 2016, doi: 10.1186/s13021-016-0044-y.
- [38] C. M. Scott-Buechler and C. H. Greene, “Role of the ocean in climate stabilization,” in *Bioenergy with Carbon Capture and Storage*, Elsevier, 2019, pp. 109–130. doi: 10.1016/B978-0-12-816229-3.00006-5.
- [39] H. Onyeaka, T. Miri, K. Obileke, A. Hart, C. Anumudu, and Z. T. Al-Sharify, “Minimizing carbon footprint via microalgae as a biological capture,” *Carbon Capture Sci. Technol.*, vol. 1, p. 100007, Dec. 2021, doi:

10.1016/j.ccst.2021.100007.

- [40] E. Nessi, A. I. Papadopoulos, and P. Seferlis, “A review of research facilities, pilot and commercial plants for solvent-based post-combustion CO₂ capture: Packed bed, phase-change and rotating processes,” *Int. J. Greenh. Gas Control*, vol. 111, p. 103474, Oct. 2021, doi: 10.1016/j.ijggc.2021.103474.
- [41] S. Seddighi, P. T. Clough, E. J. Anthony, R. W. Hughes, and P. Lu, “Scale-up challenges and opportunities for carbon capture by oxy-fuel circulating fluidized beds,” *Appl. Energy*, vol. 232, pp. 527–542, Dec. 2018, doi: 10.1016/j.apenergy.2018.09.167.
- [42] Y. W. Cheng *et al.*, “Unravelling CO₂ capture performance of microalgae cultivation and other technologies via comparative carbon balance analysis,” *J. Environ. Chem. Eng.*, vol. 9, no. 6, p. 106519, Dec. 2021, doi: 10.1016/j.jece.2021.106519.
- [43] J. Singh and D. W. Dhar, “Overview of carbon capture technology: Microalgal biorefinery concept and state-of-the-art,” *Front. Mar. Sci.*, vol. 6, no. FEB, pp. 1–9, 2019, doi: 10.3389/fmars.2019.00029.
- [44] Y. Arora, S. Sharma, and V. Sharma, “Microalgae in Bioplastic Production: A Comprehensive Review,” *Arabian Journal for Science and Engineering*, vol. 48, no. 6. Institute for Ionics, pp. 7225–7241, Jun. 01, 2023. doi: 10.1007/s13369-023-07871-0.
- [45] A. Folino, A. Karageorgiou, P. S. Calabrò, and D. Komilis, “Biodegradation of

- Wasted Bioplastics in Natural and Industrial Environments: A Review,” *Sustainability*, vol. 12, no. 15, p. 6030, Jul. 2020, doi: 10.3390/su12156030.
- [46] R. Prasad *et al.*, “Role of Microalgae in Global CO₂ Sequestration: Physiological Mechanism, Recent Development, Challenges, and Future Prospective,” *Sustainability*, vol. 13, no. 23, p. 13061, Nov. 2021, doi: 10.3390/su132313061.
- [47] A. Alavianghavanini, H. Shayesteh, P. A. Bahri, A. Vadiveloo, and N. R. Moheimani, “Microalgae cultivation for treating agricultural effluent and producing value-added products,” *Sci. Total Environ.*, vol. 912, p. 169369, Feb. 2024, doi: 10.1016/j.scitotenv.2023.169369.
- [48] R. Guo, X. Cen, B.-J. Ni, and M. Zheng, “Bioplastic polyhydroxyalkanoate conversion in waste activated sludge,” *J. Environ. Manage.*, vol. 370, p. 122866, Nov. 2024, doi: 10.1016/j.jenvman.2024.122866.
- [49] H. M. Amaro, E. M. Salgado, O. C. Nunes, J. C. M. Pires, and A. F. Esteves, “Microalgae systems - environmental agents for wastewater treatment and further potential biomass valorisation,” *J. Environ. Manage.*, vol. 337, p. 117678, Jul. 2023, doi: 10.1016/j.jenvman.2023.117678.
- [50] Y. Ren *et al.*, “Using green alga *Haematococcus pluvialis* for astaxanthin and lipid co-production: Advances and outlook,” *Bioresour. Technol.*, vol. 340, p. 125736, Nov. 2021, doi: 10.1016/j.biortech.2021.125736.
- [51] R. B. Draaisma, R. H. Wijffels, P. (Ellen) Slegers, L. B. Brentner, A. Roy, and M. J. Barbosa, “Food commodities from microalgae,” *Curr. Opin. Biotechnol.*, vol. 24,

- no. 2, pp. 169–177, Apr. 2013, doi: 10.1016/j.copbio.2012.09.012.
- [52] Q. Huang, F. Jiang, L. Wang, and C. Yang, “Design of Photobioreactors for Mass Cultivation of Photosynthetic Organisms,” *Engineering*, vol. 3, no. 3, pp. 318–329, Jun. 2017, doi: 10.1016/J.ENG.2017.03.020.
- [53] S. O. Cinar, Z. K. Chong, M. A. Kucuker, N. Wiecek, U. Cengiz, and K. Kuchta, “Bioplastic production from microalgae: A review,” *International Journal of Environmental Research and Public Health*, vol. 17, no. 11. MDPI AG, Jun. 01, 2020. doi: 10.3390/ijerph17113842.
- [54] I. B. Magalhães, J. Ferreira, J. de S. Castro, L. R. de Assis, and M. L. Calijuri, “Agro-industrial wastewater-grown microalgae: A techno-environmental assessment of open and closed systems,” *Sci. Total Environ.*, vol. 834, p. 155282, Aug. 2022, doi: 10.1016/J.SCITOTENV.2022.155282.
- [55] J. W. Roy Chong *et al.*, “Microalgae-based bioplastics: Future solution towards mitigation of plastic wastes,” *Environ. Res.*, vol. 206, p. 112620, Apr. 2022, doi: 10.1016/j.envres.2021.112620.
- [56] Y. W. Sari, K. Kartikasari, Widayani, I. Setyaningsih, and D. Lestari, “Techno-economic assessment of microalgae for biofuel, chemical, and bioplastic,” in *Microalgae: Cultivation, Recovery of Compounds and Applications*, Elsevier, 2020, pp. 409–432. doi: 10.1016/B978-0-12-821218-9.00013-X.
- [57] M. Koller, “‘Bioplastics from microalgae’—Polyhydroxyalkanoate production by cyanobacteria,” in *Handbook of Microalgae-Based Processes and Products*,

Elsevier, 2020, pp. 597–645. doi: 10.1016/B978-0-12-818536-0.00022-1.

