ASSESSMENT OF SELECTED BLUE-GREEN AND GREEN ALGAE SPECIES AS POTENTIAL LANDSCAPE ECOLOGICAL INDICATOR AGENT FOR A SUSTAINABLE ENVIRONMENT

BY

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A Thesis submitted in fulfilment of the requirement for the Doctor of Philosophy (Built Environment)

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MAY 2025

ABSTRACT

Algae have long been recognized as valuable bioindicators in environmental assessments, yet rapid urbanization and increasing industrial activities have intensified the need for innovative ecological monitoring solutions. The escalating carbon emissions and toxic metal contamination in urban environments demand effective biological mitigation strategies. This study investigates the potential of selected algae species *Chlorella vulgaris* (green algae) and two cyanobacteria species, *Phormidium* sp. and *Synechococcus* sp. as ecological indicators for environmental stress and carbon sequestration. The algae were cultured in various media formulations (BBM, BG11, Bristol, Chu-10) under different pH levels (3.0, 5.0, 7.0, 9.0, and 11.0) and photoperiods (24:0, 16:8, 12:12, and 8:16). Growth performance, total chlorophyll content, chromaticity (lightness and chroma stability), and carbon sequestration efficiency were assessed using a UV-Vis spectrophotometer and CIELAB colorimeter, while biomass accumulation was measured by dry weight analysis. To evaluate phycoremediation potential, the algae were exposed to heavy metals (Cr, Cd, and Cu) at concentrations of 1 mg/L, 2 mg/L, and 3 mg/L over four weeks, with weekly spectrophotometric analysis. The results indicate that *C. vulgaris* achieved the highest absorbance value of 1.45 in BBM medium at pH 9, demonstrating superior growth and adaptability. In terms of carbon sequestration, *C. vulgaris* recorded a peak sequestration rate of 1.675 g/L CO_2e under BBM at pH 9 with a 16:8 photoperiod, while *Synechococcus sp.* achieved a higher sequestration rate of 2.006 g/L CO_2e under similar conditions. *Phormidium sp*. showed a moderate carbon sequestration rate of 1.149 g/L CO_2e in BG11 medium at pH 11. The bioconcentration factor (BCF) analysis revealed varying metal uptake capacities, with *C. vulgaris* demonstrating the highest affinity for Cu (BCF = 12.1) by week 2, while *Synechococcus sp.* exhibited significant Cr accumulation (BCF = 7.0) by week 3. These findings highlight the species-dependent efficiency of algae in carbon sequestration and phycoremediation, reinforcing their role as potential landscape ecological indicators in urban environments. The integration of *C. vulgaris*, *Synechococcus* and *Phormidium* into environmental management strategies can enhance carbon capture, improve water quality, and provide real-time ecological monitoring, ultimately contributing to climate mitigation and sustainable urban development.

Keywords: Landscape Ecological Indicator, Phycoremediation, Carbon sequestration, Environmental monitoring, Mitigation and Sustainability

ملخص البحث.

لقد عُرفت الطحالب كمؤشرات حيوية قيّمة في التقييمات البيئية، إلا أن التوسع الحضري السريع وتزايد الأنشطة الصناعية زادا من الحاجة إلى حلول مبتكرة للرصد البيئي. وتتطلب انبعاثات الكربون المتصاعدة والتلوث بالمعادن السامة في البيئات الحضرية استراتيجيات فعالة للتخفيف البيولوجي. تبحث هذه الدراسة في إمكانات أنواع مختارة من الطحالب، مثل الكلوريلا فولغاريس (الطحالب الخضراء)، ونوعين من البكتيريا الزرقاء، مثل فورميديوم وسينيكوكوكوس، كمؤشرات بيئية للإجهاد البيئي واحتجاز الكربون. وقد زُرعت الطحالب في تركيبات متنوعة من الوسائط (BBM، BG11، بريستول، Chu-10) في ظل مستويات حموضة مختلفة (3.0، 5.0، 7.0، 9.0، و11.0) وفترات ضوئية (24:0، 16:8، 12:12، و8:16). وتم تقييم أداء النمو، ومحتوى الكلوروفيل الكلي، واللون (ثبات الإضاءة واللون)، وكفاءة عزل الكربون باستخدام مطياف الأشعة فوق البنفسجية المرئية ومقياس الألوان CIELAB، بينما تم قياس تراكم الكتلة الحيوية عن طريق تحليل الوزن الجاف. لتقييم إمكانية المعالجة النباتية، تعرضت الطحالب للمعادن الثقيلة (الكروم والكادميوم والنحاس) بتركيزات 1 ملغم/لتر، 2 ملغم/لتر، و3 ملغم/لتر على مدى أربعة أسابيع، مع التحليل الطيفي الأسبوعي. تشير النتائج إلى أن *C. vulgaris* حقق أعلى قيمة امتصاص بلغت 1.45 في وسط BBM عند درجة حموضة 9، مما يدل على نمو وتكيف فائقين. من حيث عزل الكربون، سجل *C. vulgaris* معدل عزل ذروة بلغ 1.675 غ/لتر من مكافئ ثاني أكسيد الكربون تحت BBM عند درجة حموضة 9 مع فترة ضوئية 16:8، بينما *Synechococcus sp* حقق صنف *Phormidium* معدل عزل أعلى بلغ 2.006 غ/ل من مكافئ ثاني أكسيد الكربون في ظل ظروف مماثلة. وأظهر صنف *Phormidium* معدل عزل كربون معتدل بلغ 1.149 غ/ل من مكافئ ثاني أكسيد الكربون في وسط BG11 عند درجة حموضة 11. وكشف تحليل عامل التركيز الحيوي (BCF) عن تفاوت في قدرات امتصاص المعادن، حيث أظهر صنف *C. vulgaris* أعلى درجة انجذاب للنحاس (BCF = 12.1) بحلول الأسبوع الثاني، بينما أظهر صنف *Synechococcus* sp. تراكمًا كبيرًا للكروم (BCF = 7.0) بحلول الأسبوع الثالث. وتُبرز هذه النتائج كفاءة الطحالب المعتمدة على الأنواع في عزل الكربون والمعالجة النباتية، مما يعزز دورها كمؤشرات بيئية محتملة للمناظر الطبيعية في البيئات الحضرية. ويمكن أن يُعزز دمج صنف *C. vulgaris* و*Synechococcus* و*Phormidium* في استراتيجيات الإدارة البيئية احتجاز الكربون، وتحسين جودة المياه، وتوفير مراقبة بيئية آنية، مما يُسهم في نهاية المطاف في التخفيف من آثار تغير المناخ والتنمية

الحضرية المستدامة. الكلمات المفتاحية: مؤشر بيئي للمناظر الطبيعية، المعالجة النباتية، عزل الكربون، الرصد البيئي، التخفيف والاستدامة

الكلمات المفتاحية: المؤشرات الحيوية للطحالب، المعالجة النباتية، احتجاز الكربون، المراقبة البيئية

APPROVAL PAGE

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ACKNOWLEDGEMENTS

In the name of Allah, The Most Gracious and the Most Merciful

First and foremost, I would like to extend my deepest gratitude to my supervisor, Prof. Ts. Dr. Rashidi bin Othman, for granting me this golden opportunity to embark on this incredible learning journey. His unwavering guidance, encouragement, and support have been my constant companions throughout this research, and I am profoundly grateful for the time, energy, and dedication he has invested in me. His belief in my potential and his wisdom are among the many reasons I was able to complete this work. I would also like to express my sincere thanks to my co-supervisor, Dr. Wan Syibrah, for her invaluable insights and guidance.

I am especially indebted to my beloved husband, Mohamad Noriqmal Hafiz, whose unwavering love, patience, and endless support have been the bedrock of my journey. His prayers and motivation have carried me through challenging times, and I would not be the person I am today without him by my side. To my wonderful parents and my siblings, your constant encouragement and belief in me have been a profound source of inspiration. You have been my silent strength, and I am forever grateful for your love and guidance throughout this journey. A special mention goes to my dearest son, Imran Hadif, whose innocence and boundless joy have been my ultimate inspiration, giving me the determination to complete this journey for him and our future.

I would also like to express my appreciation to my dear friends, Qurratu Aini, Ainaa Eliah, Faiq Najwan, and Nur Atiqah, who have stood by me throughout this process, assisting with sample collection and sharing their knowledge selflessly. Your companionship has lightened this journey immeasurably. Lastly, I am eternally grateful to Allah for blessing me with the strength and perseverance to meet such wonderful people and complete this journey. This thesis stands not just as a reflection of my hard work, but of the love, support, and sacrifices of everyone who believed in me.

May Allah bless all of you with His greatest protection and loves. InsyaAllah.

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CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

The increasing concern over environmental sustainability has heightened interest in the use of ecological indicators to assess ecosystem health. Among these indicators, blue-green algae (cyanobacteria) and green algae have garnered attention due to their sensitivity to environmental changes and their role in aquatic ecosystems. However, their effectiveness as ecological indicators remain insufficiently explored, necessitating further investigation.

As sustainability becomes a global priority, the need for reliable ecological indicators to monitor environmental health is increasingly urgent. Blue-green and green algae, due to their sensitivity to environmental fluctuations, serve as valuable indicators of water quality and ecosystem health. Despite their potential, existing research primarily focuses on harmful algal blooms rather than their broader ecological roles. This research gap underscores the necessity for comprehensive studies evaluating the suitability of these algae as ecological indicators in promoting sustainability.

Blue-green algae, play a crucial role in aquatic ecosystems by fixing atmospheric nitrogen, thereby influencing nutrient cycling. However, under certain conditions, they can produce toxins, adversely affecting both human and environmental health. Monitoring their abundance can provide critical insights into water quality, nutrient levels, and overall ecosystem stability. Similarly, green algae, known for their photosynthetic efficiency and bioaccumulation potential, serve as indicators of environmental pollutants, such as heavy metals and nutrient overloads. Their population dynamics offer valuable data for ecological monitoring and sustainable resource management.

Moreover, the role of algae in eutrophication and blue-green infrastructure is significant. Eutrophication, driven by excessive nutrient loads, has led to severe water

quality degradation worldwide. Research has demonstrated that blue-green algae contribute to this process through excessive biomass production, altering oxygen levels and releasing toxins (Renata et al., 2023). Their incorporation into ecological monitoring programmes can thus enhance strategies for mitigating nutrient pollution. Furthermore, the concept of blue-green infrastructure highlights the integration of these algae into urban sustainability initiatives, promoting environmental conservation alongside social well-being.

Ecological civilization is a way of approaching social and ecological reform and represents a new standard of human existence that may be sustainable well into the future. Various landscape ecology measures have been used extensively throughout the landscape ecology community (Frohn, 2018). Algae is one the new measures that have the ability as a future landscape indicator due to many reasons proven by several studies, which are:

Table 1.1 Ability of algae as a future landscape indicator

Nos	Advantages	Author	Year
1.	Algae have the potential to address several issues concurrently, which is not feasible in traditional treatments in an environmentally sustainable way.	Daneshvar et al.	2019
2.	The ability of algae to sequester carbon is a promising alternative to the risk of global warming. The oxygen emitted will then be used to help purify the atmosphere.	Lutzu et al.	2021
3.	Remediation using algae is a cost-effective treatment that does not require large number of toxic substances or electricity to operate.	Sirakov et al.	2013

Given these considerations, this study seeks to investigate the potential of blue-green and green algae as ecological indicators for landscape sustainability. The findings will contribute to a deeper understanding of ecosystem dynamics, inform environmental policies, and aid in the development of effective management strategies

1.2 RESEARCH QUESTIONS

1. How do the growth behaviours of blue-green and green algae vary under different medium formulations, photoperiods, and pH levels?
2. What are the differences in carbon sequestration efficiencies between blue-green and green algae, and how do these efficiencies contribute to environmental sustainability?
3. How effective are blue-green and green algae in sequestering heavy metals (Cd, Cr, Cu) at different concentrations and exposure durations?
4. To what extent do environmental conditions influence the pigmentation of blue-green and green algae, and how can this be utilised as an ecological indicator for environmental health assessment?

1.3 PROBLEM STATEMENT

1.3.1 Global Climate Change

Climate change remains one of the most pressing environmental challenges, driven by rising carbon emissions, biodiversity loss, and increasing global temperatures. More than 150 million people worldwide have already been affected by rising sea levels, leading to displacement and coastal flooding (Xu et al., 2019; Ekwebelem et al., 2020). Biological approaches, such as the use of algae for carbon capture, present cost-effective and sustainable solutions to mitigate these impacts.

1.3.2 Carbon Emissions

The urgency to develop sustainable solutions requires the implementation of robust ecological indicators to assess their environmental impact. Microalgae-based biological carbon capture has emerged as a viable method for reducing carbon emissions (Choi et al., 2019). Microalgae have been identified as one of the most effective carbon sequestration strategies globally, with sequestration rates 10–50 times higher than terrestrial plants (Batista & Kohler, 2020). Their rapid growth and high photosynthetic efficiency make them ideal candidates for sustainability monitoring.

1.3.3 The Need for Effective Ecological Indicators for Sustainability

Traditional industrial processes are unsustainable due to their reliance on fossil fuels and their significant environmental footprints (Arun et al., 2020). The integration of microalgae into sustainability assessment frameworks can provide valuable insights into ecological balance and carbon neutrality. Their use as bioindicators in water quality monitoring and pollution mitigation underscores their potential in advancing sustainable environmental practices (Moreira & Pires, 2016; Alami et al., 2021).

1.4 RESEARCH AIM

To explore the potential of blue-green and green algae as reliable ecological indicator for a sustainable environment.

1.5 RESEARCH OBJECTIVES

This research sets out the following objectives:

1. To investigate and compare the behavior two algae species groups under varying conditions of medium formulation, photoperiods, and pH levels.
2. To evaluate and compare carbon sequestration efficiencies between blue-green and green algae.
3. To analyze the heavy metal sequestration capabilities of green and blue green algae across different metal concentrations and exposure durations.
4. To examine the impact of on the pigmentation of blue green and green algae as potential ecological indicators for environmental health assessment.

1.6 RESEARCH HYPOTHESES

Algae can be applied in landscape ecological design to sequester and remediate, or pollutants indicators such as excess nutrients, CO2, and heavy metals from polluted or contaminated environments. It can be hypothesized that:

1. The growth behaviour of blue-green and green algae significantly differs under varying medium formulations, photoperiods, and pH levels.
2. Blue-green and green algae exhibit significantly different carbon sequestration efficiencies.
3. Blue-green and green algae show significant differences in their ability to sequester heavy metals (Cd, Cr, Cu) across varying concentrations and exposure durations.
4. Environmental conditions significantly influence the pigmentation of blue-green and green algae, supporting their role as ecological indicators for environmental health assessment.

1.7 SIGNIFICANCE OF THE STUDY

This study aims to bridge the gap in ecological assessment by exploring the role of blue-green and green algae as landscape ecological indicators. Their integration into urban sustainability initiatives offers a novel approach to mitigating environmental challenges. Recent studies highlight the effectiveness of ecological assessment methods, such as remote sensing and statistical modelling, in understanding urban ecosystem dynamics (Diep et al., 2024). Similarly, water footprint analysis in agriculture has underscored the need for sustainable water management (Mehla et al., 2023). By aligning this study with these broader sustainability frameworks, the research contributes to the ongoing discourse on environmental conservation and policy development.

Comparative analysis of blue-green and green algae further strengthens their viability as ecological indicators. While green algae are widely recognised for their oxygen production and bioaccumulation capabilities, cyanobacteria contribute uniquely to nitrogen fixation and eutrophication dynamics. Understanding their interactions with environmental stressors will enhance ecological monitoring frameworks and inform sustainability policies

1.8 RESEARCH GAP

While the use of algae in architectural design has progressed, its application within landscape architecture remains underexplored. For instance, microalgae panels on building facades have demonstrated significant potential in carbon dioxide absorption, improving urban air quality (Figure 1.1). Over a six-day period, these panels recorded an average daily CO_2 absorption rate of 22.55%, illustrating their effectiveness in reducing atmospheric carbon levels.

Figure 1.1 Comparing the south façade of Enghelab Street, Iran with (right) and without (left) microalgae panels.

However, within landscape architecture, there is a distinct research gap regarding the application of algae as ecological indicators (Rinanti & Purwadi, 2018). Further research is required to explore their potential in ecological health assessments, urban sustainability initiatives, and ecosystem restoration strategies. This study seeks to address these gaps by examining the viability of algae as sustainable landscape ecological indicators.

1.9 RESEARCH SCOPE

This study will focus on three selected algae species from two major groups:

1. Green Algae: *Chlorella vulgaris*
2. Blue-Green Algae (Cyanobacteria): *Synechococcus* and *Phormidium*

These species will be assessed under varying environmental conditions, including:

- pH Levels: 3.0, 5.0, 7.0, 9.0, and 11.0
- Culture Media: BBM, BG11, Bristol, and Chu-10
- Photoperiods: 24:0, 16:8, 12:12, and 8:16

Additionally, the study will evaluate growth performance at a neutral pH (7.0) under different nutrient strengths (0.5×, 1×, 2×, and 3×). The experiments will assess algae adaptability and tolerance to environmental stressors, including:

1. Polyethylene glycol (PEG) stress at 5 g/L, 10 g/L, and 15 g/L
2. Salicylic acid (SA) stress at 25 mg/L, 50 mg/L, and 100 mg/L

Subsequent assessments will investigate the growth behaviour of green and blue-green algae under the influence of four media (BBM, BG11, Chu-10, and Bristol) at varying pH levels (3.0, 5.0, 7.0, 9.0, and 11.0) and photoperiods (24:0, 16:8, 12:12, and 8:16). The following parameters will be measured:

1. Absorbance at 750 nm
2. Effects of pH and photoperiod on UV-Vis spectra for chlorophyll content
3. Lightness (L) of cell culture growth*
4. Chromaticity (ΔE*Lab) based on CIE Lab colour differences
5. Biomass as a carbon sequestration agent

Lastly, the species from both green and blue-green algae groups will be evaluated for their heavy metal sequestration efficiency, investigating their phycoremediation capabilities. The algae will be exposed to three toxic pollutants—Cadmium (Cd), Chromium (Cr), and Copper (Cu) at concentrations of 1 mg/L, 2 mg/L, and 3 mg/L over a four-week period.

The results from this study will contribute to the development of an ecological indicator scorecard for sustainable landscape management, facilitating the integration of algae-based bioindicators into environmental assessment frameworks.

1.10 CHAPTER SUMMARY

The role of blue-green and green algae in ecological assessments offers promising applications for sustainable landscape management. Their ability to indicate water quality, mitigate nutrient pollution, and sequester carbon makes them valuable assets in environmental monitoring. This study seeks to explore their viability as ecological indicators through three primary applications:

1. Phycoindicators: Assessing algae as indicators of ecosystem health
2. Phycoremediation: Investigating their role in pollution mitigation
3. Phycosequestration: Evaluating their carbon sequestration potential

By integrating these findings into sustainability frameworks, this study aims to enhance ecological assessment methodologies and contribute to long-term environmental conservation.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

The concept of cleanliness extends beyond religious and cultural practices, playing a fundamental role in global environmental health. However, the increasing contamination of freshwater resources due to industrialization, urbanization, and unsustainable agricultural practices presents a significant challenge (Wan Sulaiman et al., 2022; Ashraf et al., 2019). Traditional water treatment methods, such as chemical precipitation, reverse osmosis, and membrane filtration, have been widely used to mitigate water pollution. However, these approaches are often criticized for their high operational costs, energy consumption, and secondary pollution generation (Vidu et al., 2020).

Phycoremediation, which leverages the bioremediation capabilities of microalgae, has emerged as a promising alternative (Pichtel, 2016). Several studies have demonstrated its effectiveness in removing heavy metals, nitrates, and phosphates from wastewater (Rafique & Tariq, 2016; Hamzah et al., 2016). However, while the bioaccumulation potential of microalgae is well-documented, critical questions remain regarding their efficiency across different environmental conditions. For instance, the effectiveness of phycoremediation is highly dependent on species selection, environmental factors (such as pH and light exposure), and metal bioavailability, which vary significantly between studies (Chen et al., 2016; Iqbal et al., 2016).

This chapter explores the existing literature on algae-based bioremediation while critically analyzing the methodologies used, identifying limitations, and addressing conflicting findings. It highlights the importance of optimizing algal species selection and cultivation conditions to enhance sequestration efficiency.

2.2 WATER: ISSUES, CHALLENGES AND TECHNOLOGIES

Water pollution remains a critical environmental issue in Malaysia's freshwater and marine ecosystems, exacerbated by rapid urbanization, industrial activities, agricultural runoff, and improper waste management. Pollutants entering Malaysia's waterways compromise water quality, aquatic biodiversity, and human health, threatening the nation's sustainable development goals

With rapid urbanization and industrialization, the presence of heavy metals in Malaysian rivers, lakes, and coastal waters has risen significantly over the past few decades, triggering widespread environmental concerns (Wan Sulaiman et al., 2022; Ashraf et al., 2019). Heavy metals—such as mercury (Hg), arsenic (As), nickel (Ni), cadmium (Cd), lead (Pb), zinc (Zn), copper (Cu), and chromium (Cr)—originate from both anthropogenic and natural sources, including the oil and gas industry (Pichtel, 2016), phosphate fertilizers in agriculture (Hamzah et al., 2016), metal mining (Chen et al., 2016), and industrial wastewater discharge (Farahat & Linderholm, 2015). The tropical monsoon climate in Malaysia exacerbates the problem, as heavy rainfall increases surface runoff, carrying pollutants into rivers and coastal waters, further deteriorating water quality.

Despite the implementation of the Environmental Quality Act of 1974, which has successfully regulated pollution to some extent, numerous challenges remain in achieving optimal water quality. As Malaysia continues to expand its industrial base, many of its rivers and lakes are turning into de facto waste disposal sites for sewage, chemicals, and industrial effluents. Alarmingly, around 60% of Malaysia's 90 reservoirs and lakes are classified as eutrophic, primarily due to agricultural runoff, fertilizer use, and untreated wastewater discharge. These pollutants not only threaten aquatic ecosystems and biodiversity (SDG 15: Life on Land) but also compromise water security for human consumption and economic use (Wan Sulaiman, 2019).

Malaysia's tropical aquatic environments are highly sensitive to contamination, with pollutants accumulating from both natural and human sources. The bioaccumulation of heavy metals in aquatic organisms poses a significant risk, as contaminants enter the food chain, affecting fisheries, aquaculture, and human health.