- [58] G. Coppola, M. T. Gaudio, C. G. Lopresto, V. Calabro, S. Curcio, and S. Chakraborty, “Bioplastic from Renewable Biomass: A Facile Solution for a Greener Environment,” *Earth Syst. Environ.*, vol. 5, no. 2, pp. 231–251, Jun. 2021, doi: 10.1007/s41748-021-00208-7.
- [59] A. K. Sadvakasova *et al.*, “Microalgae as a key tool in achieving carbon neutrality for bioproduct production,” *Algal Res.*, vol. 72, p. 103096, May 2023, doi: 10.1016/j.algal.2023.103096.
- [60] R. G. Martins, I. Severo Gonçalves, M. G. De Moraes, and J. A. V. Costa, “Bioprocess engineering aspects of biopolymer production by the cyanobacterium spirulina strain LEB 18,” *Int. J. Polym. Sci.*, vol. 2014, pp. 1–7, 2014, doi: 10.1155/2014/895237.
- [61] Y. L. Itzel *et al.*, “Revalorization of Microalgae Biomass for Synergistic Interaction and Sustainable Applications : Bioplastic Generation,” 2022.
- [62] E. Daneshvar, R. J. Wicker, P. Show, and A. Bhatnagar, “Biologically-mediated carbon capture and utilization by microalgae towards sustainable CO₂ biofixation and biomass valorization – A review,” vol. 427, no. April 2021, 2022.
- [63] R. Madadi, H. Maljaee, and S. Serafim, “Microalgae as Contributors to Produce Biopolymers,” pp. 1–27, 2021.
- [64] B. Kunasundari and K. Sudesh, “Isolation and recovery of microbial polyhydroxyalkanoates,” *Express Polym. Lett.*, vol. 5, no. 7, pp. 620–634, 2011, doi:

10.3144/expresspolymlett.2011.60.

- [65] M. J. Pettinari and D. E. Egoburo, “Manipulation of global regulators in *Escherichia coli* for the synthesis of biotechnologically relevant products,” in *Microbial Cell Factories Engineering for Production of Biomolecules*, Elsevier, 2021, pp. 437–453. doi: 10.1016/B978-0-12-821477-0.00018-0.
- [66] S. Bayarı and F. Severcan, “FTIR study of biodegradable biopolymers: P(3HB), P(3HB-co-4HB) and P(3HB-co-3HV),” *J. Mol. Struct.*, vol. 744–747, pp. 529–534, Jun. 2005, doi: 10.1016/j.molstruc.2004.12.029.
- [67] K. Roja, D. Ruben Sudhakar, S. Anto, and T. Mathimani, “Extraction and characterization of polyhydroxyalkanoates from marine green alga and cyanobacteria,” *Biocatal. Agric. Biotechnol.*, vol. 22, no. August, p. 101358, 2019, doi: 10.1016/j.bcab.2019.101358.
- [68] M. G. A. Vieira, M. A. da Silva, L. O. dos Santos, and M. M. Beppu, “Natural-based plasticizers and biopolymer films: A review,” *Eur. Polym. J.*, vol. 47, no. 3, pp. 254–263, Mar. 2011, doi: 10.1016/j.eurpolymj.2010.12.011.
- [69] A. Shrivastav, S. K. Mishra, and S. Mishra, “Polyhydroxyalkanoate (PHA) synthesis by *Spirulina subsalsa* from Gujarat coast of India,” *Int. J. Biol. Macromol.*, vol. 46, no. 2, pp. 255–260, Mar. 2010, doi: 10.1016/j.ijbiomac.2010.01.001.
- [70] S. S. Costa *et al.*, “Efficacy of *Spirulina* sp. polyhydroxyalkanoates extraction methods and influence on polymer properties and composition,” *Algal Res.*, vol. 33, no. May, pp. 231–238, 2018, doi: 10.1016/j.algal.2018.05.016.

- [71] V. D. Mendhulkar and L. A. Shetye, “Synthesis of Biodegradable Polymer Polyhydroxyalkanoate (PHA) in Cyanobacteria *Synechococcus elongates* Under Mixotrophic Nitrogen- and Phosphate-Mediated Stress Conditions,” *Ind. Biotechnol.*, vol. 13, no. 2, pp. 85–93, Apr. 2017, doi: 10.1089/ind.2016.0021.
- [72] C. Khomlaem, H. Aloui, and B. S. Kim, “Biosynthesis of Polyhydroxyalkanoates from Defatted Chlorella Biomass as an Inexpensive Substrate,” *Appl. Sci.*, vol. 11, no. 3, p. 1094, Jan. 2021, doi: 10.3390/app11031094.
- [73] S. G. Mastropetros, K. Pispas, D. Zagklis, S. S. Ali, and M. Kornaros, “Biopolymers production from microalgae and cyanobacteria cultivated in wastewater: Recent advances,” *Biotechnol. Adv.*, vol. 60, p. 107999, Nov. 2022, doi: 10.1016/j.biotechadv.2022.107999.
- [74] S. S. Costa *et al.*, “Influence of nitrogen on growth, biomass composition, production, and properties of polyhydroxyalkanoates (PHAs) by microalgae,” *Int. J. Biol. Macromol.*, vol. 116, pp. 552–562, Sep. 2018, doi: 10.1016/j.ijbiomac.2018.05.064.
- [75] C. K. da Silva, J. A. V. Costa, and M. G. de Moraes, “Polyhydroxybutyrate (PHB) Synthesis by *Spirulina* sp. LEB 18 Using Biopolymer Extraction Waste,” *Appl. Biochem. Biotechnol.*, vol. 185, no. 3, pp. 822–833, Jul. 2018, doi: 10.1007/s12010-017-2687-x.
- [76] A. Kaewbai-ngam, A. Incharoensakdi, and T. Monshupanee, “Increased accumulation of polyhydroxybutyrate in divergent cyanobacteria under nutrient-deprived photoautotrophy: An efficient conversion of solar energy and carbon