Addressing metal pollution and eutrophication requires nature-based solutions, such as phycoremediation, which leverages Malaysia's diverse microalgae species to absorb and neutralize contaminants.

To date, the use of macrophytes and microalgae in wastewater treatment has shown promising results, demonstrating the potential of native plant species for phytoremediation (Mohd Hatta et al., 2022). However, the selection of effective phytoremediation species must align with Malaysia's environmental policies, economic feasibility, and long-term sustainability goals. One of the key factors influencing the adoption of phytoremediation is the measurement of its efficiency, scalability, and impact on carbon neutrality, which aligns with Malaysia's National Green Technology Policy and low-carbon economy aspirations (Sharma et al., 2020).

Malaysia urgently requires affordable, scalable, and low-energy environmental technologies to support SDG-driven policies and mitigate water pollution. Phytoremediation, a plant-based biotechnology, has emerged as an effective and sustainable solution for treating polluted water bodies, reducing industrial waste, and enhancing biodiversity conservation. Various biological, chemical, and physical remediation techniques exist, but many are costly and inefficient at higher pollutant concentrations (Vidu et al., 2020). Phycoremediation, in contrast, harnesses microalgae's ability to immobilize, degrade, and transform heavy metals, offering a nature-inspired solution for sustainable water management.

Microalgae play a pivotal role in Malaysia's aquatic ecosystems, climate regulation, and biodiversity preservation. As key primary producers, algae contribute to global oxygen production and carbon fixation, making them vital for maintaining ecological balance (Cenci et al., 2017). Their adaptability to extreme environmental conditions, such as fluctuating pH levels, high temperatures, and varying nutrient availability, highlights their resilience and effectiveness as bioremediation agents (Mishra et al., 2022).

In Malaysia, where tropical conditions provide year-round sunlight and high humidity, algae-based water treatment technologies present a practical, cost-effective, and eco-friendly alternative to conventional wastewater treatment methods. This

chapter explores the potential of locally available algal species for phycoremediation, focusing on their role in water purification, heavy metal removal, and ecosystem restoration, contributing to Malaysia's long-term sustainability objectives.

2.3 MICROALGAE

Microalgae exhibit vast biodiversity, with estimates suggesting there are between 200,000 and 800,000 species, of which only approximately 40,000 have been identified (Randrianarison & Ashraf, 2017). Also referred to as microphytes, microalgae are microscopic organisms found in both marine and freshwater environments. They represent a diverse group of unicellular autotrophs, primarily photosynthetic, and are classified as either prokaryotic (cyanobacteria or blue-green algae) or eukaryotic. These organisms possess simple cellular structures and require light, water, carbon dioxide, and essential nutrients such as nitrogen and phosphorus for growth and energy production (Othman et al., 2020). A defining characteristic of microalgae is the presence of chlorophyll in each cell, enabling them to photosynthesise. The group is highly diverse, encompassing various phyla with distinct physiological and morphological properties, ranging from simple prokaryotic cyanobacteria to complex multicellular eukaryotic algae (Tan et al., 2020).

Table 2.1 Classes, Characteristics and Group of Algae Tan et al., 2020).

Type of algae	Characteristic	Group
Cyanobacteria	Cyanobacteria or blue-green algae are gram-negative bacteria. Some of them are known to be able to do nitrogen fixation along with carbon fixation.	*Prochlorococcus Spirulina, Nostoc Cyanothece, Anabaena*

Glaucocystophytes	Glaucocytophytes are relatively uncommon unicellular eukaryotic algae that contain plastid which is structurally similar to cyanobacteria. Glaucocystophytes are one of the descendants of the product of early endosymbiosis.	*Cyanophora* *Peliaina* *Glaucocysti* *Gloeochaete*
Rhodophytes	Rhodophytes or red algae are comprised of mostly multicellular photosynthetic eukaryotes. They are characterized by their distinctive red colour due to the presence of red pigments such as phycobilisomes in their chloroplast.	*Batrachospermum* *Bangi,* *Chroodactylon* *Cyanidium* *Compsopogon*
Chlorophytes	Chlorophytes or green algae are photosynthetic eukaryotes which contain chlorophylls as	*Haematococcus,* *Chlorella,* *Dunaliella,* *Graesiella,* *Scenedesmus*

	their main photosynthetic pigments.	
Charophytes	Charophytes are mainly terrestrial and freshwater algae. They are notably having similar features as terrestrial plants, suggesting that the ancestors of charophytes gave rise to land plants.	*Coleochaete Penium Micrasterias, Chara Klebsormidium*
Chlorarachinophytes	Chlorarachinophytes are marine photosynthetic protists which possess secondary plastids originated from secondary endosymbiosis.	*Chlorarachnion Lotharella Bigelowella*
Euglenoids	Euglenoids are unicellular flagellated eukaryotes which exhibit both animal- and plant-like characteristics. Most Euglenoids are freshwater species while some are marine species.	*Euglena Discoplastis Phacus, Colacium Strombomonas*

Apicomplexans	Apicomplexans are a group of obligate intracellular parasites with which are responsible for causing various diseases in animals and human.	*Plasmodium* *Toxoplasma* *Cryptosporidium*
Dinoflagellates	Dinoflagellates are a class of unicellular protists and are characterized by their relatively large nuclei, golden-brown-coloured plastid and its unique method of swimming.	*Gymnodinium* *Karenia* *Dinophysis* *Alexandrium*
Heterokontophytes	Heterokontophytes are flagellated photosynthetic eukaryotes. They are characterized by their biflagellate which is different in length.	*Chrysophyacae* *Parmophyaceae* *Xanthophyacae* *Dictyophyaceae*
Haptophytes	Haptophytes are unicellular photosynthetic microalgae with its	*Chrysochromulin a Prymnesium* *Pavlova* *Diacronema*

	chloroplast originated from the endosymbiosis of red algae. It usually has 2 equal or unequal flagella which allow it to be motile.	
Cryptophytes	Cryptophytes are motile, and photosynthetic unicellular organisms characterized by the presence of 2 flagella and are typically found in freshwater and marine habitat.	*Guillardia,* *Teleaulax* *Campylomonas* *Geminigera* *Rhodomonas*

Eukaryotic microalgae belong to the Plantae kingdom and can be broadly classified into two main groups: macroalgae (large, multicellular species) and microalgae (unicellular species) (Ruggiero et al., 2015). This study focuses on microalgae, which, although often described as unicellular plants, lack the structural complexity of higher plants, such as stems, roots, and leaves. Their pigmentation varies based on the dominant photosynthetic pigments present in their cells. For instance, species rich in chlorophyll A appear green, whereas those containing high levels of carotenoids exhibit orange or red hues. Microalgae are typically classified according to their pigment composition. However, modern taxonomic systems also consider additional characteristics, including morphology, cytology, the chemical composition of storage products, and cell wall structure. Most microalgae exist as single cells, either independently or as part of colonies or filaments (Tan et al., 2020).

Table 2.2 Kingdom Classification Scheme of Microalgae

Domain	Kingdom	Phylum	Class
Prokaryota	Eubacteria	Cyanobacteria	Cyanophyceae
			Gloeobacterophyceae
Eukaryota	Protozoa	Euglenozoa	Euglenophyceae
	Chromista	Cryptista	Cryptophyceae
		Haptophyta	Coccolithophyceae
			Pavlovophyceae
		Heterokontophyta	Bacillariophyceae
			Chrysophyceae
			Eustigmatophyceae
			Dictyochophyceae
			Phaeophyceae
			Xanthophyceae
	Plantae	Glaucophyta	Glaucophyceae
		Rhodophyta	Cyanidiophyceae
		Chlorophyta	Chlorophyceae
			Trebouxiophyceae

Microalgae are commonly divided into four major classes: Chlorophyceae, Cyanophyceae, Bacillariophyceae, and Chrysophyceae. The Cyanophyceae group, also known as cyanobacteria, consists of photosynthetic bacteria that contribute significantly

to primary production in aquatic ecosystems. Chlorophyceae microalgae, which contain chlorophyll A, lack differentiated structures such as stems or leaves but remain highly efficient in photosynthesis by harnessing sunlight, water, and carbon dioxide (Tan et al., 2020). Due to their ability to fix carbon and produce valuable metabolites, microalgae are regarded as a promising resource in biotechnology, biofuel production, and environmental applications (Randrianarison & Ashraf, 2017). For example, Spirulina is widely cultivated due to its high nutritional value (Grahl et al., 2018). Additionally, microalgae contain a wide range of bioactive compounds, including proteins, lipids, carotenoids, carbohydrates, and vitamins, which are commercially valuable in various industries. However, the vast majority of microalgal species remain underutilised, and ongoing research aims to optimise their potential applications.

Microalgae can grow under three different metabolic conditions: autotrophy, heterotrophy, and mixotrophy. In autotrophic growth, microalgae rely on light energy and inorganic nutrients (such as nitrogen, carbon dioxide, and phosphorus) to synthesise essential biomolecules, including proteins, lipids, pigments, and carbohydrates. Autotrophic cultivation is typically carried out in open pond systems or closed photobioreactors. In contrast, heterotrophic growth occurs in the absence of light, where organic compounds serve as both carbon and energy sources. Glucose is the most commonly utilised carbon source, although other organic substrates such as glycerol can also support heterotrophic metabolism. Mixotrophic growth combines elements of both autotrophy and heterotrophy, allowing microalgae to switch between metabolic pathways depending on environmental conditions. This adaptability enables microalgae to utilise organic carbon sources while simultaneously photosynthesising in the presence of light, making them highly efficient in fluctuating natural environments (Khan et al., 2018).

The production of autotrophic microalgal biomass presents several advantages over traditional heterotrophic microbial systems, such as yeast and bacterial fermentation, which require organic feedstocks. Since autotrophic microalgae utilise light as an energy source and fix atmospheric carbon dioxide, they provide a sustainable means of converting solar energy into organic matter. Furthermore, mixotrophic cultivation offers a hybrid approach that enhances biomass yield, lipid accumulation, and pigment production while reducing dependency on artificial light and exogenous

carbon sources. However, only a limited number of microalgal species can effectively grow under mixotrophic or heterotrophic conditions, restricting the widespread application of these methods. Future research is needed to optimise microalgal cultivation strategies for enhanced biomass productivity, carbon sequestration, and commercial feasibility (Tan et al., 2020).

Microalgae exhibit remarkable morphological diversity, ranging from simple unicellular species, such as *Chlorella,* to more complex multicellular forms. While some algae are microscopic, measuring between 1–5 μm, others, like kelp, can grow to lengths exceeding 50 meters (Bulota & Budtova, 2015). Estimates of algal diversity vary widely, with taxonomists suggesting the existence of between 50,000 and 10 million species, depending on classification methods and taxonomic philosophy.

Algae are distinct from plants despite their shared ability to perform photosynthesis. Unlike plants, algae lack true roots, stems, or leaves, and their cellular structures are generally simpler (Mary, 2019). Most algae are single-celled organisms found in aquatic environments, where they serve as primary producers, forming the base of the food chain. All algae contain chlorophyll a, along with various other chlorophyll types and pigments that contribute to their classification.

In general, algae are categorized into two main groups (Ścieszka & Klewicka, 2018):

1. Microalgae – Classified based on their morphological structures, cell types, and the presence of specific pigments.
2. Macroalgae – Classified based on their morphological and chemical structures, pigments, and cell wall composition.

Microalgae classification is further refined based on their photosynthetic pigments, cell wall composition, storage products, and genetic characteristics. These factors help differentiate major microalgal groups such as *Chlorophyceae* (green algae), *Cyanophyceae* (cyanobacteria or blue-green algae), *Bacillariophyceae* (diatoms), and *Chrysophyceae* (golden algae).

2.3.1 Characteristics

Microalgae in the atmosphere are subjected to various environmental stressors, including:

- Humidity
- Temperature fluctuations
- Oxidative stress
- Nitrogen starvation
- Radiation exposure
- Osmotic stress

These stress factors result from evolutionary selection pressures, which influence the adaptation and distribution of microalgae. The survival of cyanobacteria and other microalgae in atmospheric and aquatic environments is largely dependent on their ability to respond to changing conditions. Their stress-response mechanisms enable them to colonise new habitats and persist under extreme environmental conditions. In response to adverse conditions, certain microalgal species can enter a dormant state, altering their metabolic activity to enhance survival.

All algae contain chlorophyll a, yet variations in accessory pigments, such as chlorophyll b, chlorophyll c, phycobilins, and carotenoids, determine the specific coloration of the cells. Cyanobacteria (blue-green algae) are prokaryotic, lacking a membrane-bound nucleus, while all other algal groups are eukaryotic. Eukaryotic microalgae possess a distinct nucleus and subcellular organelles, including chloroplasts, where photosynthesis occurs (John et al., 2020).

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Table 2.3 Summary of characteristics between blue-green and green algae

Characteristics	Green Algae	Author (Year)	Blue-Green Algae	Author (Year)
Morphology	• Unicellular, colonial, filamentous, coenocytic, or macrophytes with robust axes bearing fronds (blade, sometimes with stipe or stem)	• John et al. (2020)	• Unicellular, colonial or filamentous • do not have a membrane-bound nucleus, mitochondria or other type of membrane-bound organelle	• John et al. (2020) • Mary, (2019)
Habitat	• Marine and freshwater environments • Widely diverse, in aquatic or moist environments	• Rath et al. (2012)	• Can live under every type of environment and on every type of substrate • Fresh water, sea water, salt mashers, moist rocks, tree trunks, moist soils, hot springs, and frozen waters. • Prefer alkaline conditions, and during a bloom, the pH may rise to more than 9 • Highly tolerant of salt and grown in media with high osmolarities	• Mary,(2019)
Reproduction	• Sexual reproduction	• Mary, (2019)	• Asexual reproduction only	• Mary, (2019)
Pigment	• Cells with one to several chloroplasts, clearly green • Major accessory pigment is chlorophyll b, direct evolutionary precursors of higher plants;	• Mary, (2019)	• The main photosynthetic pigment is chlorophyll-a • May be accompanied by carotenoids, xanthophylls, and phycobilisomes, which contain mostly phycoerythrin and phycocyanin. • Some cyanobacteria possess divinyl-chlorophyll-b or chlorophyll-d	• Mary, (2019)

2.3.2. Green Algae

Chlorophyta, commonly referred to as green algae, comprise approximately 7,000 to 8,000 species and are widely distributed across diverse habitats. They can be found in soil, snow, tree bark, and in symbiotic relationships with various flora and fauna. However, their primary habitat is aquatic environments, particularly freshwater ecosystems. Chlorophyta exhibit significant morphological and structural diversity, existing in unicellular, multicellular, colonial (loose cell aggregates), or filamentous forms (Garcia-Pichel & Belnap, 2021). The Viridiplantae group includes two primary phyla of green algae: Chlorophyta and Charophyta. The evolutionary origin of green algae is traced to primary endosymbiosis, in which a heterotrophic protist engulfed a cyanobacterium, leading to the development of the green lineage, as well as glaucophytes and rhodophytes (Borowitzka, 2018).

2.3.2.1 Chlorella Vulgaris

Chlorella vulgaris is a species of microalgae that thrives in freshwater, marine, and terrestrial environments. It exhibits rapid and efficient growth depending on its metabolic conditions (Ardila-Álvarez et al., 2017). C. vulgaris belongs to the order Chlorococcales, within the family Oocytaceae and genus Chlorella. Its characteristic green colour results from its chloroplasts, which play a crucial role in photosynthesis (Coronado et al., 2020).

This unicellular microalga typically ranges in size from 2 to 10 μm and possesses a cellular structure similar to that of higher plants. It contains a rigid cell wall, mitochondria, and chloroplasts, the latter being essential for its photosynthetic function (Safi et al., 2014). Figures 2.1 and 2.2 illustrate the ultrastructure of C. vulgaris and the stages of daughter cell-wall formation, while Table 2.4 summarises its morphological characteristics.

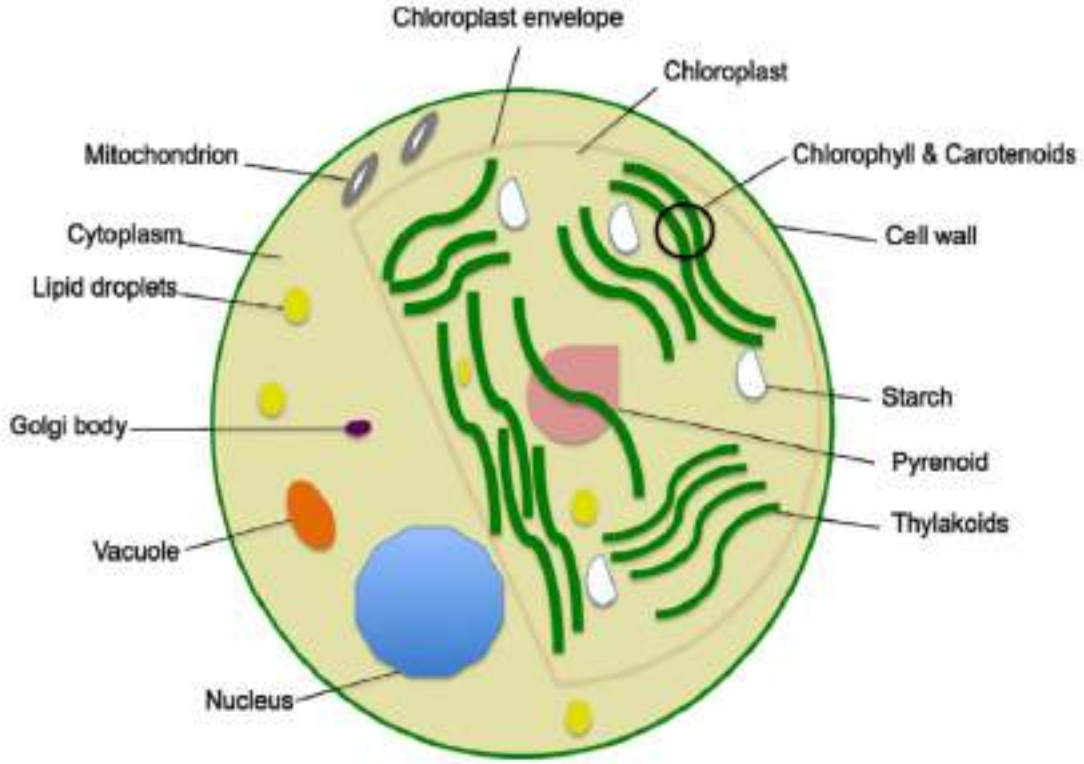

Figure 2.1 Schematic ultrastructure of C. vulgaris representing different organelles.

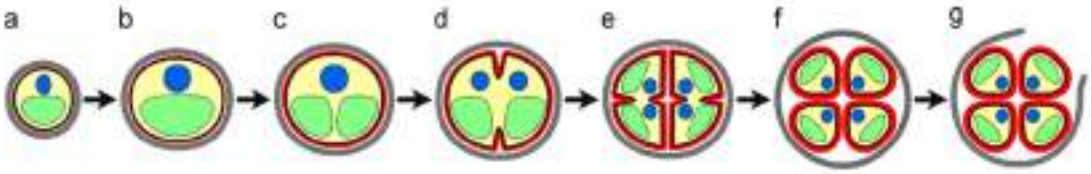

Figure 2.2 Drawings showing the different phases of daughter cell-wall formation in *C.vulgaris*

Based on the figure above, (a) indicates early cell-growth phase; (b) late cell-growth phase; (c) chloroplast dividing phase; (d) early protoplast dividing phase; (e) late protoplast dividing phase; (f) daughter cells maturation phase and (g) hatching phase (Safi et al. (2014).

Table 2.4 Morphology of *Chlorella vulgaris*

Morphology	Characteristics	Author (Year)
Cell wall	• Its thickness is approximately 2 nm and as the microalgae matures, the thickness increases until it reaches a thickness of 21 nm • C. vulgaris has a unilaminar cell wall that lacks sporopollenin, which is an extremely resistant polymerised carotenoid found on the cell wall of *Haematococcus pluvialis*	• Coronado et al., (2020) • Safi et al., (2014)
Cytoplasm	• gel-like substance • confined within the barrier of the cell membrane and it is composed of water, soluble proteins and minerals.	• Coronado et al., (2020) •
Mitochondrion	• Made up of double membranes, proteins and phospholipids.	• Coronado et al., (2020)
Chloroplast	• contains a single chloroplast • composed of phospholipids • consists of two membranes where the first is permeable to certain metabolites and some ions, but the second membrane is highly selective and its function is the transport of proteins	• Safi et al., (2014)

According to Safi et al. (2014), the algorefinery concept illustrates a platform that facilitates the fractionation of biomass components to produce multiple products. *C. vulgaris* can be cultivated using various techniques, including autotrophic, heterotrophic, and mixotrophic growth, as reported by Safi et al. (2014). The tables below summarise the production technique, optimal production and harvesting techniques for *C. vulgaris.*

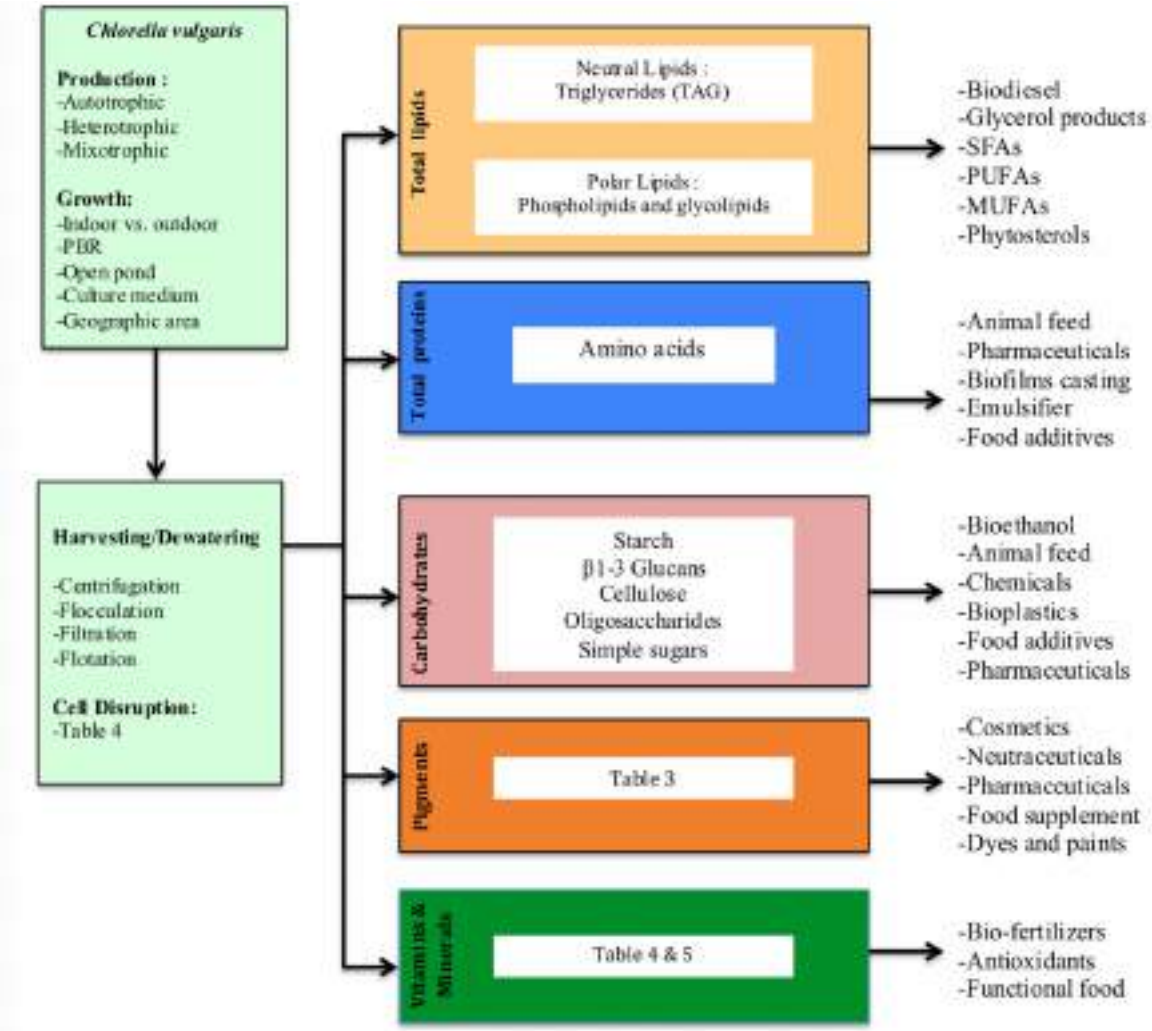

Figure 2.3 Algo-refinery concept from (1), harvesting (2) to valorisation (3) by Safi et al. (2014)

Table 2.5 Summary of production for *C.vulgaris*

Technique	Characteristics	Author (Year)
Autotrophic growth:		
• **Open pond systems**	• Open pond systems: - The most common way of production - Cheapest method for large-scale biomass production - Natural waters (lakes, lagoons and ponds) - Wastewater or artificial ponds or containers.	• Andrade et al. (2014). • Safi et al. (2014) • Coronado et al. (2020)
• **Closed photo-bioreactor**	• Closed photo-bioreactor: - To overcome some limiting factors in the open pond systems - Growing the biomass in a managed environment (pH, light, intensity, temperature, carbon dioxide concentration)	
Heterotrophic growth	• Grown in a stirred tank bioreactor or fermenter • a higher degree of growth are expected as well as low harvesting cost due to the higher dry biomass productivity • does not require light while the biomass is fed with carbon sources	• Morales-Sánchez et al. (2014).
Mixotrophic growth	• By performing photosynthesis as well as ingesting organic materials • Such as glucose, which is the most appropriate for *C.vulgaris*	• Coronado et al., (2020)

Table 2.6 Summary production between freshwater and marine *C.vulgaris*

Parameters	Freshwater *C.vulgaris*	Author (Year)	Marine *C.vulgaris*	Author (Year)
Common media	• Bold Basal Medium • MBL Medium Woods-Hole • BG-11 • (modified) CHU10	• Josephine et al. (2020))	• f/2 medium • ASP-M • ESAW • L1 • MNK • GPM	• Josephine et al. (2020)

Table 2.7 Optimal cultivation conditions for freshwater and marine *C.vulgaris*

Parameters	Freshwater and marine *C.vulgaris*	Author (Year)
Nitrogen source	• Ammonium (autotrophic) • Urea (mixotrophic)	• Gong et al. (2014) • Kong et al., (2011)
Carbon source	• 1.2g/l of NaHCO3 • 8% CO2 • 10% of CO2 with 24h light provision • 1% w/v glucose (mixotrophic) • 4758 ppm of Co2, 75 mg/l of NaHCO3 (lipid production)	• Yeh at al., (2010) • Daliry et al., (2017) • Daliry et al., (2017) • Daliry et al., (2017) • Daliry et al., (2017)
Light intensity and quality	• 100 to 200 0 µmol photons m−2 s −1 • Yellow, red and white light (achieves log phase faster) • Green light (highest amount of C16:3 and C18:3) • Red light (chlorophyll production) • In dark (polyunsaturated fatty acids)	• Halfmann, (2014) • Halfmann et al., (2014) • Fu et al., (2012) • Josephine et al., (2020)

Parameters	Freshwater and marine *C.vulgaris*	Author (Year)
pH	• pH 7.5 and pH 8.0 (contain cadmium) • pH 10.0 and 10.5	• Josephine et al., (2020) • Gong et al., (2014)
Temperature	• 30°C (biomass production) • 25°C (lipid production) • 38°C (increased oleic acid)	• Josephine et al., (2009) • Josephine et al., (2009)
Salinity	• below 0.5 M sodium chloride (NaCl) • 0.5 M of NaCl (lipid production)	• Josephine et al., (2007) • Rai et. al., (2015)
Culture conditions	• 250 rpm agitation (chlorophyll production) • Aeration 200 ml/min (lipid production)	• Halgmann et al., (2014)

Table 2.7 Type of harvesting technique for *C.vulgaris*

Technique	Characteristics	Author (Year)
1. Centrifugation	• Contributes to 20–30% of the total biomass production cost • Highly efficient (95% recovery), not time consuming, and treats large volumes • Permits high centrifugal stress without damaging its structure during the process.	• Safi et al. (2014)
2. Flocculation	• Less expensive but time consuming. • Necessary to increase the pH by adding a base • A pre-harvesting step in order to facilitate or complement other harvesting methods like centrifugation or filtration	• Wang et al. (2017) • Cheng et al. (2010)
3. Flotation	• Trapping the cells using dispersed micro-air bubbles • Can also occur naturally when the lipid content in microalgae increases.	• Safi et al. (2014) • Cheng et al. (2010)
4. Filtration	• Continuous passing of the broth with the microalga across a filter • Algal cells will concentrate constantly until it reaches a certain thickness • Due to the small size ultrafiltration or microfiltration is more efficient.	• Safi et al. (2014) • Frappart et al. (2011)

Based on the analysed research, *C. vulgaris* is a microorganism with significant biotechnological potential. Its ability to produce substantial amounts of secondary metabolites is highly dependent on the type of substrate consumed. These metabolites have diverse applications, including biodiesel production, as the synthesised lipids accumulate in large quantities. Additionally, the starch generated by *C. vulgaris* can be

utilised for the production of bioethanol at significant concentrations. Beyond its role in biofuel production, C. vulgaris serves as a valuable food supplement, emulsifier, and natural additive. Furthermore, its high protein content makes it a promising candidate for the development of functional foods enriched with essential nutrients.

2.3.3 Cyanobacteria

Cyanobacteria derive their energy through photosynthesis, a process that transforms photons captured from light, as well as water and carbon dioxide, into algal biomass (Prabha et al., 2022). Cyanobacteria contain chlorophyll *a*, and certain strains also contain phycobiliproteins, chlorophyll *d* or *b*, glycogen as a storage product, and cell walls composed of amino acids and amino sugars. Under various conditions and stress factors, microalgae and cyanobacteria produce vital bioactive compounds, such as lipids, antioxidants (e.g., carotenoids), polyunsaturated fatty acids (PUFA), and specific types of proteins. Additionally, some cyanobacterial strains can survive in dark (heterotrophic) conditions, sometimes in organic substrates or anaerobic environments, while performing oxygenic photosynthesis (Sivakami, Mahalakshmi, & Premkishore, 2015). Many species are also capable of fixing atmospheric nitrogen, reducing it to ammonia for nitrogen biosynthesis in cellular compounds. Due to these metabolic properties, cyanobacteria are considered to be microbial groups with the simplest nutritional requirements among all microorganisms. These adaptations enable cyanobacteria to thrive and compete effectively in diverse ecosystems. Along with their competitive advantages and ease of cultivation, these characteristics make cyanobacteria suitable for use in wastewater treatment systems (Othman et al., 2020).

Cyanobacteria generate energy through photosynthesis, utilising light, water, and carbon dioxide to produce biomass (Prabha et al., 2022). They contain chlorophyll a, and certain strains also possess phycobiliproteins, chlorophyll d or b, glycogen as a storage product, and cell walls composed of amino acids and amino sugars. Under varying environmental conditions, cyanobacteria and other microalgae produce essential bioactive compounds such as lipids, antioxidants (e.g., carotenoids), polyunsaturated fatty acids (PUFAs), and specific proteins. Some cyanobacteria can survive in heterotrophic (dark) conditions by metabolising organic substrates or thriving

in anaerobic environments while still performing oxygenic photosynthesis (Sivakami, Mahalakshmi, & Premkishore, 2015). Additionally, many species are capable of fixing atmospheric nitrogen, reducing it to ammonia for use in cellular biosynthesis. These characteristics make cyanobacteria highly adaptable, allowing them to colonise a wide range of ecosystems, including wastewater treatment systems (Othman et al., 2020).

One of the key features of cyanobacteria is their ability to fix atmospheric nitrogen (Vaz et al., 2020). Biological nitrogen fixation converts nitrogen gas into biologically available forms, a process that is crucial for life and plays a significant role in global nitrogen cycling (Reis et al., 2020). Before the advent of synthetic fertilisers, nitrogen fixation by bacteria and cyanobacteria was the primary means of making nitrogen available for biological use. In freshwater environments, cyanobacteria and heterotrophic bacteria are the main nitrogen-fixing organisms. Their distinct bluish-green colour is attributed to phycobilin pigments, particularly phycocyanins. Some strains can also produce phycoerythrin, a red pigment that varies in intensity depending on light conditions (Hamouda & El-Naggar, 2021).

Cyanobacteria dominate phytoplankton communities in both marine and freshwater environments and are known for their ability to form large-scale algal blooms (Granéli & Turner, 2006; Huisman et al., 2005). They are found in nearly all terrestrial and aquatic habitats, from freshwater lakes and oceans to soil, bare rocks, and even extreme environments such as deserts and temporarily moistened surfaces. Some cyanobacteria exist as free-floating plankton, while others form biofilms or live in symbiotic relationships with plants, lichens, sponges, or protists, providing energy to their hosts. A unique example includes cyanobacteria living in the fur of sloths, acting as camouflage.

Due to their adaptability and metabolic versatility, cyanobacteria have promising applications in wastewater treatment, bioremediation, biofertilisers, and various industries, including food, feed, and pharmaceuticals (Singh et al., 2019). However, further research is needed before cyanobacteria can be widely adopted for large-scale bioremediation of contaminated water bodies, industrial effluents, and natural aquatic environments.

2.3.3.1 Synechococcus and Phormidium

The genus *Synechococcus* is morphologically simple but polyphyletic, as described by Dvořák, Petr, and Casamatta (2017). These cyanobacteria are unicellular and typically less than 4 μm in size. Some species form pseudo-filaments, with certain strains displaying involuted cells. The genus is highly heterogeneous, requiring further classification through ultrastructural and molecular taxonomy techniques (Komárek et al., 2020). *Synechococcus* is considered one of the most abundant photo-oxygenic microorganisms on Earth. Together with Prochlorococcus, it accounts for approximately 25% of global oceanic net primary production. *Synechococcus* is widely studied due to its prevalence in pelagic marine picoplankton and thermal aquatic habitats.

The genus *Phormidium.* includes over 200 species, though it is polyphyletic and likely contains several unclassified genera. According to Palinska et al. (2011), *Phormidium* species are characterised by thin, hyaline sheaths that can partially dissolve, causing filaments to aggregate into mat-like structures. Found in both freshwater and marine environments, *Phormidium* species exhibit a range of morphological characteristics, including differences in cell size, proportions, apical cell shapes, and cross-wall constrictions. Approximately 170 species of *Phormidium* have been described (Komárek et al., 2020). A key morphological feature is the presence of curved trichomes, often enclosed within a colourless sheath.

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Table 2.8 Difference of characteristics between *Synechococcus* and *Phormidium*

Characteristics	***Synechococcus***	**Author (Year)**	***Phormidium***	**Author (Year)**
Morphology	• Unicellular, small (<4 μm), • forming pseudofilaments • may possess involuted cells	• Dvorak, Petr and Casamatta (2017)	• Thin, hyaline, partly diffluent • Completely dissolved sheaths • Filaments - stick together in mat like layers	• Palinska et al. (2011)

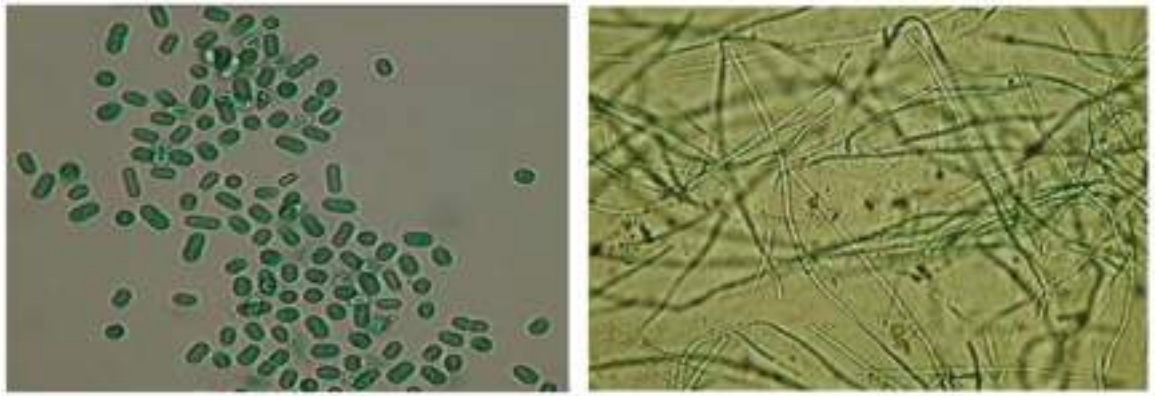

Figure 2.3 *Synechococcus* (left) and *Phormidium* (right)

According to Jeevanantham et al. (2019), cyanobacteria have been extensively studied for their role in understanding the origin of life, photosynthesis, nitrogen fixation, and primary metabolic pathways. Both *Synechococcus* and *Phormidium* share these fundamental characteristics and can be cultivated using various production techniques. As reported by Safi et al. (2014), these techniques include autotrophic, heterotrophic, and mixotrophic growth methods, similar to those used for the production of *C. vulgaris.*

Table 2.9 Technique used for production

Technique	Characteristics	Author (Year)
Autotrophic growth: • **Open pond systems** • **Closed photo-bioreactor**	• Open pond systems: - The most common way of production - Cheapest method for large-scale biomass production - Natural waters (lakes, lagoons and ponds) - Wastewater or artificial ponds or containers. • Closed photo-bioreactor: - To overcome some limiting factors in the open pond systems - Growing the biomass in a managed environment (pH, light, intensity, temperature, carbon dioxide concentration)	• Andrade et al. (2014). • Safi et al. (2014) • Coronado et al. (2020)
Heterotrophic growth	• Grown in a stirred tank bioreactor or fermenter • a higher degree of growth are expected as well as low harvesting cost due to the higher dry biomass productivity • does not require light while the biomass is fed with carbon sources	• Morales-Sánchez et al. (2014).
Mixotrophic growth	• By performing photosynthesis as well as ingesting organic materials • Such as glucose, which is the most appropriate for *C.vulgaris*	• (Moon et al., 2 013)

Table 2.10 Common media used for culturing

Parameters	*Synechococcus*	Author (Year)	*Phormidium*	Author (Year)
Common media	• Bold Basal Medium • BG-11 • (modified) CHU10 • ASM-1 • ASN III • Walne's medium enriched	• Saravanan et al. (2021)	• Bold Basal Medium • BG-11 • (modified) CHU10 • ASM-1 • ASN III • Walne's medium enriched	• Saravanan et al. (2021)

Table 2.11 Culture conditions for *Synechoccos* and *Phormidium*

Parameters	*Synechococcus* *Phormidium*	Author (Year)
Nutrient	• Media enriched with nitrate and phosphate	• Saravanan et al., (2021)
Light intensity	• High light (8000 lux), growth was considerably higher than with low light (2000 lux)	• Saravanan et al., (2021)
pH Range	• 6.5 to 8.5 • With variation of in their degree tolerance • Grow well in pH range 7.5 to 9	• Jeevanantham et al., (2019)
Temperature	• Room temperature (29 ±)	• Saravanan et al., (2021)
Salinity	• *Phormidium* : Salinity concentration of 25% • *Synechococcus* : salinity concentration of 15%	• Jeevanantham et al., (2019)

Cyanobacteria contribute significantly to various industries by producing food materials, fixing atmospheric nitrogen, and serving as sources of natural colorants, bioplastics, biofuels, and fine chemicals. They also generate bioactive substances and essential compounds such as lipids, pigments, enzymes, polysaccharides, and glycerol. Additionally, they contain substantial amounts of carotenoids, phycocyanin, vitamins, flavonoids, and phenolic compounds—potent antioxidants that can scavenge highly reactive free radicals responsible for tissue damage (Jeevanantham et al., 2019).

In conclusion, microalgae represent a promising field of research due to their remarkable growth and production potential. Cyanobacteria, in particular, can synthesize a wide array of valuable products, ranging from food to bioenergy, based on their biochemical composition. More importantly, their adaptability to extreme environmental conditions makes them highly versatile organisms. Their role as ecological indicators further enhances their significance in sustainability efforts, contributing to environmental conservation and the development of biological and sustainable solutions.

2.4 PHYCOTECHNOLOGY: ALGAE BIOTECHNOLOGICAL APPLICATIONS

In Malaysia, the increasing number of untreated contaminated urban water bodies remains a pressing concern due to the high costs of conventional water treatment and insufficient funding for water management (Abu Yazid, 2017). Compounding the issue, untreated sludge is often discharged directly into the environment due to the lack of proper sludge treatment facilities, further deteriorating water quality. Consequently, these polluted water bodies become less accessible and pose significant health risks to humans and aquatic ecosystems.

Moreover, only a limited number of plant species have demonstrated the potential to remediate heavy metal contamination in aquatic environments. In Malaysia, the use of constructed wetlands is largely restricted to mangroves and aquatic plant species, which may not be practical for urban water bodies. Unlike in more technologically advanced countries, green technologies such as phycotechnology

remain underutilized, leading to a lack of public awareness regarding their potential. This limited understanding has hindered the widespread adoption of sustainable alternatives, as both public and private sectors hesitate to invest in eco-friendly solutions, fearing a lack of consumer willingness to pay for raw water treated through green methods.

Phycoremediation, the use of algae (macro- or microalgae) to remove or biotransform contaminants—including nutrients, heavy metals, and xenobiotics—from polluted water, has emerged as a viable and cost-effective solution (Koul & Taak, 2018; Upadhyay et al., 2018). Algae serve as excellent carbon dioxide sinks, mitigating atmospheric carbon levels that contribute to global warming and pollution (Arbib et al., 2014; Ding et al., 2020). Their ubiquity and adaptability to diverse habitats make them particularly effective for bioremediation efforts. Among them, microalgae exhibit extraordinary biodiversity, with an estimated 200,000–800,000 species worldwide (Abinandan & Shanthakumar, 2015; Hussain et al., 2021; Poonia et al., 2022).

Beyond pollutant removal, algae-based biological treatment enhances oxygen availability for heterotrophic aerobic bacteria, promoting the breakdown of organic contaminants (Chaudhary et al., 2018; Jiang et al., 2011). Since the effectiveness of phycoremediation is directly linked to microalgal growth, optimizing their biomass production can further expand their applicability in industries such as nutrition, biofuels, aquaculture, and pharmaceuticals (Badwy et al., 2008; Chisti, 2008; Jais et al., 2017; Shields & Lupatsch, 2012; Sydney et al., 2011). Microalgae require optimal nitrogen and phosphorus levels to synthesize nucleic acids, phospholipids, and proteins, which constitute 45–60% of their dry weight (Apandi et al., 2017). Additionally, they play a crucial role in tertiary wastewater treatment by effectively removing PO_4^{3-}, NO_3^-, and ammonium (Rawat et al., 2011). Through mechanisms such as bioaccumulation, biosorption, biotransformation, and assimilation, microalgae also eliminate hydrocarbons, heavy metals, and pesticides from wastewater (Chekroun & Baghour, 2013; Rath, 2012; Leong & Chang, 2020).

Advancements in molecular and functional genomics have enabled the genetic engineering of algal strains for enhanced bioremediation. These modifications improve their resistance to extreme conditions, photosynthetic efficiency, and pollutant

detoxification capabilities (Lutzu et al., 2020; Zeng et al., 2011). Compared to conventional water treatment methods such as reverse osmosis, membrane separation, ion exchange, electrodialysis, and activated carbon adsorption, phycoremediation offers several advantages (Abdel-Shafy & El Saharty, 2015; Hussain et al., 2021). These include:

- Assimilation of phosphorus and nitrogen into biomass
- Low operational costs
- Oxygenation of effluents before discharge
- Elimination of sludge handling requirements
- Environmental sustainability, as algal biomass can be repurposed as fertilizer without producing secondary contaminants (Liu et al., 2016)

Microalgae have demonstrated high efficiency in removing both inorganic and organic pollutants, including agro-industrial wastewater with high nutrient loads (Das et al., 2019; López-Serrano et al., 2020). Various species—including Botryococcus sp., Phormidium sp., Scenedesmus sp., Chlorella sp., Chlamydomonas sp., and Oscillatoria sp.—have been widely studied for wastewater treatment applications.

Despite the significant potential of microalgae in pollutant removal, most studies have focused on their ability to eliminate organic compounds and heavy metals, with limited research on their efficacy in addressing multimetallic pollutants. Given that heavy metal concentrations in microalgae can be up to twice as high as those in surrounding water, certain species serve as effective biosorption agents and environmental indicators. The strong correlation between metal concentrations in algae and aquatic environments further supports their use as biomonitors.