- dioxide to polyhydroxybutyrate by *Calothrix scytonemicola* TISTR 8095,” *Bioresour. Technol.*, vol. 212, pp. 342–347, Jul. 2016, doi: 10.1016/j.biortech.2016.04.035.
- [77] T. Monshupanee and A. Incharoensakdi, “Enhanced accumulation of glycogen, lipids and polyhydroxybutyrate under optimal nutrients and light intensities in the cyanobacterium *Synechocystis* sp. PCC 6803,” *J. Appl. Microbiol.*, vol. 116, no. 4, pp. 830–838, Apr. 2014, doi: 10.1111/jam.12409.
- [78] B. Panda, P. Jain, L. Sharma, and N. Mallick, “Optimization of cultural and nutritional conditions for accumulation of poly- β -hydroxybutyrate in *Synechocystis* sp. PCC 6803,” *Bioresour. Technol.*, vol. 97, no. 11, pp. 1296–1301, Jul. 2006, doi: 10.1016/j.biortech.2005.05.013.
- [79] M. Serif, G. Dubois, A.-L. Finoux, M.-A. Teste, D. Jallet, and F. Daboussi, “One-step generation of multiple gene knock-outs in the diatom *Phaeodactylum tricornutum* by DNA-free genome editing,” *Nat. Commun.*, vol. 9, no. 1, p. 3924, Sep. 2018, doi: 10.1038/s41467-018-06378-9.
- [80] R. Bhati, S. Samantaray, L. Sharma, and N. Mallick, “Poly- β -hydroxybutyrate accumulation in cyanobacteria under photoautotrophy,” *Biotechnol. J.*, vol. 5, no. 11, pp. 1181–1185, Nov. 2010, doi: 10.1002/biot.201000252.
- [81] S. Samantaray and N. Mallick, “Production and characterization of poly- β -hydroxybutyrate (PHB) polymer from *Aulosira fertilissima*,” *J. Appl. Phycol.*, vol. 24, no. 4, pp. 803–814, Aug. 2012, doi: 10.1007/s10811-011-9699-7.

- [82] F. H. P. Tan, N. Nadir, and K. Sudesh, “Microalgal Biomass as Feedstock for Bacterial Production of PHA: Advances and Future Prospects,” *Front. Bioeng. Biotechnol.*, vol. 10, May 2022, doi: 10.3389/fbioe.2022.879476.
- [83] M. Simonazzi *et al.*, “Production of polyhydroxybutyrate by the cyanobacterium cf. *Anabaena* sp.,” *Int. J. Biol. Macromol.*, vol. 191, pp. 92–99, Nov. 2021, doi: 10.1016/j.ijbiomac.2021.09.054.
- [84] G. García, J. E. Sosa-Hernández, L. I. Rodas-Zuluaga, C. Castillo-Zacarías, H. Iqbal, and R. Parra-Saldívar, “Accumulation of PHA in the Microalgae *Scenedesmus* sp. under Nutrient-Deficient Conditions,” *Polymers (Basel)*, vol. 13, no. 1, p. 131, Dec. 2020, doi: 10.3390/polym13010131.
- [85] S. K. Das, A. Sathish, and J. Stanley, “Production of Biofuel and Bioplastic from *Chlorella Pyrenoidosa*,” *Mater. Today Proc.*, vol. 5, no. 8, pp. 16774–16781, 2018, doi: 10.1016/j.matpr.2018.06.020.
- [86] G. Kavitha *et al.*, “Optimization of polyhydroxybutyrate production utilizing waste water as nutrient source by *Botryococcus braunii* Kütz using response surface methodology,” *Int. J. Biol. Macromol.*, vol. 93, pp. 534–542, Dec. 2016, doi: 10.1016/j.ijbiomac.2016.09.019.
- [87] M. Gomes *et al.*, “Natural Polymers in Tissue Engineering Applications,” in *Handbook of Biopolymers and Biodegradable Plastics*, Elsevier, 2013, pp. 385–425. doi: 10.1016/B978-1-4557-2834-3.00016-1.
- [88] D. Plackett and I. Siró, “Polyhydroxyalkanoates (PHAs) for food packaging,” in

- Multifunctional and Nanoreinforced Polymers for Food Packaging*, Elsevier, 2011, pp. 498–526. doi: 10.1533/9780857092786.4.498.
- [89] B. D. Beckstrom, M. H. Wilson, M. Crocker, and J. C. Quinn, “Bioplastic feedstock production from microalgae with fuel co-products: A techno-economic and life cycle impact assessment,” *Algal Res.*, vol. 46, p. 101769, Mar. 2020, doi: 10.1016/J.ALGAL.2019.101769.
- [90] S. K. Das, A. Sathish, and J. Stanley, “Production Of Biofuel And Bioplastic From *Chlorella Pyrenoidosa*,” *Mater. Today Proc.*, vol. 5, no. 8, pp. 16774–16781, 2018, doi: 10.1016/j.matpr.2018.06.020.
- [91] J.-G. Rosenboom, R. Langer, and G. Traverso, “Bioplastics for a circular economy,” *Nat. Rev. Mater.*, vol. 7, no. 2, pp. 117–137, Jan. 2022, doi: 10.1038/s41578-021-00407-8.
- [92] A. D. A. Bin Abu Sofian, H. R. Lim, S. Manickam, W. L. Ang, and P. L. Show, “Towards a Sustainable Circular Economy: Algae-Based Bioplastics and the Role of Internet-of-Things and Machine Learning,” *ChemBioEng Rev.*, Nov. 2023, doi: 10.1002/cben.202300028.
- [93] F. Frongia, L. Arru, M. R. Cramarossa, and L. Forti, “Microalgae potential in the capture of CO₂ emission,” *Acta Innov.*, no. 41, pp. 19–27, Dec. 2021, doi: 10.32933/ActaInnovations.41.2.
- [94] W. L. Filho *et al.*, “Low carbon futures: assessing the status of decarbonisation efforts at universities within a 2050 perspective,” *Energy. Sustain. Soc.*, vol. 13, no.

1, p. 5, Feb. 2023, doi: 10.1186/s13705-023-00384-6.

- [95] G. R. Conceição, J. R. de J. Santos, C. S. da Silva, and F. A. Chinalia, “Microalgae as agents for upcycling industrial wastewater: energy patents and scientific literature overview,” *Res. Soc. Dev.*, vol. 11, no. 15, p. e230111537097, Nov. 2022, doi: 10.33448/rsd-v11i15.37097.
- [96] P. Kaur *et al.*, “Recent Development in Urban Polyhydroxyalkanoates Biorefineries,” *ChemBioEng Rev.*, vol. 10, no. 4, pp. 441–461, Aug. 2023, doi: 10.1002/cben.202200045.
- [97] J. Y. Boey, L. Mohamad, Y. Sen Khok, G. S. Tay, and S. Baidurah, “A Review of the Applications and Biodegradation of Polyhydroxyalkanoates and Poly(lactic acid) and Its Composites,” *Polymers (Basel)*, vol. 13, no. 10, p. 1544, May 2021, doi: 10.3390/polym13101544.
- [98] J. E. Keffer and G. T. Kleinheinz, “Use of *Chlorella vulgaris* for CO₂ mitigation in a photobioreactor,” *J. Ind. Microbiol. Biotechnol.*, vol. 29, no. 5, pp. 275–280, Nov. 2002, doi: 10.1038/sj.jim.7000313.
- [99] D. Moreira and J. C. M. Pires, “Atmospheric CO₂ capture by algae: Negative carbon dioxide emission path,” *Bioresour. Technol.*, vol. 215, pp. 371–379, Sep. 2016, doi: 10.1016/j.biortech.2016.03.060.
- [100] H. Roh *et al.*, “Improved CO₂-derived polyhydroxybutyrate (PHB) production by engineering fast-growing cyanobacterium *Synechococcus elongatus* UTEX 2973 for potential utilization of flue gas,” *Bioresour. Technol.*, vol. 327, p. 124789, May