2.4.1 Phycoindicator

Algae play a critical role in environmental monitoring and management, serving as primary producers in aquatic ecosystems and responding rapidly to environmental fluctuations. According to Saber et al. (2020), pollution from various sources remains a major global challenge. While pollutants can be detected using technological tools, one

of the most eco-friendly and cost-effective methods involves the use of biological indicators, including plants, microorganisms, and animals.

Algae have been widely recognised as biological indicators of environmental pollution since the mid-19th century (Dokulil, 2013). Their ability to detect contamination in specific habitats has become increasingly valuable as environmental degradation continues to escalate. According to Hosmani (2013), algae such as *Euglena sp., Chlamydomonas sp., Scenedesmus sp.,* and *Chlorella sp*. effectively identify pollutants in aquatic ecosystems. Similarly, Zancan et al. (2006) report that diatoms are commonly used as indicators in polluted water bodies.

In marine ecosystems, an increase in algal species diversity may indicate environmental decline. Certain species, including *Trachelomonas sp., Phacus tortus, and Euglena clastica*, are frequently observed in degraded marine habitats (Zoghloul et al., 2020). Additionally, algal blooms in lakes and rivers, often caused by excessive nitrate and phosphate levels, reinforce the role of algae as pollution indicators.

Microalgae are particularly sensitive to pollutants and environmental stressors. For example, *Euglena gracilis*, a freshwater microalga, exhibits clear physiological responses to pollution, such as reduced mobility and altered orientation behaviour, making it a useful tool for ecotoxicological assessments. Its gravitational orientation is highly responsive to pollution, allowing for rapid environmental evaluations (Zoghloul et al., 2020).

Relying solely on chemical or physical indicators may not always detect irregular pollution patterns. Saber et al. (2016) highlight that sudden bursts of pollutants often go unnoticed by chemical indicators alone. Zaghloul et al. (2019) further emphasise that integrating biological, chemical, and physical indicators provides a more comprehensive and accurate evaluation of pollution levels. Khatri and Tyagi (2015) stress the importance of natural variables such as light, moisture, temperature, and suspended particles when interpreting biological indicators.

In terrestrial environments, pollution can significantly alter pH and nutrient levels. Singh et al. (2019) state that pH often shifts towards acidity, depleting essential

nutrients. Blue-green algae can serve as bioindicators in these environments, as they are rarely found in conditions where the pH drops below 5.

Certain macroalgae, such as *Padina sp*., have also been identified as effective bioindicators of contamination. According to Ansari (2019), Padina can absorb pollutants onto both its fresh and dried biomass. This species is particularly responsive to changes in light, temperature, and nutrient levels. In aquatic ecosystems, rising cadmium (Cd) concentrations can reduce *Padina's* growth rate and chlorophyll levels, indicating heavy metal contamination.

Algae, as photosynthetic prokaryotic and eukaryotic organisms, are widely distributed across diverse environments. Their long-established role as biomonitors and bioindicators makes them invaluable for detecting environmental changes and pollution. Beyond their function in biomonitoring, algae also contribute to phycoremediation, actively removing contaminants and improving ecosystem health.

2.4.2 Phycoremediation

Phycoremediation is a technology that uses photosynthetic algae to remediate and detoxify contaminants through the biological transformation of waste into harmless compounds (Krishnamoorthy et al., 2021). The approach of using algae for remediation to eliminate nutrients and other pollutants from the environment, particularly in wastewater, is gaining growing interest among researchers. Eutrophication, contamination, and depletion are common examples of the issues affecting surface water and water bodies worldwide. Treating wastewater sustainably using algae is important, as it is more effective compared to conventional remediation methods. According to Priyadharshini et al. (2021), phycoremediation is an intriguing method for treating wastewater, as it provides tertiary bio-treatment while producing valuable biomass that can be potentially used for a variety of applications. Additionally, phycoremediation can remove heavy metals and harmful organic substances while preventing environmental contamination.

In comparing bioremediation and phycoremediation, bioremediation is a process that uses living organisms such as bacteria, fungi, and algae to remove or reduce

the effects of pollutants through their metabolic processes. According to Corpuz et al. (2021), there is a common misconception that bioremediation and phycoremediation are the same. However, both methods are entirely different. Table 2.10 below compares both remediation methods, as compiled by several authors.

Table 2.12 The phycoremediation and bioremediation keypoint by several authors

Nos	**Phycoremediation**	**Author (Year)**	**Bioremediation**	**Author (Year)**
1.	Algae release oxygen which it promotes the growth of local bacteria.	Corpuz et al. (2021)	Bacteria used in bioremediation break down the organic material found in sewage, dead algae, and weeds.	Corpuz et al. (2021)
2.	The growth of the local bacteria can elevate water quality, marine life and biodiversity	Yadav et al. (2021)	Aerators may be installed to produce the required amount of oxygen because bacteria absorb it.	Tanabe et al. (2015)
3.	Aquatic fish and zooplankton consume photosynthethic algae, hence algae were released into the ocean as fish biomass	Wang et al. (2020)	While some organic waste is removed, dead bacteria accumulate and must be collected.	Azman et al. (2014)
4.	Only a small number of algae perish and fall to the pool's bottom therefore preserves the lake's natural food chain.	Cardoso et al. (2021)	Regular inoculations and cultures of bacteria are done at a high expense compared to algae.	Ryu et al. (2014)

5.	Local bacteria break down the nutrients while algae remove them from the water by taking up the nutrient and carbon dioxide to generate oxygen.	John et al. (2020)	Solid waste disposal is difficult to remove, and the sludge created by bacterial treatment plants is more harmful.	John et al. (2020)

Due to rapid urbanisation, industrialisation, intensive agricultural practices, and increasing household water consumption, water quality has declined significantly worldwide (Li et al., 2021). Consequently, algae-based biological remediation has emerged as a more effective, cost-efficient, and environmentally sustainable alternative to conventional remediation technologies. Algae facilitate contaminant removal through various biological mechanisms, including bioabsorption, bioaccumulation, biocoagulation, and bioconversion (Han et al., 2019). Table 2.13 presents the benefits and limitations of using algae for phycoremediation, as documented by various authors.

Table 2.13 The advantages and disadvantages of using algae as phycoremediation

Advantages	**Author (Year)**	**Disadvantages**	**Author (Year)**
Depending on the kind of wastewater or pollutants, different type of algae species can be identified and utilised to detoxify and remediate environmental contaminants.	Han et al. (2019)	The quantity of space required to grow algae and the system's speed of operation are the main drawbacks of contemporary phycoremedition technique.	Kumar et al. (2020)
Algal species are widely known to be huge therefore it can be transformed into wide range of remediation tools. As a result, a significant number of bioactive	Brar et al. (2017)	An efficient photobioreactor needs a lot of input to grow algae, including a steady supply of carbon dioxide and frequent monitoring while collecting and recovering secondary	Gupta et al. (2019)

chemicals had been generated using sufficient biomass of genetically modified strains.	metabolites in algal cells are expensive.

In conclusion, both microalgae and macroalgae have been extensively studied for their potential in environmental remediation. Phycoremediation can occur naturally within ecosystems, while its application in remediation technologies offers valuable byproducts, including food, animal feed, compost, biodiesel, and a means to reduce environmental carbon emissions. The unique characteristics of algae are therefore essential in advancing research and the development of algae-based remediation strategies for addressing environmental pollution.

2.4.3 Phycosequestration

The rising concentration of carbon dioxide (CO_2) is a major contributor to global warming and climate change. CO_2, a significant greenhouse gas, is primarily emitted through the combustion of fossil fuels, particularly coal (Viswanathan et al., 2022). This escalating issue has driven extensive research into CO_2 management strategies, including collection, storage, and sequestration. As noted by Viswanathan et al. (2022), algal biomass effectively captures CO_2 emissions from various industries and converts it into valuable metabolites, such as polysaccharides, amino acids, fatty acids, pigments, and vitamins. Biological sequestration, particularly through algae, is gaining recognition due to its integration with carbon capture and wastewater treatment. Algae can thrive in extreme environments, including seawater, polluted lakes, and industrial wastewater with high levels of organic and inorganic nutrients. This makes them a promising tool for tertiary wastewater treatment and an effective phycosequestration agent in fostering a sustainable environment.

According to Bhola et al. (2014), CO_2 biofixation using microalgae offers several advantages as a potential sequestration method:

1. Rapid Growth and Environmental Resilience – Microalgae exhibit fast growth rates, high tolerance to harsh conditions, and adaptability to changing environmental factors, with a cell doubling time of just 24 hours.
2. Versatile Growth Medium – They can be cultivated in various low-quality water sources, including seawater, industrial wastewater, and municipal sewage.
3. High CO_2 Fixation Ability – Through photosynthesis, microalgae exhibit CO_2 fixation rates at least ten times higher than terrestrial plants.
4. Efficient Carbon Capture – Microalgae can utilise approximately 10% of solar energy to capture 513 tonnes of CO_2, converting it into 280 tonnes of dry biomass per hectare annually.
5. Significant Carbon Sequestration Potential – Algae are estimated to fix over 65 gigatonnes (Gt) of carbon annually, equivalent to the carbon output of 65,000 500 MW power plants.
6. CO_2 Extraction from Industrial Sources – Microalgae naturally extract CO_2 from flue gases produced by power plants, making them highly suitable for large-scale CO_2 sequestration applications.

Cheah et al. (2016) identified several microalgal species with exceptional CO_2 fixation abilities, including *Botryococcus braunii, Chlorella sorokiniana, Chlorella vulgaris, Chlorococcum littorale, Dunaliella tertiolecta, Nannochloropsis oculata, Scenedesmus dimorphus, Scenedesmus obliquus*, and *Spirulina platensis.* These species are highly regarded for their potential as phycosequestration agents. Table 2.14 below summarises the microalgal species and their potential for CO_2 sequestration.

Table 2.14 The Microalgae studies for their potential to fix CO2

Microalgae species	**C02 Concentration (%)**	**CO_2 Fixation Rate (g L^{-1} day^{-1})**	**References**
***Chlamydomonas* sp**	15	-	Viswanaathan et al. (2022)
***Chlorella* sp.**	40 0.03 15 5 5 -	- 1.62 - 0.46 0.7 1.38	Viswanaathan et al. (2022)
Chlorella (marine)	2-15	2.14-4.69	Viswanaathan et al. (2022)
Chlorella pyrenoidosa SJTU-2	5-50	0.029-0.71	Viswanaathan et al. (2022))
C.vulgaris	10 2 18	0.25 0.43 -	Sydney et al. (2010) Yeh & Chang (2011)
Chlorococcum littorale	20 60	0.246 -	Viswanaathan et al. (2022) Viswanaathan et al. (2022)
Cyanidium caldarium	100	-	Viswanaathan et al. (2022)
***Dunaliella* sp.**	3	0.31	Viswanaathan et al. (2022)
***Eudorina* sp.**	20	-	Viswanaathan et al. (2022)
Euglena gracilis	45	-	Viswanaathan et al. (2022)

Microcystis aeruginosa	15	0.134	Viswanaathan et al. (2022)
Microcystis ichthyoblabe	15	0.142	Viswanaathan et al. (2022)
***Nannochloris* sp.**	15	-	Viswanaathan et al. (2022)
***Scenedesmus* sp.**	80 15	- 0.61	Viswanaathan et al. (2022) Viswanaathan et al. (2022)
Scenedesmus dimorphus	0.03	1.27	Fulke et al. (2013)
Scenedesmus incrassatulus	0.03	1.50	Fulke et al. (2013)
Scenedesmus obliquus	15 10 10 2.5 18	4.6 0.55 0.29 1.19 -	Viswanaathan et al. (2022) Viswanaathan et al. (2022) Tang et al. (2011)
Synechococcus elongatus	60	-	Viswanaathan et al. (2022)
***Spirulina* sp.**	20	0.14	Sivakumar et al. (2014)
***Tetraselmis* sp.**	14	-	Viswanaathan et al. (2022)

In conclusion, various microalgal species have demonstrated a broad range of CO_2 fixation rates and tolerance levels, ranging from 200 to 1000 mg L^{-1} day^{-1}. Phycosequestration has proven to be a highly effective strategy for mitigating environmental contaminants, including excessive nutrient levels, heavy metals, chemical oxygen demand (COD), biological oxygen demand (BOD), coliforms, and other pollutants commonly found in wastewater (Krishnamoorthy et al., 2021).

2.4.3.1 Future Tools for Carbon Sequestration

The elevated levels of carbon dioxide (CO_2) in the atmosphere have contributed to the accumulation of greenhouse gases, leading to significant global temperature increases. According to Molazedeh et al. (2019), CO_2 bio-fixation primarily occurs through photosynthesis in plants and trees. However, plants can only remove 5–6% of atmospheric CO_2, whereas microorganisms such as eukaryotic algae and cyanobacteria can sequester CO_2 10–50 times faster. This disparity arises due to the slower growth rate of plants compared to microorganisms. Table 2.15 presents evidence from various authors on the suitability of microalgae as future tools for carbon sequestration.

Table 2.15 Microalgae's suitability for becoming future tools for carbon sequestration

Nos	Summarisation	Author	Year
1.	• During photosynthesis, chloroplasts plays a crucial role in the process of converting light energy into chemical energy. • In microalgae, the chloroplasts use light energy for photosynthesis and the conversion of carbon dioxide into organic compounds	Daneshvar et al.	2022
2.	• Carbon sequestration absorption using microalgae has number of advantage due to the high efficiency of photosynthesis, faster growth rate, adaptability to environmental conditions and reduction in usage of energy.	Politaeva et al.	2023
3.	• 100 tons of microalgae could fix 183 tons of carbon dioxide during the period of maximum growth of biomass • But this value may varies depending on many factors such as temperature, C02 supply method, species of microalgae, design of cultivator, etc.	Chauvy et al.	2019

4.	• To enhanced the maximum growth in microalgae, the optimal of C02 enriched gases is required and supplied. • Air pumping to the medium is required for better production of microalgae	Politaeva et al.	2023
5.	• Biomass such as plants, algae produces more than 100 billion tons of organic matters annually. • This resulted in absorption about 200 billion tons of C02 and releases about 145 billion tons of oxygen into the environment. • Microalgae is proved to be photosynthesised-efficiently 10-20% higher than plants.	Politaeva et al	2023

Based on the findings summarised in the table, one of the most effective methods for utilising carbon dioxide is through absorption and adsorption. CO_2 absorption using a biofilter, where microalgal suspensions serve as the bioload, has been identified as a cost-effective and efficient approach to reducing carbon emissions (Razzak et al., 2013).

Furthermore, Ismail et al. (2016) highlighted that microalgae, particularly those of the genus Chlorella, exhibit the highest potential for carbon capture. Research has demonstrated that cultivation with higher cell density (0.0325 g/dm³) enhances resistance to carbon dioxide, with an intake of 48.17 g/h, a carbon fixation rate of 37.95 g/dm³/day (58%), and biomass production of 0.82 g/dm³/day.

Thus, carbon sequestration using microalgae presents a promising method for CO_2 emissions reduction, particularly in the energy sector, from both economic and environmental perspectives.

2.5 PHYCOTECHNOLOGY AND BUILT ENVIRONMENT

Algae are considered a promising renewable fuel source due to their rapid growth and ability to store lipids. Additionally, their high energy potential makes them an attractive alternative, as they do not require complex or expensive conditions for cultivation and storage. Algal cultivation can also take place in natural environments (Iglina et al., 2022).

According to Cenci et al. (2017), phycotechnology has been utilised for centuries in environmental assessments, particularly in aquatic ecosystems, where algae serve as indicators of ecosystem health. While algae can pose potential challenges to ecosystems, they also play a crucial role in carbon sequestration by capturing carbon dioxide through photosynthesis. Notably, algae produce more oxygen than terrestrial plants, contributing approximately 50% of Earth's total photosynthesis.

In architectural applications, algae offer innovative solutions to mitigate environmental challenges. Compared to conventional treatment technologies, which are often costly, algal-based solutions present several advantages in tackling environmental problems. Table 2.16 below illustrates these benefits.

Table 2.16 Comparison between algae and traditional method as a solution to environmental problems.

Nos	Algae as a solution	Traditional Method	Author (Year)
1.	Algae are utilised to reduce environmental contaminant in an eco-friendly manner.	Uses large resources and emits enormous amounts of hazardous by-products.	Priyadharshini et al., (2021)
2.	Under typical operating	Most of the terrestrial plants	Pacheco et al., (2020)

	conditions, carbon capture efficiency can range from 80% to 99% with gas residence times of less than two seconds.	have complex cellular structure and less effective compared to algae due to their straightforward cellular structure which can be more effective in term of absorbing nutrients, water, and carbon dioxide.	
3.	Have high photosynthetic and carbon mitigation efficiency which causes them to reduce atmospheric carbon emissions quicker.	Terrestrial organism takes a longer time to reduce the atmospheric carbon emissions rather than algae.	Pacheco et al., (2020

From the data summarised in Table 2.16, ecological solutions can be enhanced and adapted to ensure long-term sustainability. Within the landscape ecology community, several measures have been extensively explored to promote sustainable environmental practices (Frohn, 2018). Phycotechnology, a relatively emerging concept in this field, holds significant promise as a bio-ecological indicator due to its wide-ranging applications. This is supported by various studies, as detailed in Table 2.17 below.

Table 2.17 Phycotechnology as bio-ecological indicator

Nos	Advantages	Author	Year
1.	Algae have the ability to treat multiple problems at once, which is not possible with conventional treatments in a way that is environmentally sustainable.	Daneshvar et al.	2019
2.	A viable alternative to the threat of global warming is algae's capacity to trap carbon. The atmosphere will thereafter be cleaned with the help of the released oxygen.	Lutzu et al.	2021
3.	Algae-based remediation is a practical method that doesn't need a lot of electricity or harmful materials to work.	Sirakov et al.	2013

The table demonstrates that phycotechnology can serve as a future landscape indicator, contributing to more sustainable environments. Microalgae, as primary producers in aquatic ecosystems, are emerging as genetic model systems (Hopes et al., 2016). Beyond their crucial role in human nutrition (FAO, 2014), algae contribute to approximately half of Earth's oxygen production through photosynthesis (Cenci et al., 2017). Microalgae also thrive in extreme environments, demonstrating resilience to both natural and anthropogenic pressures (Mishra et al., 2022).

Despite limitations in light availability, temperature, and nutrient concentrations in their natural habitats, algae can survive in environments enriched with salts, toxic metals, pesticides, fluctuating temperatures, unfavourable pH, UV-B radiation, and high light intensity. From an ecological perspective, their ability to withstand diverse stress conditions is critical, and their metabolism is more efficient than that of terrestrial plants.

According to Haoyang (2018), microalgae are among the most effective bio-ecological indicators and environmental solutions, particularly due to their high capacity for atmospheric CO_2 reduction. While traditional carbon recycling processes absorb only small amounts of CO_2, injecting CO_2 into oceanic reservoirs presents

potential risks, especially when excessive greenhouse gases accumulate. In contrast, ocean-based algae absorb 45–50% of total CO_2 sequestered by Earth's biosphere, compared to 52% absorbed by terrestrial plants. This highlights the superior effectiveness of algae in carbon sequestration, despite their small size and short life cycles. By optimising environmental conditions—such as temperature, nutrient availability (particularly iron), and sunlight—algae can further enhance CO_2 reduction. Thus, cultivating algae offers a viable, low-cost strategy for mitigating greenhouse gas emissions.

A study conducted by researchers at the Alfred Wegener Institute for Polar and Marine Research demonstrated the effectiveness of algae in CO_2 absorption. On 13 February 2004, 7 tonnes of $FeSO_4$ were applied to 167 square kilometres of acidic seawater. Over the following five weeks, six species of diatoms rapidly proliferated. Most diatoms eventually perished and sank to the seafloor, suggesting that algal growth can be partially regulated. This method's low cost makes it an attractive large-scale approach for absorbing substantial greenhouse gas emissions in oceans worldwide.

While this approach may have potential ecological drawbacks, it highlights the untapped potential of algae. Further research on microalgae aims to optimise their applications in:

1. Phycoindicators – to assess ecosystem health,
2. Phycoremediation – to clean contaminated environments, and
3. Phycosequestration – to sequester carbon through algal processes.

2.5.1 Role of Algae in Creating Sustainable Environment

Microalgae have emerged as promising bio-prospective tools for promoting sustainability. According to Meeranayak et al. (2020), advancements in phycotechnology have accelerated efforts towards achieving sustainable environmental solutions. Algae offer eco-friendly, cost-effective, and innovative strategies to tackle pressing sustainability challenges, including:

Pollution Control and Carbon Neutrality. Algae play a crucial role in carbon sequestration, capturing atmospheric CO_2 through photosynthesis. Many algal species exhibit high CO_2 fixation capabilities, making them a viable solution for reducing greenhouse gas emissions. With their rapid growth rates and high biomass productivity per unit area, algae offer an effective method for carbon capture. Additionally, algae-based wastewater treatment helps mitigate pollution by removing contaminants while simultaneously sequestering carbon.

Toxic chemicals, heavy metals, and organic/inorganic waste from industrial and agricultural effluents pose severe threats to aquatic ecosystems and public health. Wastewater typically contains varying pollutant concentrations, which can lead to environmental degradation. Algae naturally thrive in polluted water bodies and have demonstrated the ability to absorb pollutants, reduce toxic loads, and improve water quality. Their biomass production and algal blooms can be strategically utilised in wastewater treatment, presenting a sustainable alternative to conventional water purification techniques.

Certain cyanobacteria (blue-green algae) enhance nitrogen fixation in agricultural environments, significantly improving soil fertility and crop productivity. These nitrogen-fixing cyanobacteria play a critical role in sustainable farming by reducing the need for synthetic fertilisers while also offering natural pest resistance. Additionally, some cyanobacteria species have the ability to degrade harmful pesticides and herbicides, preventing long-term environmental accumulation of agricultural pollutants.

While microalgae hold tremendous potential for sustainable applications, challenges such as scalability, cost-effectiveness, and technological advancements must be addressed to fully realise their environmental benefits. Continued research and innovation in phycotechnology will be essential in harnessing algae’s full potential for pollution control, carbon sequestration, and sustainable agriculture.

2.6 ISLAMIC PRINCIPLES AND ENVIRONMENT

Science, technology, and Islamic teachings are deeply interconnected, with the Qur'an offering insights long before modern scientific discoveries. One striking example is the proportion of water and land on Earth, a fact mentioned in the Qur'an over 1,400 years ago. The Qur'an references the word "ocean" 32 times and "land" 13 times. A simple calculation of this ratio gives 28.89% land and 71.11% ocean, a value that closely aligns with modern scientific estimates. According to Ahmed et al. (2019), Earth's surface is composed of 29% land and 71% water, demonstrating the remarkable accuracy of this ancient revelation.

Despite the abundance of water, only 3% of Earth's total water is freshwater, and an even smaller fraction (0.01%) is directly accessible for human use (Sayqal & Ahmed, 2021). This limited freshwater supply is increasingly under pressure due to rapid urbanization, population growth, and rising water demand in agriculture and industry.

Understanding and implementing Islamic environmental principles can guide sustainable resource management, ensuring that water conservation and environmental stewardship remain key priorities in addressing global ecological challenges.

Table 2.16 Pathological Impacts of Heavy Metals

Heavy Metal	Source	Effect	MCL (mg/L)
Hg	Coal, electrical batteries. vinyl chlorides	Rheumatoid arthritis, kidneys damage, circulatory and nervous systems.	-
Pb	Plastic, paint, pipe, batteries, gasoline, auto exhaust	Neurotoxic, fetal brain damage, kidneys disease, skin manifestation, visceral cancers, vascular disease.	0.006
Ar	Pesticides, coal, detergents	Liver cirrhosis, mental disturbance, cancer, ulcer, and hyperkeratosis.	0.05
Cd	Fertilizers, plastic, pigment	Kidney damage, injury in CNS and mental retardation.	0.01

Cr	Tanning, paints, pigment, fungicide	Nephritic, cancer, and ulceration.	0.05
Zn	Fertilizer	Vomiting, renal damage, and cramps.	0.8
Co	Vitamin B12.	Diarrhoea, low blood pressure, and paralysis.	-
Ni	Electroplating.	Dermatitis, nausea, chronic asthma, Teratogenic, carcinogenic, genotoxic, and mutagen.	0.2
Cu	Mining, electrical manufacturing, agricultural, leather processing.	Liver damage, Wilson disease, and insomnia.	0.25

MCL - Maximum contaminant level

Muslim scholars have long recognized the importance of environmental conservation, emphasizing its impact on all living beings. Throughout history, Islamic teachings have addressed sustainable resource management, leading to the issuance of fatwas (religious rulings) on environmental issues. In 2009, the International Islamic Fiqh Academy (IIFA), under the Organization of Islamic Cooperation (OIC), issued a fatwa prohibiting actions that lead to environmental destruction or the unsustainable use of natural resources. These rulings align with the fundamental Islamic principles of stewardship (khalifah), which encourage green technology adoption and sustainable environmental practices.

One such promising green technology is phycoremediation, a branch of phytotechnology that utilizes algae to remove pollutants from the environment. While certain heavy metals—such as calcium (Ca), iron (Fe), zinc (Zn), and magnesium (Mg)—are essential for human health and daily dietary intake, others, including cadmium (Cd), arsenic (As), methylated mercury (Hg), and lead (Pb), are toxic even at low concentrations, posing severe health risks (Mohd Hatta et al., 2022). Table 1 provides an overview of these hazardous heavy metals and their associated pathological impacts on both human health and the environment.

Similar to many developing nations, Malaysia is facing increasing challenges related to waste generation and disposal. On average, the country produces 0.5 to 1.9

kg of municipal solid waste (MSW) per capita per day, amounting to approximately 25,000 tonnes daily—a figure expected to exceed 30,000 tonnes per day by 2030 (Mohd Latiff, 2019). Malaysia's waste composition consists of 24% plastic, 45% food waste, 6% metal, 7% paper, 3% glass, and 4% wood. Unfortunately, 80–90% of MSW is disposed of in landfills, while only 5% is recycled. Practices such as composting and energy recovery remain underutilized, highlighting the urgent need for sustainable waste management solutions.

Heavy metal pollution poses a significant threat to human and animal life due to its presence in contaminated food chains. Plants and animals can absorb heavy metals from polluted soil, water, and air, leading to bioaccumulation—a process in which toxic substances build up in organisms over time at a rate faster than they are excreted (Othman et al., 2020). This gradual accumulation of toxic metals not only disrupts ecosystems but also exacerbates health risks as these substances progress up the food chain. To combat environmental toxicity, bioremediation techniques—such as metal-accumulating plants—are increasingly employed to remove heavy metals from contaminated soils (Khalid et al., 2017). These approaches offer sustainable solutions to address the growing threat of heavy metal contamination.

2.7 CHAPTER SUMMARY

Microalgae have developed efficient metabolic adaptations that enable rapid growth under favourable conditions, even in environments where nutrients are intermittently or sparsely available. Consequently, algae often surpass terrestrial plants in photosynthetic efficiency, resulting in a higher capacity for biomass production (Benedetti et al., 2018). Their ability to thrive in diverse and often extreme environments while maintaining optimal growth rates and carbon fixation capabilities underscores their significance in sustainable development. Additionally, microalgae serve as a valuable renewable biomass resource for producing a wide range of high-value products, including biodiesel, bioethanol, pigments, health supplements, and pharmaceuticals. These attributes highlight the potential of microalgae in advancing green technologies and addressing global environmental challenges.

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 INTRODUCTION

The production of microalgal biomass is significantly influenced by multiple factors, including the growth medium composition, nutrient concentration, and nutrient type (Alam, 2022). Several strategies have been employed to optimise microalgal growth, pigmentation, and biochemical composition, which are crucial for their role as ecological indicators. These strategies include refining the medium composition (e.g., nutrient sources, salts, vitamins, and carbon supply), adjusting physical parameters (e.g., temperature, pH, and light intensity), and selecting the appropriate metabolic system (e.g., autotrophic, heterotrophic, or mixotrophic growth modes) to enhance their adaptability to environmental conditions (Bernard et al., 2016; Suyadi, 2020).

This study was designed as a controlled laboratory experiment to assess the potential of *Chlorella vulgaris, Synechococcus* sp., and *Phormidium* sp. as landscape ecological indicator agents. The microalgae were cultured in different growth media, pH levels, and photoperiod conditions to evaluate their response in terms of biomass production, biochemical composition, and chromaticity. with three biological replicates per treatment to ensure statistical validity. The parameters selected for this study were based on their ecological relevance and their influence on microalgal physiological responses: Growth Media: The choice of media formulations (BBM, BG-11, Bristol, and Chu-10) was guided by their widespread use in algal cultivation and their distinct nutrient compositions. These formulations were selected to determine which medium optimises growth and pigmentation in each species. pH Levels: The pH range (3.0, 5.0, 7.0, 9.0, and 11.0) was chosen to assess the tolerance and adaptability of microalgae to different acidic and alkaline conditions, reflecting potential environmental stressors in natural ecosystems and photoperiods: Light cycles of 24:0, 16:8, 12:12, and 8:16 (light:dark) were tested to examine their effect on growth rate and pigment stability. These conditions mimic natural variations in light exposure, which influence photosynthetic efficiency and metabolic activity.

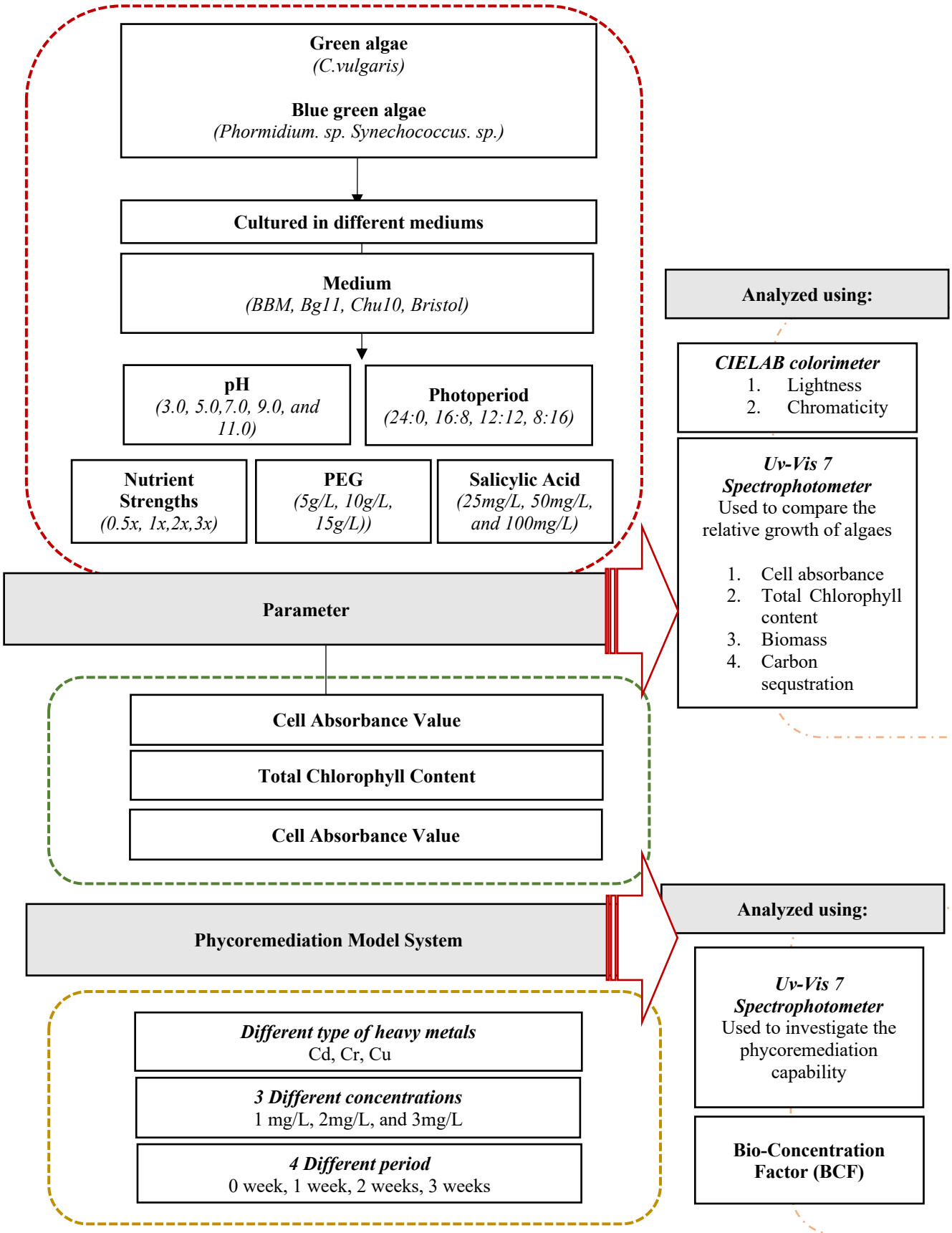

Figure 3.1 Experimental Design

3.2 OPTIMIZATION OF SPECIES OF GREEN ALGAE AND BLUE-GREEN ALGAE

3.2.1 Selection of Microalgae

For green algae, the same species were selected but with different background cultures, and a total of two species of cyanobacteria were chosen for the optimization analysis, as outlined below.

Table 3.1 Selection of Microalgae

Species name	Description	Reference
C.vulgaris	Family: Chlorellaceae Class: Trebouxiophyceae Genus: Chlorella Phylum: Chlorophyta	Jennifer et al. (2014).
Synechococcus	Family: Synechococcaceae Class: Cyanophyceae Genus: Synechococcus; Phylum: Cyanobacteria	Komárek et al. (2020).
Phormidium. amphigranulata	Family: Phormidiaceae Class: Myxophyceae Genus: Phormidesmis Phylum: Cyanobacteria	Komárek et al. (2020).

1. Green algae: *Chlorella vulgaris*
2. Blue-green algae: *Synechococcus sp*. and *Phormidium sp.*

All species were bought from Algatech Sdn. Bhd. and Universiti Sains Malaysia (USM), Pulau Pinang. The microalgae were subcultured in Bold Basal's Medium (BBM) at 24 °C, under 16:8 of light and dark cycle.

3.2.2 Preparation of Different Mediums, pH, and Photoperiods

The four media BBM, BG-11, Bristol, and Chu-10 were chosen due to their distinct nutrient compositions, which influence microalgal growth, pigment production, and biomass accumulation:

- BBM (Bold's Basal Medium): Widely used for culturing green algae such as *C. vulgaris*, BBM is nutrient-rich, supporting high biomass productivity and chlorophyll content. It provides an ideal baseline for assessing growth efficiency in *C. vulgaris* (Gupta et al., 2019).
- BG-11: Designed for cyanobacterial species such as *Synechococcus* sp. and *Phormidium* sp., BG-11 is nitrogen-rich, promoting optimal cyanobacterial growth. Its formulation supports nitrogen fixation and enhances the production of pigments such as phycocyanin, a key indicator of cyanobacterial metabolic activity.
- Bristol Medium: A commonly used medium for freshwater microalgae, Bristol contains fewer nutrients than BBM but is suitable for assessing how algae respond to nutrient-limited conditions, simulating natural water bodies with lower nutrient availability (Sharma et al., 2017).
- Chu-10 Medium: Primarily formulated for nitrogen-fixing algae, Chu-10 is suitable for evaluating how *Synechococcus* sp. and *Phormidium* sp. perform in conditions with limited combined nitrogen sources. It allows for the investigation of alternative nitrogen uptake mechanisms and their influence on biomass production (González López et al., 2019).

Microalgae naturally inhabit environments with varying pH conditions, which can significantly influence their metabolic activity, enzyme functionality, and pigment stability. The selected pH levels (3.0, 5.0, 7.0, 9.0, and 11.0) represent acidic, neutral, and alkaline conditions to examine how different species adapt to environmental stressors:

- Acidic Conditions (pH 3.0 and 5.0): These levels simulate polluted or naturally acidic environments, such as acid rain-impacted water bodies, where certain microalgae can serve as bioindicators of acidification (García-Camacho et al., 2016).

- Neutral Condition (pH 7.0): As many freshwater algae thrive at neutral pH, this condition serves as the control, representing a stable and optimal growth environment (Markou & Nerantzis, 2013).
- Alkaline Conditions (pH 9.0 and 11.0): These levels mimic highly eutrophic or alkaline environments, where some species may exhibit adaptive physiological mechanisms such as increased carbon sequestration efficiency or pigment adjustments to counteract oxidative stress (Hounslow et al., 2020).

Light availability is a critical factor influencing photosynthesis, biomass accumulation, and pigment synthesis in microalgae. The selected photoperiods (24:0, 16:8, 12:12, and 8:16 light:dark cycles) represent varying natural and artificial lighting conditions:

- Continuous Light (24:0): Simulating artificial culture conditions where light is continuously supplied, this setting tests whether uninterrupted photosynthesis enhances biomass production or induces photoinhibition (Singh et al., 2021).
- 16:8 Light:Dark Cycle: Representative of typical daylight exposure in tropical regions, this condition provides insight into optimal growth under natural light-dark fluctuations (Hosseini Tafreshi & Shariati, 2009).
- 12:12 Light:Dark Cycle: Mimicking seasonal variations in temperate regions, this condition assesses how algae regulate their metabolism in response to equal light-dark intervals (Sánchez et al., 2008).
- 8:16 Light:Dark Cycle: Simulating shaded environments or winter photoperiods, this setting examines whether reduced light exposure affects photosynthetic efficiency and biomass accumulation (Masojídek et al., 2011).

By systematically evaluating these parameters, this study aims to determine the optimal growth conditions for *C. vulgaris, Synechococcus* sp., and *Phormidium* sp. while assessing their effectiveness as ecological indicators in varying environmental conditions

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Table 3.2 Medium ingredients

Chemical component	CHU 10	BG 11	BBM	Bristol
Calcium nitrate ($Ca(NO_3)24H_2O$)	0.04 g	–	–	–
Sodium nitrate ($NaNO_3$)	–	1.5 g	0.25 g	0.25 g
Potassium phosphate monobasic (KH_2PO_4)	–	–	0.175 g	0.175 g
Potassium phosphate dibasic (K_2HPO_4)	–	0.04 g	0.075 g	0.075 g
Magnesium sulfate ($MgSO_4$)	0.05 g	0.075 g	0.075 g	0.075 g
Calcium chloride dihydrate ($CaCl_22H_2O$)	–	0.036 g	0.025 g	0.025 g
Sodium chloride (NaCl)	–	–	0.025 g	0.025 g
Sodium carbonate (Na_2CO_3)	0.02 g	0.02 g	–	–
Sodium silicate (Na_2SiO_3)	0.025 g	–	–	–
Disodium ethylenediaminetetraacetate dihydrate (Na2EDTA)	–	0.001 g	0.1 g	–
Ferric chloride ($FeCl_3$)	0.0008 g	–	–	–
Potassium hydroxide (KOH)	–	0.062 g	–	–
Boric acid (H_3BO_3)	–	–	0.12 g	–
Ferrous sulfate heptahydrate ($FeSO_47H_2O$)	–	0.05 g	0.05 g	–
Manganese(II) chloride tetrahydrate ($MnCl_24H_2O$)	–	–	–	–
Ammonium molybdate tetrahydrate ($(NH_4)6Mo7O244H_2O$)	0.1 mg	–	–	–
Citric acid (H_2SO_4)	–	0.006g		–
Ferric ammonium citrate	–	0.006g		–
pH	6.8	6.8	6.8	6.8
Trace metal mix A	–	1 mL	1 mL	–
Trace metal mix B	–	1 mL	1 mL	–
Total volume	1 L	1 L	1 L	1 L

Trace metal mix A		Trace metal mix B	
Boric acid (H_3BO_3)	2.86 g	NH4NO3	22.96 g
Manganese(II) chloride tetrahydrate ($MnCl_24H_2O$)	1.81 g	K2Cr2(SO4)4.24H2O)	96.00 g
Zinc Sulphate ($ZnSO_47H_2O$)	0.222 g	NiSO4.7H2O	47.85 g
Molybdic acid sodium salt dihydrate ($NaMoO_42H_2O$)	0.39 g	Na2SO4_2H2O	17.94 g
Copper(II) sulfate pentahydrate ($CuSO_45H_20$)	0.079 g	Ti(SO4)3	40.00 g
Cobalt(II) Nitrate Hexahydrate ($Co(NO_3)26H_2O$)	49.4 g		
Distilled water	1.0 L	Distilled water	1.0 L

To prepare the growth medium, salts were first weighed according to the prescribed formulation and dissolved in distilled water in a 3 mL Schott bottle. The total volume was adjusted to 3 mL, ensuring homogeneity of the solution. The pH was then

adjusted to the designated levels (3.0, 5.0, 7.0, 9.0, and 11.0) using 1.0 M potassium hydroxide (KOH) or hydrochloric acid (HCl), with continuous stirring to prevent precipitation. The pH was monitored using a Mettler Toledo pH meter, ensuring precision and consistency across treatments. Following pH adjustment, the medium was autoclaved at 121°C and 15 psi for 20 minutes to eliminate potential microbial contaminants. The sterilised medium was then allowed to cool under aseptic conditions before inoculation (Preisig & Andersen, 2005).

Each culture was inoculated with 1 mL of algae suspension containing 3×10^4 cells/mL of *C. vulgaris*, *Phormidium* sp. or *Synechococcus* sp. The cultures were transferred into sterilised 100 mL conical flasks, serving as growth chambers. These flasks were maintained under controlled laboratory conditions with Philips fluorescent lighting, providing an intensity of 1,800 lux or 25 μmol photons·m^{-2}·s^{-1}, depending on the designated photoperiod (24:0, 16:8, 12:12, and 8:16 light:dark cycles). The cultures were incubated at 24 ± 1°C for two weeks, ensuring stable environmental conditions to assess the impact of different treatments on algal growth and physiological responses.

3.2.3 Determination of the Growth of Algae, Pigmentation, and Biochemical Composition

The assessment of algal growth, pigmentation, and biochemical composition was conducted using multiple analytical techniques to ensure accurate and comprehensive measurements.Algal growth was quantified using optical density (OD) measurements at 750 nm (OD_{750}), recorded every 48 hours using a spectrophotometer. This method provides a reliable estimate of biomass concentration, as absorbance at this wavelength correlates with cell density while minimising interference from chlorophyll and other pigments (Xia et al., 2020).

The chlorophyll content were determined using a spectrophotometric method, following the protocol established by Wellburn (1994). This technique enables precise quantification of photosynthetic pigments, which serve as key indicators of algal physiological status and biomass production.

To assess pigment stability and colour variation under different environmental conditions, the CIE Lab* colour space values were measured using a colourimeter. This method provides objective colourimetric data, facilitating the evaluation of chromaticity changes resulting from pigment alterations due to varying growth conditions (Kim et al., 2017).

Beyond growth and pigmentation analysis, spectral variations were monitored to evaluate algal responses to environmental stressors such as heavy metal contamination and ultraviolet (UV) radiation. Previous studies have demonstrated that exposure to heavy metals significantly alters algal metabolism, leading to changes in absorbance spectra that reflect physiological stress (Machado et al., 2015; Machado & Soares, 2014). These findings highlight the significance of integrating optical and spectrophotometric measurements in assessing algal health, environmental adaptability, and ecological indicator potential.