- 2021, doi: 10.1016/j.biortech.2021.124789.
- [101] N. J. Berger, M. A. Masri, T. Bru, D. Garbe, C. Pfeifer, and J. Lindorfer, “Utilizing a CHP Power Plant ’ s Energy and CO₂ Emissions for the Manufacture of Affordable and Carbon Neutral Algae Bioplastic for Re-Useable Packaging,” 2023, doi: 10.1021/acs.iecr.3c00590.
- [102] V. G. Sharmila, J. Rajesh Banu, M. Dinesh Kumar, S. Adish Kumar, and G. Kumar, “Algal biorefinery towards decarbonization: Economic and environmental consideration,” *Bioresour. Technol.*, vol. 364, p. 128103, Nov. 2022, doi: 10.1016/j.biortech.2022.128103.
- [103] J. W. Richardson, M. D. Johnson, X. Zhang, P. Zemke, W. Chen, and Q. Hu, “A financial assessment of two alternative cultivation systems and their contributions to algae biofuel economic viability,” *Algal Res.*, vol. 4, pp. 96–104, Apr. 2014, doi: 10.1016/j.algal.2013.12.003.
- [104] “Challenges related to biomass,” *Eur. Biomass Ind. Assoc.*, 2021, [Online]. Available: <https://www.eubia.org/cms/wiki-biomass/biomass-resources/challenges-related-to-biomass/>
- [105] “Bio-waste in Europe — turning challenges into opportunities,” *European Enviroment Agency*, 2020.
- [106] P. Mandawat, “Hydrolysis of Algal Biomass To Recover Nutrients and Sugar,” no. September, pp. 1–13, 2016.
- [107] S. Khan, M. Naushad, J. Iqbal, C. Bathula, and G. Sharma, “Production and

- harvesting of microalgae and an efficient operational approach to biofuel production for a sustainable environment,” *Fuel*, vol. 311, p. 122543, Mar. 2022, doi: 10.1016/j.fuel.2021.122543.
- [108] S. Fal, A. Smouni, and H. El Arroussi, “Integrated microalgae-based biorefinery for wastewater treatment, industrial CO₂ sequestration and microalgal biomass valorization: A circular bioeconomy approach,” *Environ. Adv.*, vol. 12, no. March, p. 100365, 2023, doi: 10.1016/j.envadv.2023.100365.
- [109] P. Shetty, M. M. Gitau, and G. Maróti, “Salinity Stress Responses and Adaptation Mechanisms in Eukaryotic Green Microalgae,” *Cells*, vol. 8, no. 12, p. 1657, Dec. 2019, doi: 10.3390/cells8121657.
- [110] E. Rueda, E. Gonzalez-Flo, L. Roca, J. Carretero, and J. García, “Accumulation of polyhydroxybutyrate in *Synechocystis* sp. isolated from wastewaters: Effect of salinity, light, and P content in the biomass,” *J. Environ. Chem. Eng.*, vol. 10, no. 3, p. 107952, Jun. 2022, doi: 10.1016/j.jece.2022.107952.
- [111] A. McMahon, H. Lu, and I. A. Butovich, “The Spectrophotometric Sulfo-Phospho-Vanillin Assessment of Total Lipids in Human Meibomian Gland Secretions,” *Lipids*, vol. 48, no. 5, pp. 513–525, May 2013, doi: 10.1007/s11745-013-3755-9.
- [112] S. K. Mishra *et al.*, “Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric method,” *Bioresour. Technol.*, vol. 155, pp. 330–333, Mar. 2014, doi: 10.1016/j.biortech.2013.12.077.
- [113] L. Zhang *et al.*, “The role of microtubules in microalgae: promotion of lipid

- accumulation and extraction,” *Biotechnol. Biofuels Bioprod.*, vol. 16, no. 1, p. 7, Jan. 2023, doi: 10.1186/s13068-023-02257-8.
- [114] F. A. Ansari, P. Singh, A. Guldhe, and F. Bux, “Microalgal cultivation using aquaculture wastewater: Integrated biomass generation and nutrient remediation,” *Algal Res.*, vol. 21, pp. 169–177, Jan. 2017, doi: 10.1016/j.algal.2016.11.015.
- [115] S. A. Razzak, “Biomass and Lipid Productivity of *Neochloris oleoabundans* for CO₂ Biofixation and Biodiesel Application,” *Chem. Eng. Technol.*, vol. 41, no. 11, pp. 2177–2185, Nov. 2018, doi: 10.1002/ceat.201800330.
- [116] R. G. Martins, I. Severo Gonçalves, M. G. de Moraes, and J. A. V. Costa, “Bioprocess Engineering Aspects of Biopolymer Production by the Cyanobacterium *Spirulina* Strain LEB 18,” *Int. J. Polym. Sci.*, vol. 2014, pp. 1–6, 2014, doi: 10.1155/2014/895237.
- [117] S. S. Costa *et al.*, “Efficacy of *Spirulina* sp. polyhydroxyalkanoates extraction methods and influence on polymer properties and composition,” *Algal Res.*, vol. 33, pp. 231–238, Jul. 2018, doi: 10.1016/j.algal.2018.05.016.
- [118] R. P. Mahato, S. Kumar, and P. Singh, “Optimization of Growth Conditions to Produce Sustainable Polyhydroxyalkanoate Bioplastic by *Pseudomonas aeruginosa* EO1,” *Front. Microbiol.*, vol. 12, Oct. 2021, doi: 10.3389/fmicb.2021.711588.
- [119] P. Phalanisong, P. Plangklang, and A. Reungsang, “Photoautotrophic and Mixotrophic Cultivation of Polyhydroxyalkanoate-Accumulating Microalgae Consortia Selected under Nitrogen and Phosphate Limitation,” *Molecules*, vol. 26,

no. 24, p. 7613, Dec. 2021, doi: 10.3390/molecules26247613.