Figure 3.2 Uv-Vis Spectrophomoter

3.2.4 CIELAB Analysis

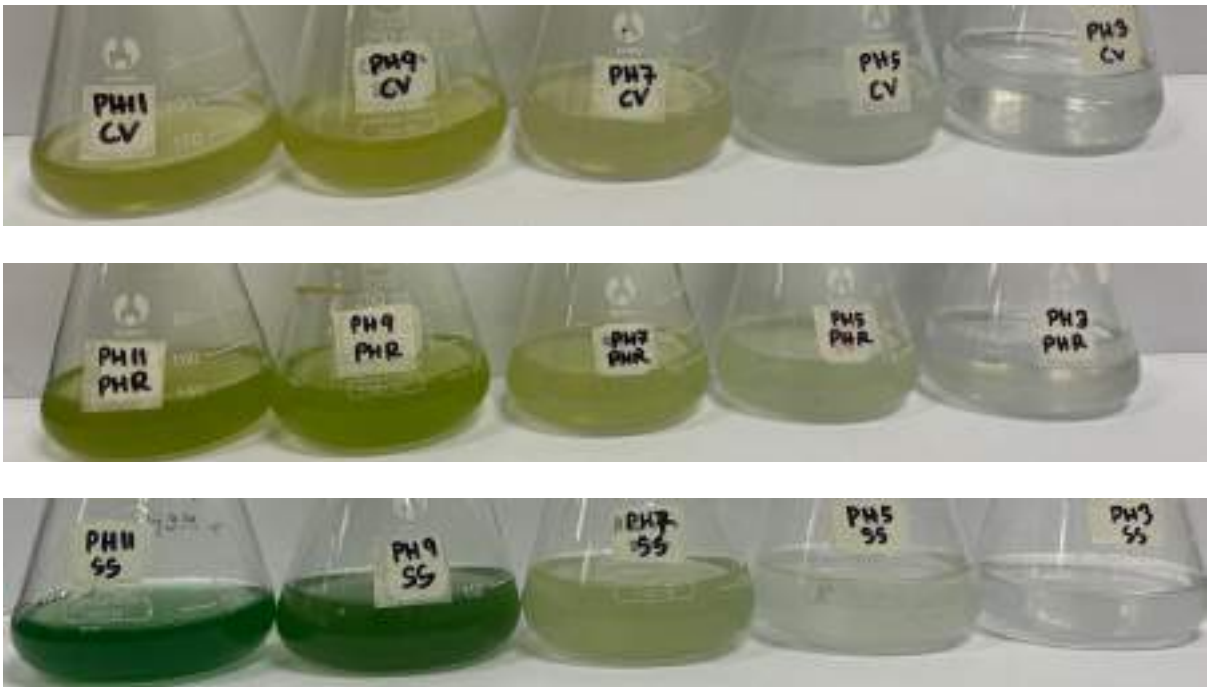


Figure 3.3 Cultured algae in different species and pH

CIELAB Color Analysis

The colour intensity of microalgae was measured by using a Metler-Toledo UV7 spectrophotometer. CIE refers to the Commission Internationale de l'É-clairage (International Commission on Illumination) or in the CIE 1976 (L*, a*, b*) colour system, abbreviated CIELAB, the lightness coefficient, L*, ranges from black (0) to white (100), positive a* indicates a hue of red purple and negative a*, of bluish green. Whereas positive b* indicates yellow and negative b* blue. The colour of achromatic or gray is when a* and b* = 0 (Othman, 2009).

3.3 THE EFFICIENCY OF GREEN ALGAE AND BLUE-GREEN ALGAE IN CARBON SEQUESTRATION

The development of carbon capture, utilisation, and storage (CCUS) technologies is considered a promising approach to reducing greenhouse gas emissions, particularly carbon dioxide (CO_2), which plays a critical role in achieving carbon neutrality (Politaeva et al., 2023). Among the various biological methods for CO_2 sequestration, microalgae have gained significant attention due to their high photosynthetic efficiency and ability to convert atmospheric CO_2 into biomass while producing valuable bioproducts.

3.3.1 Stock Culture Preparation

To ensure optimal growth conditions, three selected algae species (C. vulgaris, Synechococcus sp., and Phormidium sp.) were cultivated for 14 days in sterile conical flasks under controlled environmental conditions. The cultures were maintained at a temperature of 24 ± 1°C and exposed to a light intensity of 1800 lux using fluorescent lamps. The flasks were manually agitated three times a day to promote homogeneous mixing and enhance gas exchange, which is essential for CO_2 absorption (Preisig & Andersen, 2005).

The pH of the culture medium was maintained at 6.8, adjusted as necessary using potassium hydroxide (KOH) or hydrochloric acid (HCl) to ensure a stable alkaline environment conducive to algal growth. This pH level is optimal for enhancing photosynthesis and maximising CO_2 fixation efficiency.

3.3.2 Growth measurement

The concentration of 1 x 10^5 cells/ml isolated strains were cultured in the 48-well microplate for 15 days set at 25 ± 1°C with light intensity ranging from 60 to 70 μmol m^{-2} s^{-1} with different medium formulations, pH and photoperiods. The growth absorbance was determined every day according to an optical density at 750 nm

wavelength (OD_{750} nm) using Metler-Toledo UV7 spectrophotometer (Kasan et al., 2020).

3.3.3 Determination of Microalgae Biomass and Carbon Sequestration Rate

The dry weight (DW) of harvested biomass of microalgae species cultured in different medium formulation, pH and photoperiod was measured after 14 days of culture period. The dewatered biomass was freeze-dried for 3 days, after which the samples were weighed and stored at -80 °C until further analysis (Kumar and Mallick, 2023).

The carbon sequestered by the microalgae species was estimated on the basis of total carbon content in the dry weight biomass (Kumari et al., 2023). The total carbon sequestered was calculated by multiplying the dry weight biomass with the conversion factor 0.427. The quantity of total CO_2 sequestered was calculated by multiplying the carbon content with 3.67 (ratio of molecular weight of carbon dioxide and carbon).

3.4 ANALYSIS OF HEAVY METALS BIOSORPTION BY SPECIES OF GREEN ALGAE AND BLUE-GREEN ALGAE

Microalgae are sensitive organisms when exposed to different pollutants (Zhu et al., 2020) in toxicity bioassays, likely due to their high surface-to-volume ratio. Microalgae have developed peptides that can bind heavy metals, but the binding efficiency is species-dependent and varies based on cell size, shape, and cell wall composition (Ranjbar & Malcata, 2022). Algal growth and nutrient uptake are not only affected by the availability of nutrients but are also influenced by the complex interactions of physical factors such as pH, light intensity, temperature, and biotic factors (Abdel-Raouf et al., 2012; Xing, 2019). Thus, in the following study, different species in the chosen media were developed for the preparation of phycoremediation for heavy metal agents.

3.4.1 Stock Preparation

The selected algae species were cultured for 14 days in flasks under controlled conditions to ensure optimal growth. The cultures were maintained at a light intensity of 1800 lux, with a temperature of 24 ± 1°C, and manually agitated three times daily to promote uniform growth (Andersen, 2005). The pH of the medium was adjusted and maintained at 6.8 using potassium hydroxide (KOH) or hydrochloric acid (HCl). This optimized stock culture was then used for heavy metal biosorption experiments.

3.4.2 Preparation of Metals Stock Solution

The metal compounds used in this study included cadmium chloride ($CdCl_2$), copper sulfate ($CuSO_4$), and chromium chloride ($CrCl_2$). These three metals were prepared in three concentrations: 1 mg/L, 2 mg/L and 3 mg/L. First, the salts were weighed accordingly. Then, a 1000 mg/L stock solution of each heavy metal was prepared by dissolving the salts in 1000 mL of distilled water. The samples then underwent an acid digestion process before further analysis.

3.4.3 Acid Digestion

Samples from the treatment after 1, 2, and 3 weeks were centrifuged at 8000 rpm for 3 minutes to harvest the microalgae cells. A weight of 0.4 g of each sample was accurately measured and placed into a container made of perfluoroalkoxy polymer (PFA), which was then transferred into polytetrafluoroethylene (TFM) Teflon tubes. All microalgae samples were digested using Microwave Digestion Ethos EZ (Milestone, 2009). 5 ml of 65% nitric acid (HNO_3) was added into the tube and retained in the digestion vessel. A torque wrench was used to tighten the vessel tubes, which were then placed in the rotor segment. The first vessel was connected to a temperature sensor. Temperature, power, and time were adjusted according to the US EPA 3015 method from the application book of the SK-10 High-Pressure Rotor for Water, as shown in Table 5 below.

Table 3.3 Temperature, Power, and Time Setting on Micro Digester Method

Step	Time	T1	T2	P	Power
1	0:10:00	170 ºC	95 ºC	45 bar	1500 (max)
2	0:10:00	170 ºC	95 ºC	45 bar	1500 (max)

After digestion was completed, the vessels were cooled down for 30 minutes before the samples were transferred to centrifuge tubes. Three replicates were prepared in 15 mL centrifuge tubes for each sample. Further analyses were conducted using a UV-Vis spectrophotometer at the respective wavelengths for each heavy metal.

3.4.5 Determination of Heavy Metals Content.

Three heavy metals cadmium (Cd), chromium (Cr), and copper (Cu) were analyzed using a Mettler Toledo UV-Vis spectrophotometer. For accurate detection, specific wavelengths corresponding to each metal were selected from the broad range emitted by the spectrophotometer's light source.

Before beginning the measurement, turn on the spectrophotometer and allow the lamps to warm up for an appropriate time to stabilize. Prepare a blank by filling a clean cuvette with the sample solvent, then wipe the outside with lint-free paper to remove any fingerprints. Ensure that the cuvette is aligned properly, with any grooved sides facing out of the beam path and insert it into the spectrophotometer. Secure the lid to prevent ambient light from entering the system. Measure the absorbance of the blank at a single wavelength or over a wavelength range. Record or save the absorbance value, as it must be subtracted from the sample absorbance.

Next, discard the blank and rinse the cuvette twice with the sample. Fill the cuvette about ¾ full with the sample and wipe the outside again to ensure it is clean and free of fingerprints. Place the cuvette in the spectrophotometer in the correct orientation, secure the lid, and collect the absorbance measurement or spectrum at the same

wavelength or range used for the blank. If the instrument does not automatically subtract the blank measurement, manually subtract it from the sample absorbance.

3.4.6 Bio-concentration factor (BCF)

The bioconcentration factor (BCF) is the result of a balance between the rate of chemical intake through the test organism's respiratory surface and the rate of chemical elimination. It is also known as the ratio of the absorption rate constant (k1) to the depuration rate constant (k2). The BCF was calculated to determine the efficiency of heavy metal uptake by algae (Du, 2022). The formula for calculating BCF is as follows:

BCF = Heavy metal concentration in algae (mg/kg) / Initial heavy metal concentration in water (mg/L)

This study uses the BCF as a measure of heavy metal sequestration. BCF values will be calculated to assess the efficiency of different algae species in sequestering heavy metals such as cadmium (Cd), chromium (Cr), and copper (Cu). This metric is crucial for evaluating the potential of algae as agents for phycoremediation.

3.5 STATISTICAL ANALYSIS

The dataset was processed using XLSTAT-Basic+ (2024) statistical software (Addinsoft, Lumivero, France) for outlier treatment, dataset transformation, and Kaiser-Meyer-Olkin (KMO) test. All the datasets were standardised and subjected to outliers at a significant level (α) of 0.05. The distance between the extreme and centre values was computed for the Grubbs test, and values with too large distances were identified as outliers. Dixon's approach computed the lowest and highest sample values, divided the range into subranges, and then identified the outliers (Coucke et al., 2012). The analysis of variance (ANOVA) test of Tukey's test was carried out to determine the significant difference between the means of all dataset at α of 0.05. The dataset was transformed using standardise (n-1), standardise (n), standard deviation^{-1} (n-1), standard deviation^{-1} (n), centre, 0 to 1 rescaling, 0 to 100 rescaling, Pareto and log methods to

ensure normal distribution is followed. Transformed dataset distribution was reevaluated using the same normality measure and subjected to the KMO test. The KMO test evaluated the dataset for adequacy at α of 0.05 to ensure the dataset is adequate for the Multivariate Data Analysis. The average KMO value was ranked as: KMO < 0.5 = inadequate, 0.5 < KMO < 0.7 = mediocre, 0.7 < KMO < 0.8 = good, 0.8 < KMO < 0.9 = very good and KMO > 0.9 excellent to indicate the dataset adequacy and only KMO > 0.5 was acceptable for PCA (Ismail et al., 2021). A principal component analysis (PCA) of Pearson correlation at α of 0.05 was employed to describe the correlation and distribution of significant. The dataset was transformed into sets of independent variables known as principal components (PCs). Cumulative variability (CV) of two dimensional PCs entailing PC1 and PC2 were computed for the datasets profile exploratory. The variables with strong, moderate and weak factor loading (FL) were identified. Based on these FLs, the variable correlations and their contributions to algae cell culture growth treatments were assessed and clustered.

3.6 CHAPTER SUMMARY

The study evaluated both green algae (*C.vulgaris*) and blue-green algae (*Phormidium* and *Synechococcus*) under specific culture conditions. The media used for cultivation included BBM, BG11, Chu 10, and Bristol, with pH levels set at 3.0, 5.0, 7.0, 9.0, and 11.0. Different photoperiods were also tested for each medium, including BBM, BG11, Bristol, and Chu 10. To further investigate the algae's responses, various elicitors were applied. These included nutrient strengths at concentrations of 0.5x, 1x, 2x, and 3x, as well as Polyethylene Glycol (PEG) at concentrations of 5 g/L, 10 g/L, and 15 g/L. Additionally, salicylic acid was introduced at concentrations of 25 mg/L, 50 mg/L, and 100 mg/L.

The study also examined the potential of these algae as ecological indicator agents. Analytical methods included the use of a CIE LAB colorimeter to measure lightness and chromaticity, as well as a UV-Vis spectrophotometer for assessing cell absorbance values, total chlorophyll content, biomass, and carbon sequestration.

The efficiency of the algae as potential carbon sequestration agents was analyzed using the UV-Vis spectrophotometer, which provided a comparative analysis of relative growth and absorbance.

In terms of heavy metal sequestration, the study focused on three heavy metals: cadmium (Cd), chromium (Cr), and copper (Cu). The algae were exposed to these metals at concentration levels of 1 mg/L, 2 mg/L, and 3 mg/L over periods of 0 weeks, 1 week, 2 weeks, and 3 weeks. The efficiency of heavy metal uptake was analyzed using a UV-Vis spectrophotometer to evaluate the phycoremediation capability, alongside the calculation of the bioconcentration factor (BCF) to quantitatively assess heavy metal accumulation.

CHAPTER FOUR

RESULTS & DISCUSSION

4.1 INTRODUCTION

C.vulgaris are green, spherical unicellular eukaryotic microalgae with a thick cell wall (100–200 nm) as the main characteristic. This cell wall provides mechanical and chemical protection and it is reported its relation to heavy metals resistance, which explains why *C. vulgaris* is one of the most used microorganisms for waste treatment as well as carbon fixation. The green colour comes from the chloroplasts that the algae use to make food through photosynthesis. Chlorella comes from a combination of the Greek 'chloros', which means green, with the Latin conjugation 'ella', which means small. Vulgaris means common or not special. So by name, *C.vulgaris* is just a common little green cell. However, this does not fit its great potential. *C. vulgaris* has a high protein and nutrient content, and is cultivated for future green food source. Besides for food, *C. vulgaris* is a promising biofuel source. It is regarded as a possible substitute for crops that are currently used for this. In the past decade, however, interest in Chlorella has grown again

4.2 RESULTS AND DISCUSSIONS

4.2.1 Cell Culture Growth of *C. vulgaris* in different medium formulation, PEG and SA

Concentrations

In this experiment, four types of medium formulation which are BBM, BG11, Bristol, and Chu10 were used. The cell culture growth performance of *C. vulgaris* across the various media formulations were measured through their absorbance at the wavelength of 750 nm each day for 2 weeks. By optimizing the medium composition, it is possible to enhance cell density and potentially improve the overall productivity and bioactivity of *C. vulgaris* in various applications.

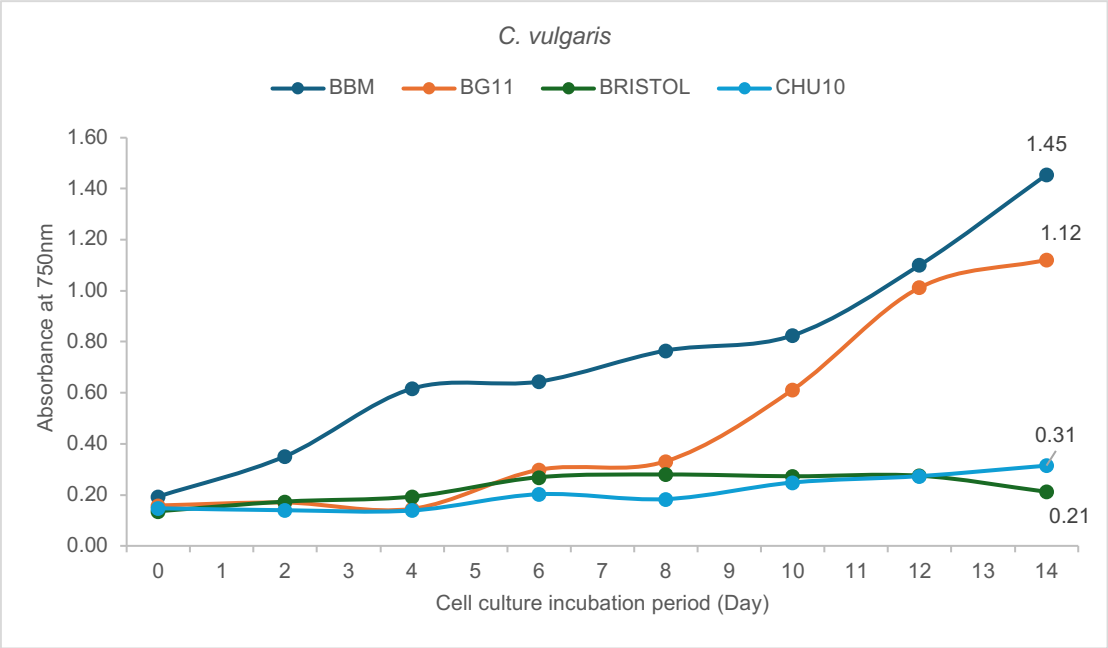


Figure 4.1 Distribution of cell culture growth of *C. vulgaris* in different medium formulations

Table 4.1 The optimum absorbance value of *C. vulgaris* cell culture growth in different media formulation

Green Algae	**Medium**	**Abs**
C. vulgaris	BBM	1.45
	BG11	1.12
	BRISTOL	0.31
	CHU10	0.21

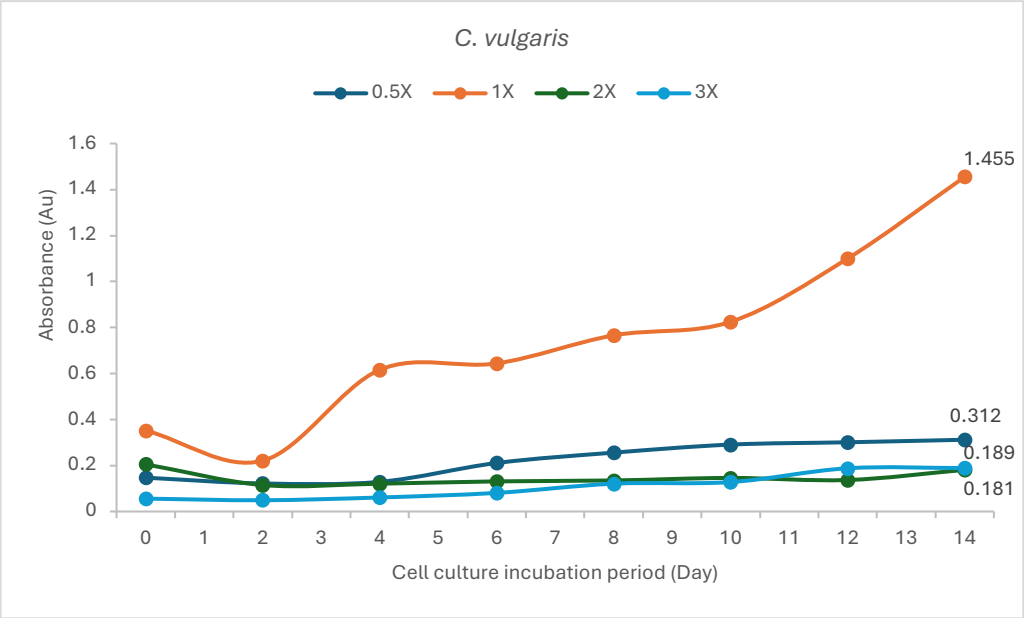


Figure 4.2 Distribution of cell culture growth of *C. vulgaris* in different nutrient strength.

Table 4.2 The highest absorbance value of *C. vulgaris* cell culture growth in different BBM medium formulation strength

Green Algae	**Medium strength**	**Abs**
C. vulgaris	0.5	0.31
	1x	1.45
	2x	0.18
	3x	0.19

The optimum absorbance values for *C. vulgaris* cultured in four different media, measured at 750 nm. The results indicate that BBM medium exhibited the highest absorbance value of 1.45, suggesting that it provided the most favourable conditions for *C. vulgaris* growth. The BG11 medium showed an absorbance of 1.12, indicating moderately favourable conditions. In contrast, the Bristol medium recorded a significantly lower absorbance of 0.31, while the Chu medium demonstrated the lowest absorbance of 0.21, suggesting that these media were less optimal for supporting C. vulgaris growth. A second analysis was conducted to compare *C. vulgaris* absorbance

in BBM medium of varying strengths. The results showed that 1× BBM yielded the highest absorbance (1.45), confirming its suitability for optimal growth. In contrast, 0.5× BBM resulted in a reduced absorbance of 0.31, while increasing the BBM strength beyond 1× did not enhance growth. The absorbance values for 2× BBM and 3× BBM were 0.18 and 0.19, respectively, indicating that higher nutrient concentrations did not further support cell proliferation.

Previous studies have reported that BBM provides optimal growth conditions for *C. vulgaris,* particularly at a pH of approximately 6, which facilitates nutrient uptake and metabolic activity (Adochite & Andronic, 2020; Adam et al., 2022). The high absorbance values observed in BBM medium correlate with increased cell density, attributed to its balanced nutrient composition. Similarly, BG11 medium is known for its high nitrogen content, which has been associated with enhanced biomass accumulation and chlorophyll content when *C. vulgaris* is cultivated under optimal light conditions (Amouri, 2023). Conversely, although Bristol medium is capable of supporting moderate growth rates, its overall effectiveness remains lower than that of BBM and BG11. While specific absorbance values may vary, Bristol medium typically does not achieve the same cell proliferation levels as BBM or BG11. Similarly, Chu medium, which is primarily formulated for freshwater algae, has demonstrated variable growth outcomes. Growth performance in Chu medium can be inconsistent due to nutrient concentration variations and environmental factors. The lower absorbance values recorded in this study align with previous findings, indicating that while Chu medium can sustain growth, it is less effective for maximising biomass production compared to BBM and BG11.

Next experiment, *C. vulgaris* was measured by absorbance at 750nm over a 14-day incubation period, in two separate studies: one with different PEG concentrations and the other with varying salicylic acid concentrations.

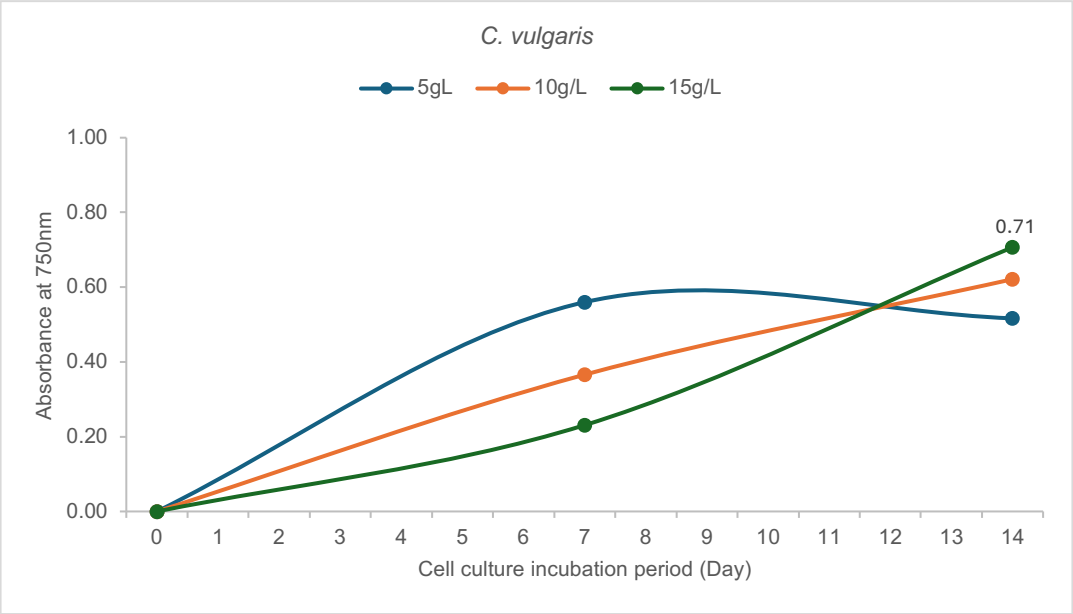


Figure 4.3 Distribution of cell culture growth of *C. vulgaris* in different PEG concentrations

Table 4.3 The highest absorbance value of *C. vulgaris* cell culture growth in different PEG concentrations

Green Algae	**PEG Conc. (g/L)**	**Peak (Day)**	**Abs**
C. vulgaris	5	D14	0.71
	10	D14	0.62
	15	D7	0.56

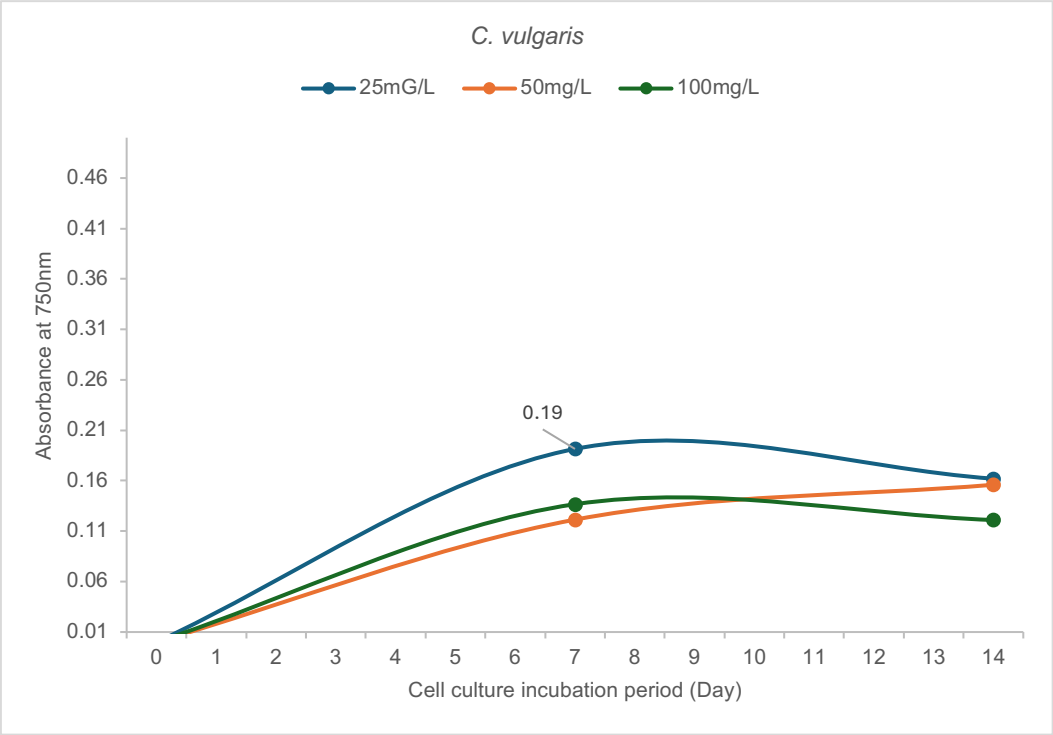


Figure 4.4 Distribution of cell culture growth of *C. vulgaris* in different SA concentrations.

Table 4.4 The highest absorbance value of *C. vulgaris* cell culture growth in different SA concentrations

Green Algae	**SA Conc. (mg/L)**	**Peak (Day)**	**Abs**
C. vulgaris	25	D7	0.19
	50	D14	0.16
	100	D7	0.14

The highest absorbance values for *C. vulgaris* cultured under different polyethylene glycol (PEG) concentrations. The 5 g/L PEG concentration achieved the highest absorbance of 0.71 on day 14, indicating the most favourable conditions for *C. vulgaris* growth. The 10 g/L PEG concentration recorded a slightly lower absorbance of 0.62, also peaking on day 14, while the 15 g/L concentration exhibited a maximum absorbance of 0.56 on day 7. Similarly, Table 4.4 summarises the highest absorbance values for *C. vulgaris* at varying salicylic acid (SA) concentrations. The 25 mg/L SA

concentration recorded the highest absorbance value of 0.19, peaking on day 7. In comparison, the 50 mg/L and 100 mg/L concentrations demonstrated lower absorbance values of 0.16 and 0.14, peaking on day 14 and day 7, respectively. This analysis suggests that the 5 g/L PEG concentration and the 25 mg/L salicylic acid concentration provide the most optimal conditions for C. vulgaris growth, as indicated by the absorbance values at 750 nm.

Salicylic acid has been shown to positively influence *C. vulgaris* growth. Research indicates that the application of salicylic acid can significantly enhance biomass production. For instance, Zhang et al. (2022) demonstrated that the addition of 10 mg/L salicylic acid resulted in increased biomass in *C. vulgaris* strains, highlighting its role in promoting algal growth under stress conditions. Naaz et al. (2021) further corroborated these findings, noting that salicylic acid enhances the growth of C. vulgaris by acting as a signalling molecule that regulates various metabolic processes essential for algal development. This aligns with the broader understanding that salicylic acid plays a crucial role in cellular stress responses, facilitating metabolic adaptations that contribute to improved algal growth. On the other hand, PEG, a synthetic polymer, is widely utilised as an osmotic agent in plant studies. However, its effects on *C. vulgaris* have been less extensively documented compared to salicylic acid. Studies have indicated that PEG can influence algal growth by inducing osmotic stress, which may trigger adaptive responses in algal cells (Wu et al., 2018). The interaction between PEG and algal cells can lead to alterations in metabolic pathways, potentially affecting growth rates and biomass accumulation. For example, Wijanarko et al. (2010) discussed how osmotic stress induced by PEG can influence CO_2 fixation and overall metabolic activity in C. vulgaris. While PEG may not directly enhance growth, it can stimulate metabolic adaptations that could be beneficial under certain conditions, particularly in environments with fluctuating osmotic pressures.

4.2.2 Effect of pH, and medium formulation and photoperiod on *C. vulgaris* Cell Culture Growth, biomass, Chromaticity, and chlorophyll Content

This section revealed the behaviour of green algae *C. vulgaris* cell culture growth in four different mediums of BBM, BG11, Chu-10, and Bristol under different pH (3.0, 5.0, 7.0, 9.0 and 11.0) and photoperiod (24:0, 16:8, 12:12 and 8:16). The parameter will include:

1. Absorbance (750nm) of *C. vulgaris.*
2. Effects of pH and photoperiod on UV-vis spectra absorbance values for chlorophyll content.
3. The lightness (L*) of *C. vulgaris* cell culture growth.
4. The CIE L*a*b* colour difference or Chromaticity (ŒîE*Lab) of *C. vulgaris* cell culture growth.
5. Assessment of *C. vulgaris* Biomass as Carbon Sequestration Agent.

Effect of pH, BBM medium formulation and photoperiod on C. vulgaris Cell Culture Growth, biomass, Chromaticity, and Chlorophyll content

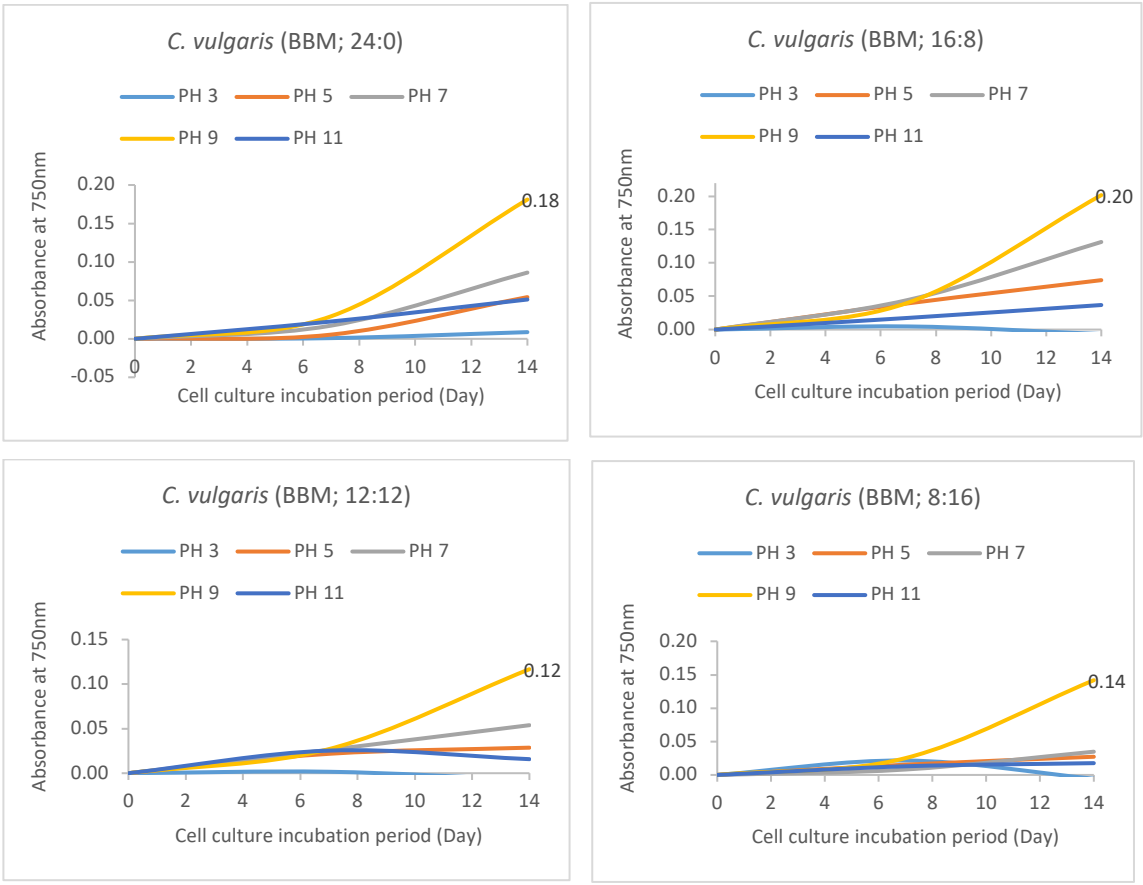


Figure 4.5 Effect of pH on cell culture growth of *C. vulgaris* in BBM medium.

Table 4.5 The optimum cell density and day of growth of *C. vulgaris* in BBM medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**Abs**
C. vulgaris	24:0	pH 9 (Day 14)	0.18
	16:8	pH 9 (Day 14)	0.20
	12:12	pH 9 (Day 14)	0.12
	8:16	pH 9 (Day 14)	0.14

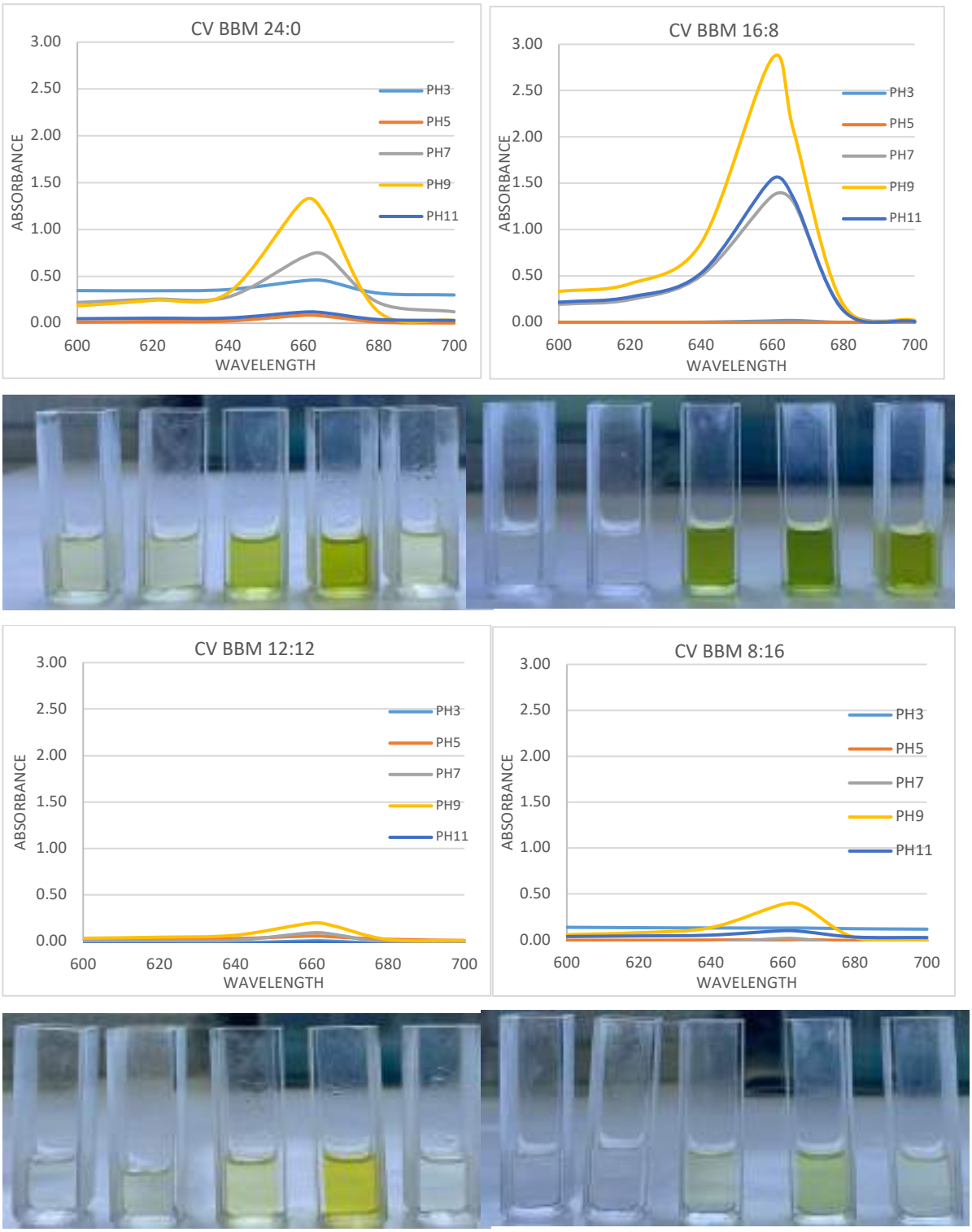


Figure 4.6 Effects of pH and photoperiod on chlorophyll content

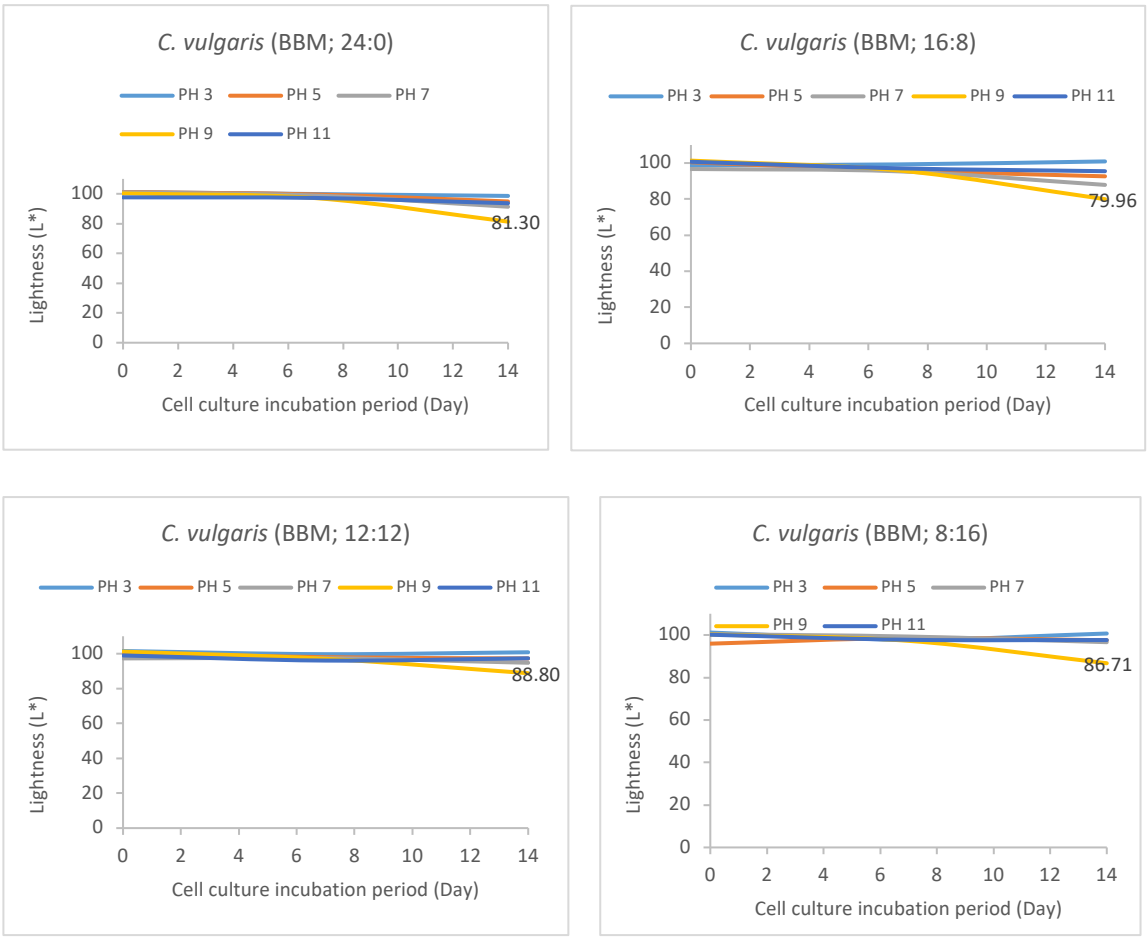


Figure 4.7 Effect of pH on lightness of *C. vulgaris* in BBM medium.

Table 4.6 The lightness (L*) of *C. vulgaris* cell culture growth in BBM medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**L***
C. vulgaris	24:0	pH 9 (Day 14)	81.30
	16:8	pH 9 (Day 14)	79.96
	12:12	pH 9 (Day 14)	88.80
	8:16	pH 9 (Day 14)	86.71

L, ranges from black (0) to white (100)*

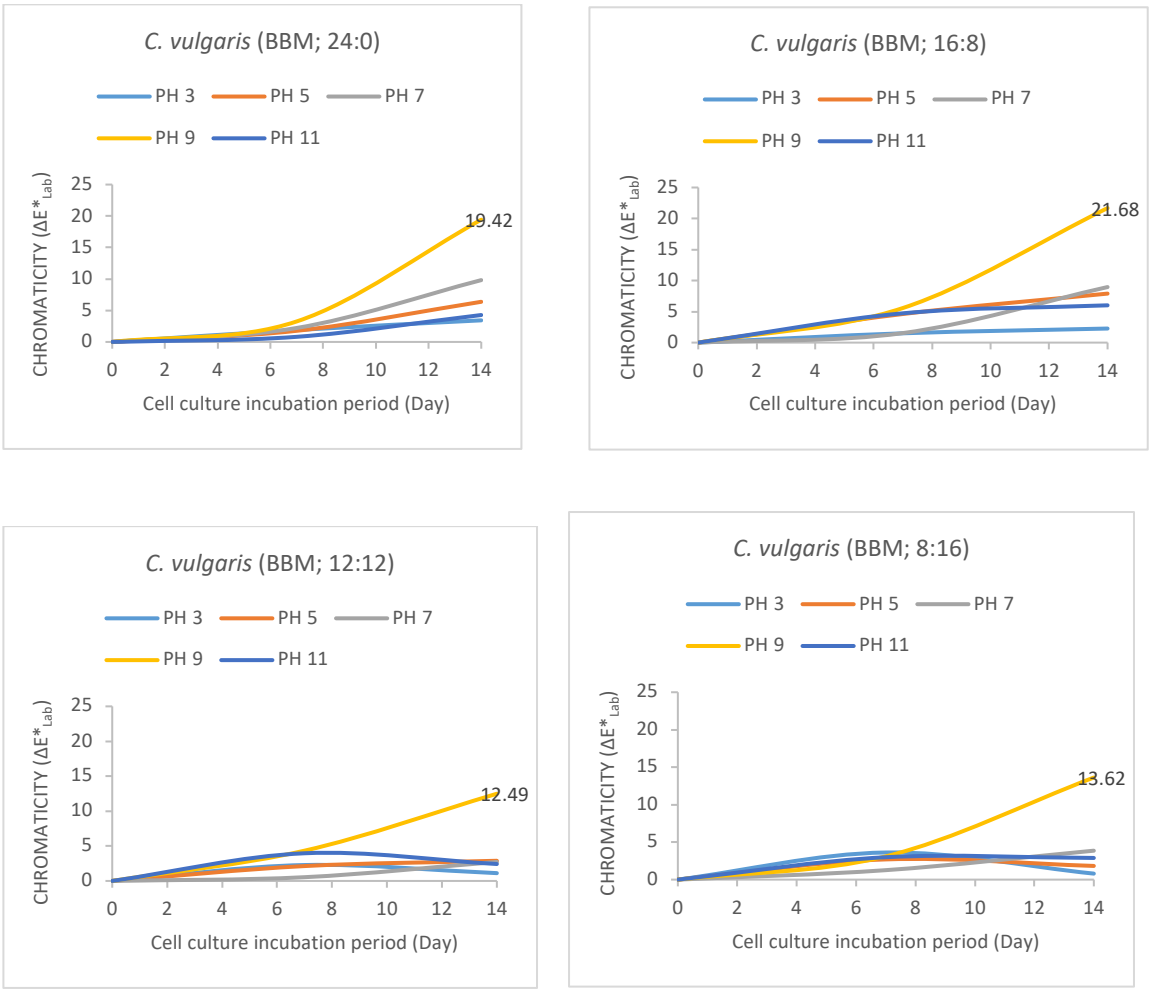
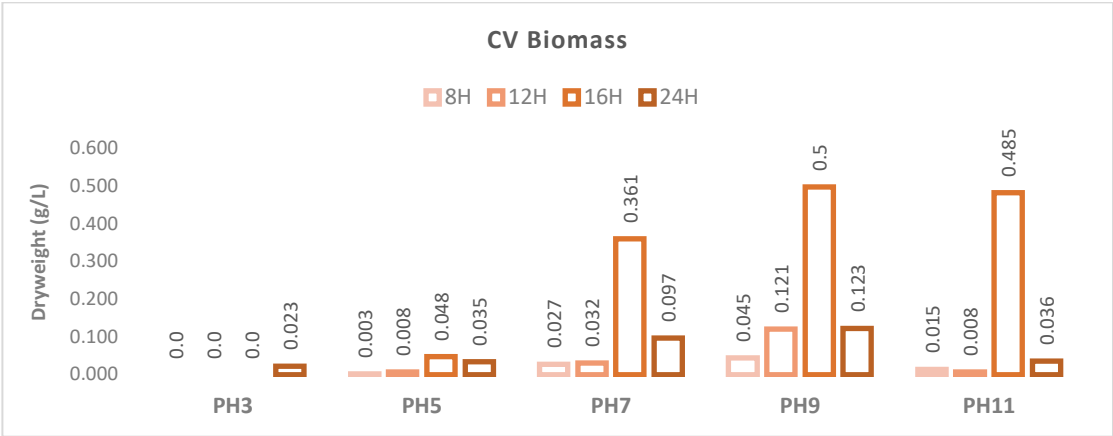


Figure 4.8 Effect of pH on cell chromaticity of *C. vulgaris* in BBM medium.