- [120] A. Adetunji and M. Erasmus, “Green Synthesis of Bioplastics from Microalgae: A State-of-the-Art Review,” *Polymers (Basel)*, vol. 16, no. 10, p. 1322, May 2024, doi: 10.3390/polym16101322.
- [121] A. Werker, L. Lorini, M. Villano, F. Valentino, and M. Majone, “Modelling Mixed Microbial Culture Polyhydroxyalkanoate Accumulation Bioprocess towards Novel Methods for Polymer Production Using Dilute Volatile Fatty Acid Rich Feedstocks,” *Bioengineering*, vol. 9, no. 3, p. 125, Mar. 2022, doi: 10.3390/bioengineering9030125.
- [122] W. Zhou, D. I. Colpa, B. Geurkink, G.-J. W. Euverink, and J. Krooneman, “The impact of carbon to nitrogen ratios and pH on the microbial prevalence and polyhydroxybutyrate production levels using a mixed microbial starter culture,” *Sci. Total Environ.*, vol. 811, p. 152341, Mar. 2022, doi: 10.1016/j.scitotenv.2021.152341.
- [123] H. Eladel, A. E.-F. Abomohra, M. Battah, S. Mohmmmed, A. Radwan, and H. Abdelrahim, “Evaluation of *Chlorella sorokiniana* isolated from local municipal wastewater for dual application in nutrient removal and biodiesel production,” *Bioprocess Biosyst. Eng.*, vol. 42, no. 3, pp. 425–433, Mar. 2019, doi: 10.1007/s00449-018-2046-5.
- [124] A. Farkas *et al.*, “Salinity stress provokes diverse physiological responses of eukaryotic unicellular microalgae,” *Algal Res.*, vol. 73, p. 103155, Jun. 2023, doi: 10.1016/j.algal.2023.103155.

- [125] Y. Pan *et al.*, “Effects of salinity on the growth, physiological and biochemical components of microalga *Euchlorocystis marina*,” *Front. Mar. Sci.*, vol. 11, May 2024, doi: 10.3389/fmars.2024.1402071.
- [126] T. Qiao, Y. Zhao, D. Zhong, and X. Yu, “Hydrogen peroxide and salinity stress act synergistically to enhance lipids production in microalga by regulating reactive oxygen species and calcium,” *Algal Res.*, vol. 53, p. 102017, Mar. 2021, doi: 10.1016/j.algal.2020.102017.
- [127] X. Ji *et al.*, “The effect of NaCl stress on photosynthetic efficiency and lipid production in freshwater microalga—*Scenedesmus obliquus* XJ002,” *Sci. Total Environ.*, vol. 633, pp. 593–599, Aug. 2018, doi: 10.1016/j.scitotenv.2018.03.240.
- [128] S. Venkata Mohan and M. P. Devi, “Salinity stress induced lipid synthesis to harness biodiesel during dual mode cultivation of mixotrophic microalgae,” *Bioresour. Technol.*, vol. 165, pp. 288–294, Aug. 2014, doi: 10.1016/j.biortech.2014.02.103.
- [129] D. Pal, I. Khozin-Goldberg, Z. Cohen, and S. Boussiba, “The effect of light, salinity, and nitrogen availability on lipid production by *Nannochloropsis* sp.,” *Appl. Microbiol. Biotechnol.*, vol. 90, no. 4, pp. 1429–1441, May 2011, doi: 10.1007/s00253-011-3170-1.
- [130] B.-H. Kim, R. Ramanan, Z. Kang, D.-H. Cho, H.-M. Oh, and H.-S. Kim, “*Chlorella sorokiniana* HS1, a novel freshwater green algal strain, grows and hyperaccumulates lipid droplets in seawater salinity,” *Biomass and Bioenergy*, vol. 85, pp. 300–305, Feb. 2016, doi: 10.1016/j.biombioe.2015.12.026.

- [131] E. Daneshvar, C. Santhosh, E. Antikainen, and A. Bhatnagar, “Microalgal growth and nitrate removal efficiency in different cultivation conditions: Effect of macro and micronutrients and salinity,” *J. Environ. Chem. Eng.*, vol. 6, no. 2, pp. 1848–1854, Apr. 2018, doi: 10.1016/j.jece.2018.02.033.
- [132] L. Xin, H. Hong-ying, G. Ke, and S. Ying-xue, “Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp.,” *Bioresour. Technol.*, vol. 101, no. 14, pp. 5494–5500, Jul. 2010, doi: 10.1016/j.biortech.2010.02.016.
- [133] J. Beardall, S. Roberts, and J. A. Raven, “Regulation of inorganic carbon acquisition by phosphorus limitation in the green alga *Chlorella emersonii*,” *Can. J. Bot.*, vol. 83, no. 7, pp. 859–864, Jul. 2005, doi: 10.1139/b05-070.
- [134] M. D. Popa, I.-A. Simionov, S. M. Petrea, P.-L. Georgescu, G. A. Ifrim, and C. Iticescu, “Efficiency of Microalgae Employment in Nutrient Removal (Nitrogen and Phosphorous) from Municipal Wastewater,” *Water*, vol. 17, no. 2, p. 260, Jan. 2025, doi: 10.3390/w17020260.
- [135] S. A. Razzak, M. Ilyas, S. A. M. Ali, and M. M. Hossain, “Effects of CO₂ Concentration and pH on Mixotrophic Growth of *Nannochloropsis oculata*,” *Appl. Biochem. Biotechnol.*, vol. 176, no. 5, pp. 1290–1302, Jul. 2015, doi: 10.1007/s12010-015-1646-7.
- [136] W. Xie, Y. Han, and H. Wang, “Magnetic Fe₃O₄/MCM-41 composite-supported sodium silicate as heterogeneous catalysts for biodiesel production,” *Renew. Energy*, vol. 125, pp. 675–681, 2018, doi: 10.1016/j.renene.2018.03.010.

- [137] N. Haris, H. Manan, M. Jusoh, H. Khatoon, T. Katayama, and N. A. Kasan, “Effect of different salinity on the growth performance and proximate composition of isolated indigenous microalgae species,” *Aquac. Reports*, vol. 22, p. 100925, Feb. 2022, doi: 10.1016/j.aqrep.2021.100925.
- [138] A. N. Kumbhar *et al.*, “Supplementation of Kelp Waste Extract with Different Nitrogen Sources as a Promising Technique to Enhance Growth and Lipid Accumulation in *Chlorella sorokiniana*,” vol. XX, no. X, pp. 1–10, 2024, doi: 10.15244/pjoes/190596.
- [139] L. Tavares, M. H. Nudi, P. A. Arroyo, R. F. B. Godoy, and E. Trevisan, “Effect of different concentrations of phosphorus and nitrogen on the growth of the microalgae *Chlorella vulgaris*,” *Int. J. Energy Environ. Eng.*, vol. 14, no. 4, pp. 563–572, Dec. 2023, doi: 10.1007/s40095-022-00535-z.
- [140] G. Policastro, S. Ebrahimi, D. G. Weissbrodt, M. Fabbicino, and M. C. M. van Loosdrecht, “Selecting for a high lipid accumulating microalgae culture by dual growth limitation in a continuous bioreactor,” *Sci. Total Environ.*, vol. 912, p. 169213, Feb. 2024, doi: 10.1016/j.scitotenv.2023.169213.
- [141] F.-F. Chu, P.-N. Chu, P.-J. Cai, W.-W. Li, P. K. S. Lam, and R. J. Zeng, “Phosphorus plays an important role in enhancing biodiesel productivity of *Chlorella vulgaris* under nitrogen deficiency,” *Bioresour. Technol.*, vol. 134, pp. 341–346, Apr. 2013, doi: 10.1016/j.biortech.2013.01.131.
- [142] M. J. Zarrinmehr *et al.*, “Effect of nitrogen concentration on the growth rate and biochemical composition of the microalga, *Isochrysis galbana*,” *Egypt. J. Aquat.*

Res., vol. 46, no. 2, pp. 153–158, Jun. 2020, doi: 10.1016/j.ejar.2019.11.003.

- [143] L. Gao *et al.*, “Effects of different nitrogen/phosphorus ratios on the growth and metabolism of microalgae *Scenedesmus obliquus* cultured in the mixed wastewater from primary settling tank and sludge thickener,” *Process Saf. Environ. Prot.*, vol. 170, pp. 824–833, Feb. 2023, doi: 10.1016/j.psep.2022.12.059.
- [144] X. Li *et al.*, “¹³C-metabolic flux analysis of lipid accumulation in the green microalgae *Tetradismus obliquus* under nitrogen deficiency stress,” *Bioresour. Technol.*, vol. 388, p. 129740, Nov. 2023, doi: 10.1016/j.biortech.2023.129740.
- [145] F. Yang, W. Xiang, T. Li, and L. Long, “Transcriptome analysis for phosphorus starvation-induced lipid accumulation in *Scenedesmus* sp,” *Sci. Rep.*, vol. 8, no. 1, p. 16420, Nov. 2018, doi: 10.1038/s41598-018-34650-x.
- [146] T.-S. Le *et al.*, “Influence of nitrogen loading rate on nutrient removal and algal biomass production using revolving algae biofilm reactor,” *J. Taiwan Inst. Chem. Eng.*, vol. 166, p. 105417, Jan. 2025, doi: 10.1016/j.jtice.2024.105417.
- [147] Y. Wang, J. Yu, P. Wang, S. Deng, J. Chang, and Z. Ran, “Response of energy microalgae *Chlamydomonas reinhardtii* to nitrogen and phosphorus stress,” *Environ. Sci. Pollut. Res.*, vol. 25, no. 6, pp. 5762–5770, Feb. 2018, doi: 10.1007/s11356-017-0931-0.
- [148] T.-S. Dao *et al.*, “Salinity tolerance and nutrient uptake of the freshwater microalga *Scenedesmus protuberans*,” *Case Stud. Chem. Environ. Eng.*, vol. 10, p. 100803, Dec. 2024, doi: 10.1016/j.cscee.2024.100803.

- [149] H. Xu *et al.*, “Impoundment-induced stoichiometric imbalance exacerbated phosphorus limitation in a deep subtropical reservoir: Implications for eutrophication management,” *Water Res.*, vol. 269, p. 122787, Feb. 2025, doi: 10.1016/j.watres.2024.122787.
- [150] A. Figler, K. Márton, V. B-Béres, and I. Bácsi, “Effects of Nutrient Content and Nitrogen to Phosphorous Ratio on the Growth, Nutrient Removal and Desalination Properties of the Green Alga *Coelastrum morus* on a Laboratory Scale,” *Energies*, vol. 14, no. 8, p. 2112, Apr. 2021, doi: 10.3390/en14082112.
- [151] S. Fal, A. Aasfar, R. Rabie, A. Smouni, and H. E. Arroussi, “Salt induced oxidative stress alters physiological, biochemical and metabolomic responses of green microalga *Chlamydomonas reinhardtii*,” *Heliyon*, vol. 8, no. 1, p. e08811, Jan. 2022, doi: 10.1016/j.heliyon.2022.e08811.
- [152] T. Murwanashyaka, L. Shen, J. D. Ndayambaje, Y. Wang, N. He, and Y. Lu, “Kinetic and transcriptional exploration of *Chlorella sorokiniana* in heterotrophic cultivation for nutrients removal from wastewaters,” *Algal Res.*, vol. 24, pp. 467–476, Jun. 2017, doi: 10.1016/j.algal.2016.08.002.
- [153] J. Wang and W. R. Curtis, “Proton stoichiometric imbalance during algae photosynthetic growth on various nitrogen sources: toward metabolic pH control,” *J. Appl. Phycol.*, vol. 28, no. 1, pp. 43–52, Feb. 2016, doi: 10.1007/s10811-015-0551-3.
- [154] Y. Maltsev, M. Kulikovskiy, and S. Maltseva, “Nitrogen and phosphorus stress as a tool to induce lipid production in microalgae,” *Microb. Cell Fact.*, vol. 22, no. 1, p.

239, Nov. 2023, doi: 10.1186/s12934-023-02244-6.

- [155] V. C. Liyanaarachchi, M. Premaratne, T. U. Ariyadasa, P. H. V. Nimarshana, and A. Malik, “Two-stage cultivation of microalgae for production of high-value compounds and biofuels: A review,” *Algal Res.*, vol. 57, p. 102353, Jul. 2021, doi: 10.1016/j.algal.2021.102353.
- [156] M. M. A. Aziz *et al.*, “Two-stage cultivation strategy for simultaneous increases in growth rate and lipid content of microalgae: A review,” *Renew. Sustain. Energy Rev.*, vol. 119, p. 109621, Mar. 2020, doi: 10.1016/j.rser.2019.109621.
- [157] K. K. Sharma, H. Schuhmann, and P. M. Schenk, “High Lipid Induction in Microalgae for Biodiesel Production,” *Energies*, vol. 5, no. 5, pp. 1532–1553, May 2012, doi: 10.3390/en5051532.
- [158] S. Anto, A. Pugazhendhi, and T. Mathimani, “Lipid enhancement through nutrient starvation in *Chlorella* sp. and its fatty acid profiling for appropriate bioenergy feedstock,” *Biocatal. Agric. Biotechnol.*, vol. 20, p. 101179, Jul. 2019, doi: 10.1016/j.bcab.2019.101179.
- [159] A. Roopnarain, V. M. Gray, and S. D. Sym, “Phosphorus limitation and starvation effects on cell growth and lipid accumulation in *Isochrysis galbana* U4 for biodiesel production,” *Bioresour. Technol.*, vol. 156, pp. 408–411, Mar. 2014, doi: 10.1016/j.biortech.2014.01.092.
- [160] J. S. Arcila, D. Céspedes, and G. Buitrón, “Influence of wavelength photoperiods and N/P ratio on wastewater treatment with microalgae–bacteria,” *Water Sci.*

- Technol.*, vol. 84, no. 3, pp. 712–724, Aug. 2021, doi: 10.2166/wst.2021.257.
- [161] R. Bossa, M. Di Colandrea, G. Salbitani, and S. Carfagna, “Phosphorous Utilization in Microalgae: Physiological Aspects and Applied Implications,” *Plants*, vol. 13, no. 15, p. 2127, Aug. 2024, doi: 10.3390/plants13152127.
- [162] D. A. Gabrielyan *et al.*, “Optimization of CO₂ Supply for the Intensive Cultivation of *Chlorella sorokiniana* IPPAS C-1 in the Laboratory and Pilot-Scale Flat-Panel Photobioreactors,” *Life*, vol. 12, no. 10, p. 1469, Sep. 2022, doi: 10.3390/life12101469.
- [163] S.-H. Ho, C.-Y. Chen, D.-J. Lee, and J.-S. Chang, “Perspectives on microalgal CO₂-emission mitigation systems — A review,” *Biotechnol. Adv.*, vol. 29, no. 2, pp. 189–198, Mar. 2011, doi: 10.1016/j.biotechadv.2010.11.001.
- [164] C. Montoya-Vallejo, F. L. Guzmán Duque, and J. C. Quintero Díaz, “Biomass and lipid production by the native green microalgae *Chlorella sorokiniana* in response to nutrients, light intensity, and carbon dioxide: experimental and modeling approach,” *Front. Bioeng. Biotechnol.*, vol. 11, May 2023, doi: 10.3389/fbioe.2023.1149762.
- [165] Z. Wang *et al.*, “Effects of salinity regulation strategies on the enrichment of polyhydroxyalkanoate (PHA) producing mixed cultures: Microbial community succession and metabolic mechanisms,” *Chem. Eng. J.*, vol. 506, p. 160001, Jan. 2025, doi: 10.1016/j.cej.2025.160001.
- [166] S. Ansari and T. Fatma, “Cyanobacterial Polyhydroxybutyrate (PHB): Screening, Optimization and Characterization,” *PLoS One*, vol. 11, no. 6, p. e0158168, Jun.

2016, doi: 10.1371/journal.pone.0158168.

- [167] S. Ilhami, S. N. S. A. Rahman, M. Iqhrammullah, Z. Hamid, Y. H. Chai, and M. K. Lam, “Polyhydroxyalkanoates production from microalgae for sustainable bioplastics: A review,” *Biotechnol. Adv.*, vol. 79, p. 108529, Mar. 2025, doi: 10.1016/j.biotechadv.2025.108529.
- [168] M. Wan *et al.*, “The effect of iron on growth, lipid accumulation, and gene expression profile of the freshwater microalga *Chlorella sorokiniana*,” *Appl. Microbiol. Biotechnol.*, vol. 98, no. 22, pp. 9473–9481, Nov. 2014, doi: 10.1007/s00253-014-6088-6.
- [169] J. R. Tanoeiro, G. W. Fehrenbach, P. Murray, R. Pedrosa, and Y. Chen, “Evaluation of *Dunaliella salina* Growth in Different Salinities for Potential Application in Saline Water Treatment and Biomass Production,” *Aquac. J.*, vol. 4, no. 3, pp. 92–103, Jul. 2024, doi: 10.3390/aquacj4030007.
- [170] S. Negi *et al.*, “Impact of nitrogen limitation on biomass, photosynthesis, and lipid accumulation in *Chlorella sorokiniana*,” *J. Appl. Phycol.*, vol. 28, no. 2, pp. 803–812, Apr. 2016, doi: 10.1007/s10811-015-0652-z.
- [171] G.-Q. Chen, X.-Y. Chen, F.-Q. Wu, and J.-C. Chen, “Polyhydroxyalkanoates (PHA) toward cost competitiveness and functionality,” *Adv. Ind. Eng. Polym. Res.*, vol. 3, no. 1, pp. 1–7, Jan. 2020, doi: 10.1016/j.aiepr.2019.11.001.
- [172] S. Anto, M. Premalatha, and T. Mathimani, “N:P ratio and salinity as keys: A study on optimizing biomass and lipid production in marine *Chlorella* sp. NITT 02 and

- Picochlorum sp. NITT 04 for biodiesel production,” *Biomass and Bioenergy*, vol. 190, p. 107409, Nov. 2024, doi: 10.1016/j.biombioe.2024.107409.
- [173] S. Ruangsomboon, M. Ganmanee, and S. Choochote, “Effects of different nitrogen, phosphorus, and iron concentrations and salinity on lipid production in newly isolated strain of the tropical green microalga, *Scenedesmus dimorphus* KMITL,” *J. Appl. Phycol.*, vol. 25, no. 3, pp. 867–874, Jun. 2013, doi: 10.1007/s10811-012-9956-4.
- [174] K. Thepsuthammarat, T. Imai, P. Plangklang, S. Sittijunda, and A. Reungsang, “Two-step polyhydroxybutyrate production from hydrogenic effluent by freshwater microalgae *Coelastrella* sp. KKU-P1 and *Acutodesmus* sp. KKU-P2 under mixotrophic cultivation,” *Heliyon*, vol. 10, no. 17, p. e37261, Sep. 2024, doi: 10.1016/j.heliyon.2024.e37261.
- [175] H.-W. Yen and J.-T. Chang, “A two-stage cultivation process for the growth enhancement of *Chlorella vulgaris*,” *Bioprocess Biosyst. Eng.*, vol. 36, no. 11, pp. 1797–1801, Nov. 2013, doi: 10.1007/s00449-013-0922-6.
- [176] S. S. Costa, A. L. Miranda, M. G. de Moraes, J. A. V. Costa, and J. I. Druzian, “Microalgae as source of polyhydroxyalkanoates (PHAs) — A review,” *Int. J. Biol. Macromol.*, vol. 131, pp. 536–547, Jun. 2019, doi: 10.1016/j.ijbiomac.2019.03.099.
- [177] Y. Yang *et al.*, “Screening of microalgae species and evaluation of algal-lipid stimulation strategies for biodiesel production,” *Sci. Total Environ.*, vol. 857, p. 159281, Jan. 2023, doi: 10.1016/j.scitotenv.2022.159281.

- [178] S. A. Razzak, “Biomass and Lipid Productivity of *Neochloris oleoabundans* for CO₂ Biofixation and Biodiesel Application,” *Chem. Eng. Technol.*, vol. 41, no. 11, pp. 2177–2185, 2018, doi: 10.1002/ceat.201800330.
- [179] A. Udayan *et al.*, “Production of microalgae with high lipid content and their potential as sources of nutraceuticals,” *Phytochem. Rev.*, vol. 22, no. 4, pp. 833–860, Aug. 2023, doi: 10.1007/s11101-021-09784-y.
- [180] X.-M. Sun, L.-J. Ren, Q.-Y. Zhao, X.-J. Ji, and H. Huang, “Microalgae for the production of lipid and carotenoids: a review with focus on stress regulation and adaptation,” *Biotechnol. Biofuels*, vol. 11, no. 1, p. 272, Dec. 2018, doi: 10.1186/s13068-018-1275-9.
- [181] G. T. Abrha, A. Makaranga, and P. P. Jutur, “Enhanced lipid accumulation in microalgae *Scenedesmus* sp. under nitrogen limitation,” *Enzyme Microb. Technol.*, vol. 182, p. 110546, Jan. 2025, doi: 10.1016/j.enzmictec.2024.110546.
- [182] T.-Q. Shi, L.-R. Wang, Z.-X. Zhang, X.-M. Sun, and H. Huang, “Stresses as First-Line Tools for Enhancing Lipid and Carotenoid Production in Microalgae,” *Front. Bioeng. Biotechnol.*, vol. 8, Jul. 2020, doi: 10.3389/fbioe.2020.00610.
- [183] T. Liu *et al.*, “Biochemical and Morphological Changes Triggered by Nitrogen Stress in the Oleaginous Microalga *Chlorella vulgaris*,” *Microorganisms*, vol. 10, no. 3, p. 566, Mar. 2022, doi: 10.3390/microorganisms10030566.
- [184] Y. Feng *et al.*, “Using high CO₂ concentrations to culture microalgae for lipid and fatty acid production: Synthesis based on a meta-analysis,” *Aquaculture*, vol. 594,

- p. 741386, Jan. 2025, doi: 10.1016/j.aquaculture.2024.741386.
- [185] J. Li, X. Tang, K. Pan, B. Zhu, Z. Wang, and Y. Zhao, “Application study for the high CO₂ tolerant *Chlorella* strain by flue gas culture: Evaluation of growth performance and adaptive mechanisms,” *Chem. Eng. J.*, vol. 479, p. 147700, Jan. 2024, doi: 10.1016/j.cej.2023.147700.
- [186] L. A. Schwendler and M. A. Lansarin, “Polyhydroxybutyrate-co-hydroxyvalerate/titanium dioxide composite films: green synthesis, characterization, and photocatalytic assessment,” *J. Polym. Res.*, vol. 30, no. 5, p. 169, May 2023, doi: 10.1007/s10965-023-03556-2.
- [187] A. Kovalcik *et al.*, “Enzymatic Hydrolysis of Poly(3-Hydroxybutyrate-co-3-Hydroxyvalerate) Scaffolds,” *Materials (Basel)*, vol. 13, no. 13, p. 2992, Jul. 2020, doi: 10.3390/ma13132992.
- [188] M. Abbasi, D. Pokhrel, E. R. Coats, N. M. Guho, and A. G. McDonald, “Effect of 3-Hydroxyvalerate Content on Thermal, Mechanical, and Rheological Properties of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) Biopolymers Produced from Fermented Dairy Manure,” *Polymers (Basel)*, vol. 14, no. 19, p. 4140, Oct. 2022, doi: 10.3390/polym14194140.
- [189] M. Wojtczak, A. Galeski, and M. Pracella, “Inhibited crystallization of polyhydroxybutyrate by blending with aliphatic-aromatic copolyester,” *Eur. Polym. J.*, vol. 103, pp. 133–144, Jun. 2018, doi: 10.1016/j.eurpolymj.2018.04.007.
- [190] M. I. Ibrahim, D. Alsafadi, K. A. Alamry, and M. A. Hussein, “Properties and

Applications of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) Biocomposites,” *J. Polym. Environ.*, vol. 29, no. 4, pp. 1010–1030, Apr. 2021, doi: 10.1007/s10924-020-01946-x.

- [191] T. T. H. Pham, T. L. A. Nguyen, T. T. Duong, O. T. Doan, H. T. T. Tran, and L. T. T. Tran, “Selection of microalgae and cyanobacteria to produce polyhydroxyalkanoates (PHAs) - A case study in Vietnam,” *Case Stud. Chem. Environ. Eng.*, vol. 10, p. 100808, Dec. 2024, doi: 10.1016/j.cscee.2024.100808.
- [192] V. Cánovas *et al.*, “Analysis of Polyhydroxyalkanoates Granules in *Haloferax mediterranei* by Double-Fluorescence Staining with Nile Red and SYBR Green by Confocal Fluorescence Microscopy,” *Polymers (Basel)*, vol. 13, no. 10, p. 1582, May 2021, doi: 10.3390/polym13101582.

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Catalytic co-pyrolysis and kinetic study of microalgae biomass with solid waste feedstock for sustainable biofuel production (<https://doi.org/10.1016/j.jaap.2024.106755>) Journal of Analytical and Applied Pyrolysis (<https://www.sciencedirect.com/journal/journal-of-analytical-and-applied-pyrolysis>) Shaikh Abdur Razzak, Minahil Khan, Fatima Irfan, Mudasir Akbar shah, Ahmad Nawaz, Mohammad Mozahar Hossain

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Accumulation of Biopolymer in the Microalgae *Chlorella Sorokiniana* Cultivated Under Petroleum-Derived Produced Water; Mohammad Omar Faruque, Fatima Irfan, Shaikh Abdur Razzak (AIChE Annual Meeting 2024)