Table 4.7 The CIE L*a*b* colour difference or Chromaticity (ΔE^*_{Lab}) of *C. vulgaris* cell culture growth in BBM medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**ΔE^*_{Lab}**
C. vulgaris	24:0	pH 9 (Day 14)	19.42
	16:8	pH 9 (Day 14)	21.68
	12:12	pH 9 (Day 14)	12.49
	8:16	pH 9 (Day 14)	13.62

ΔE^*_{Lab} > 5.0 - indicate clearly two different colours



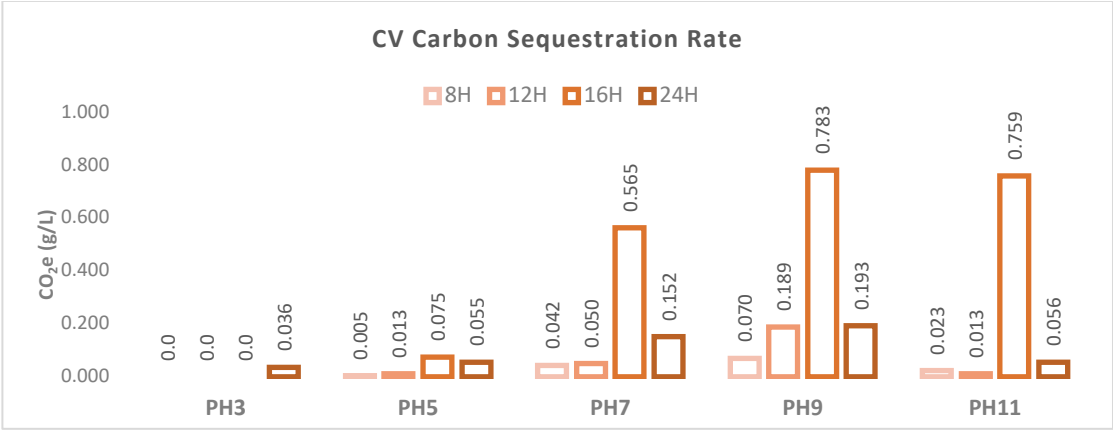


Figure 4.9 The biomass and carbon sequestration rate of *C. vulgaris*

Table 4.8 The biomass and carbon sequestration rate of *C. vulgaris* cell culture growth in BBM medium at different pH and photoperiod.

Green Algae	**pH**	**Biomass (g/L)**	**CSR (CO2e)**
C. vulgaris	pH 3 (24H)	0.023	0.036
	pH 5 (16H)	0.048	0.075
	pH 7 (16H)	0.361	0.565
	pH 9 (16H)	0.5	0.783
	pH 11 (16H)	0.485	0.759

Firstly, the highest absorbance was consistently recorded at pH 9, peaking on day 14. Among the photoperiod conditions, the 16:8 photoperiod exhibited the highest absorbance (0.20), followed by 24:0 (0.18), 8:16 (0.14), and 12:12 (0.12). These findings suggest that continuous light exposure (24:0) or an extended light period (16:8) is more favourable for *C. vulgaris* growth compared to shorter photoperiods (12:12 and 8:16).

Secondly, UV-Vis spectral analysis revealed that pH 9 consistently exhibited the highest absorbance peaks (660–670 nm), corresponding to chlorophyll content. In contrast, lower pH levels (3 and 5) demonstrated significantly reduced absorbance, indicating lower chlorophyll production. Visual observations further corroborated these results, as cultures grown at pH 9 appeared more intensely green, supporting the conclusion that chlorophyll is enhanced under this pH condition.

Thirdly, chromaticity measurements (ΔE*Lab) indicated that pH 9 resulted in the greatest colour differences across all photoperiods. The 16:8 photoperiod exhibited the largest colour difference (ΔE*Lab = 21.68), followed by 24:0 (19.42). In contrast, shorter photoperiods (12:12 and 8:16) produced lower colour differences (12.49 and 13.62, respectively).

Furthermore, biomass production and carbon sequestration were highest at pH 9 with a 16-hour photoperiod, followed closely by pH 11 under the same conditions. In contrast, lower pH levels (3 and 5) resulted in minimal biomass accumulation and carbon sequestration, indicating that acidic conditions are unfavourable for *C. vulgaris* growth and carbon capture. These findings highlight that alkaline conditions (pH 9) combined with an extended photoperiod (16:8) are optimal for biomass production and carbon sequestration.

C. vulgaris growth and chlorophyll production are maximised at pH 9 under a photoperiod of 16:8 or 24:0. Extended or continuous light exposure significantly enhances cell density and pigment production, making these the most optimal cultivation conditions in BBM medium. Conversely, shorter photoperiods (12:12 and 8:16) result in reduced growth and chlorophyll synthesis, as demonstrated by lower absorbance and chromaticity values.

Effect of pH, BG11 medium formulation and photoperiod on C. vulgaris Cell Culture Growth, biomass, Chromaticity, and Chlorophyll content

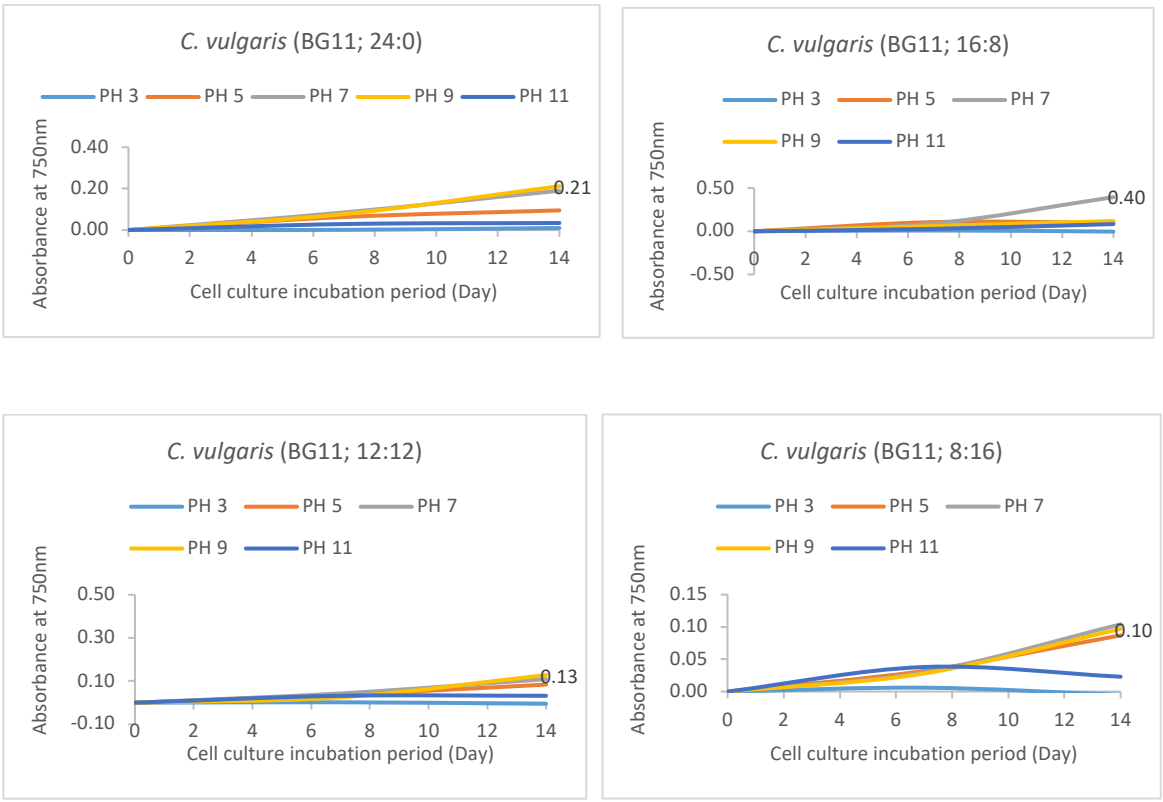


Figure 4.10 Effect of pH on cell culture growth of *C. vulgaris* in BG11 medium.

Table 4.9 The optimum cell density and day of growth of *C. vulgaris* in BG11 medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**Abs**
C. vulgaris	24:0	pH 9 (Day 14)	0.21
	16:8	pH 7 (Day 14)	0.40
	12:12	pH 9 (Day 14)	0.13
	8:16	pH 7 (Day 14)	0.10

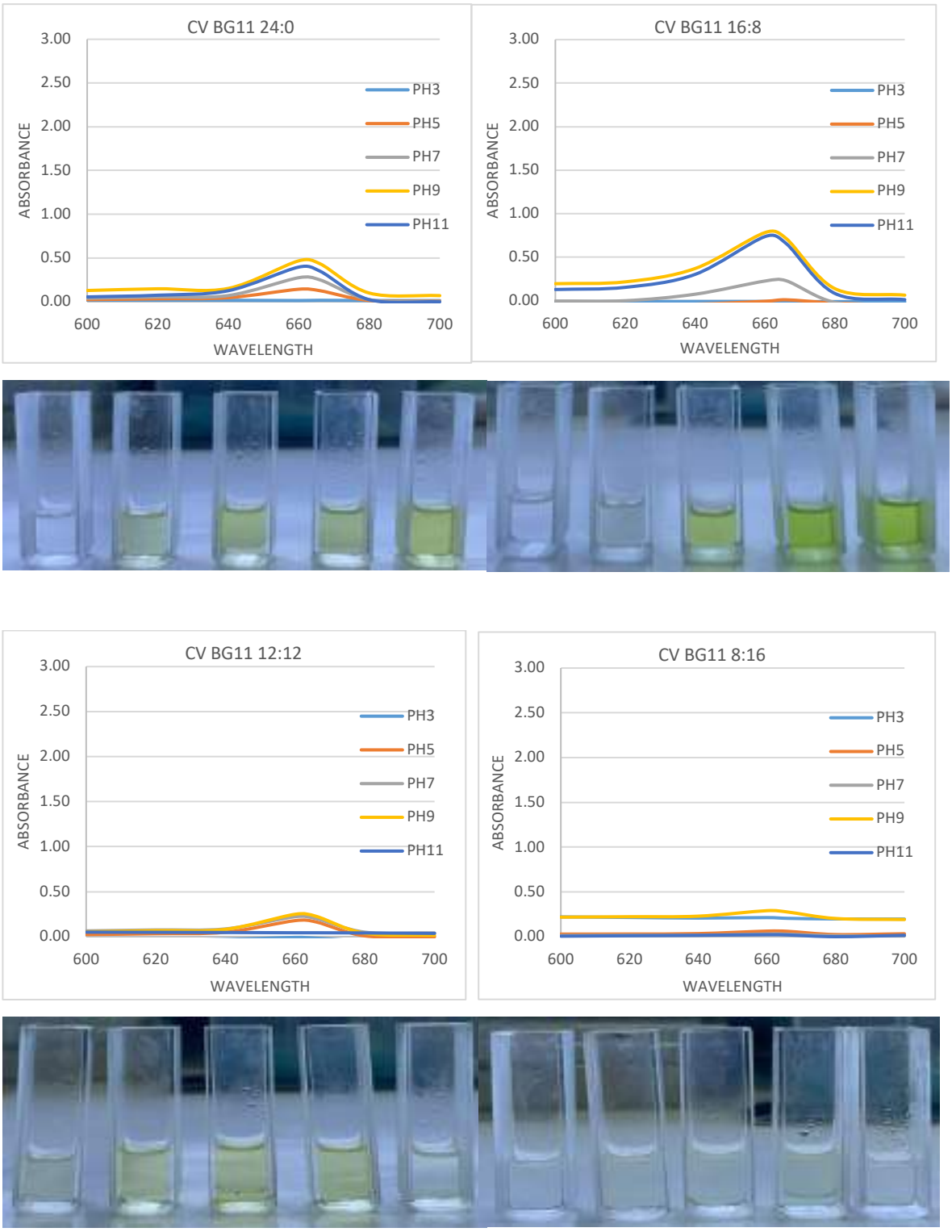


Figure 4.11: Effects of pH and photoperiod on UV-vis on chlorophyll content

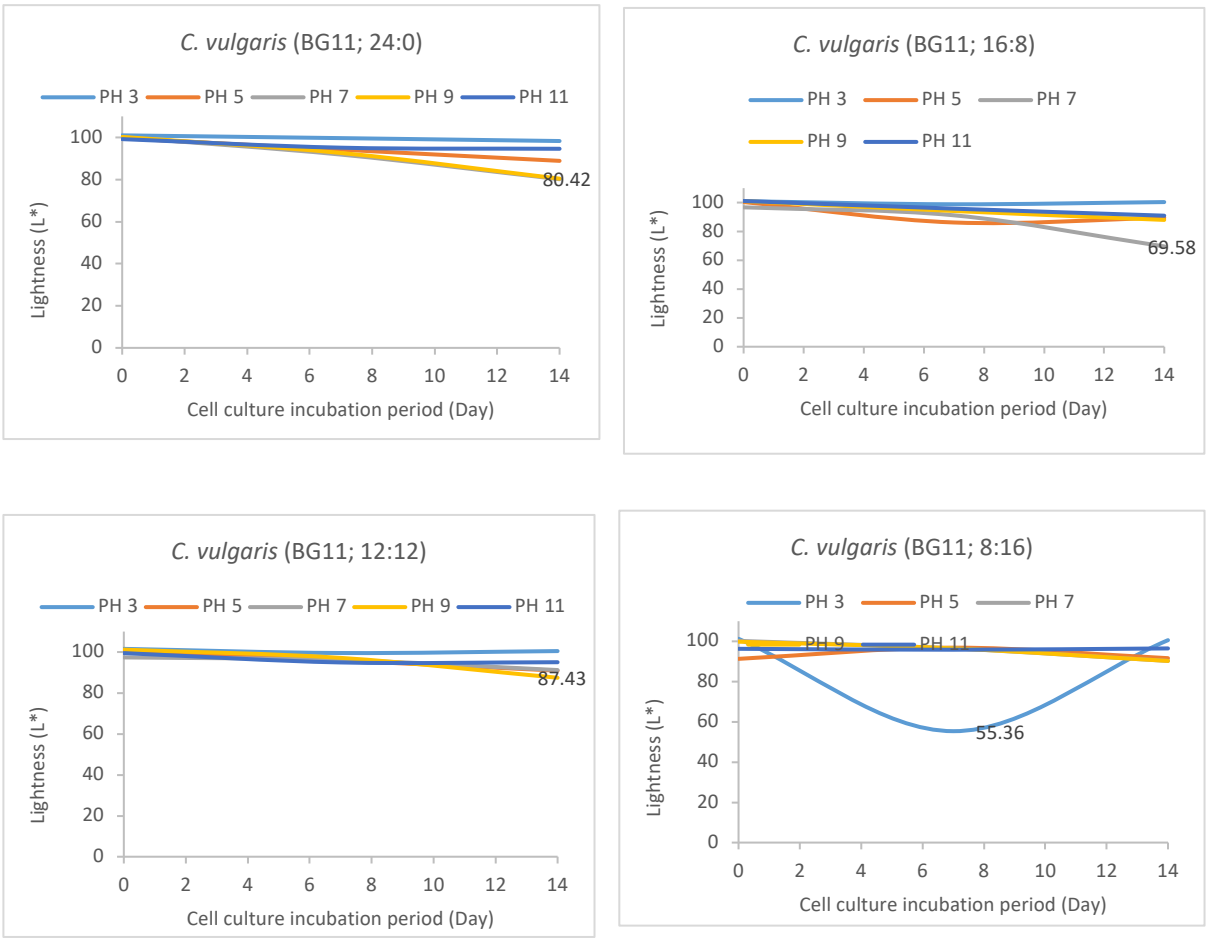


Figure 4.12 Effect of pH on lightness of *C. vulgaris* in BG11 medium.

Table 4.10 The lightness (L*) of *C. vulgaris* cell culture growth in BG11 medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**L***
C. vulgaris	24:0	pH 9 (Day 14)	80.42
	16:8	pH 7 (Day 14)	69.58
	12:12	pH 9 (Day 14)	87.43
	8:16	pH 3 (Day 7)	55.36

L, ranges from black (0) to white (100)*

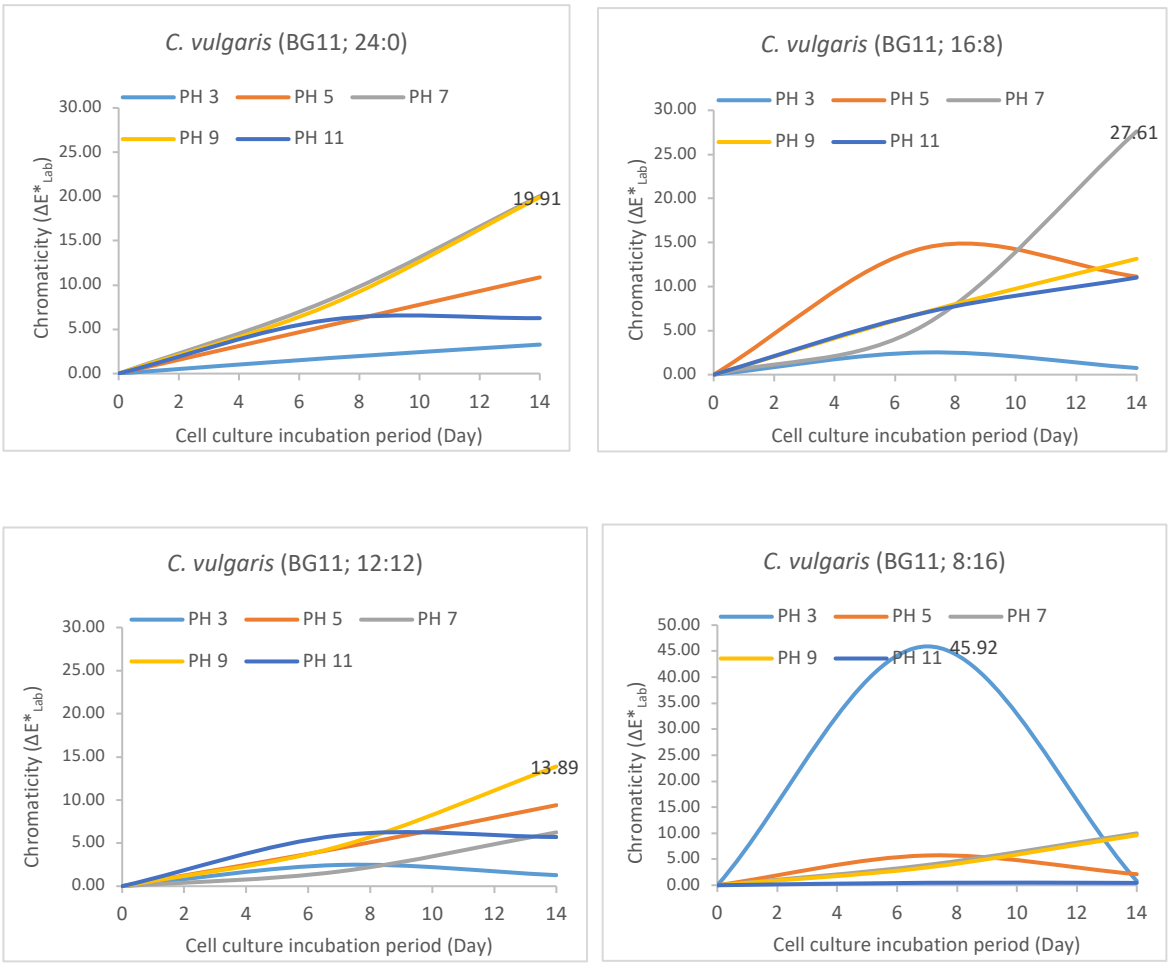


Figure 4.13 Effect of pH on cell chromaticity of *C. vulgaris* in BG11 medium.

Table 4.11 The CIE L*a*b* colour difference or Chromaticity (ΔE^*_{Lab}) of *C. vulgaris* cell culture growth in BG11 medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**ΔE^*_{Lab}**
C. vulgaris	24:0	pH 7 (Day 14)	19.91
	16:8	pH 7 (Day 14)	27.61
	12:12	pH 9 (Day 14)	13.89
	8:16	pH 3 (Day 7)	45.92

ΔE^*_{Lab} > 5.0 - indicate clearly two different colours

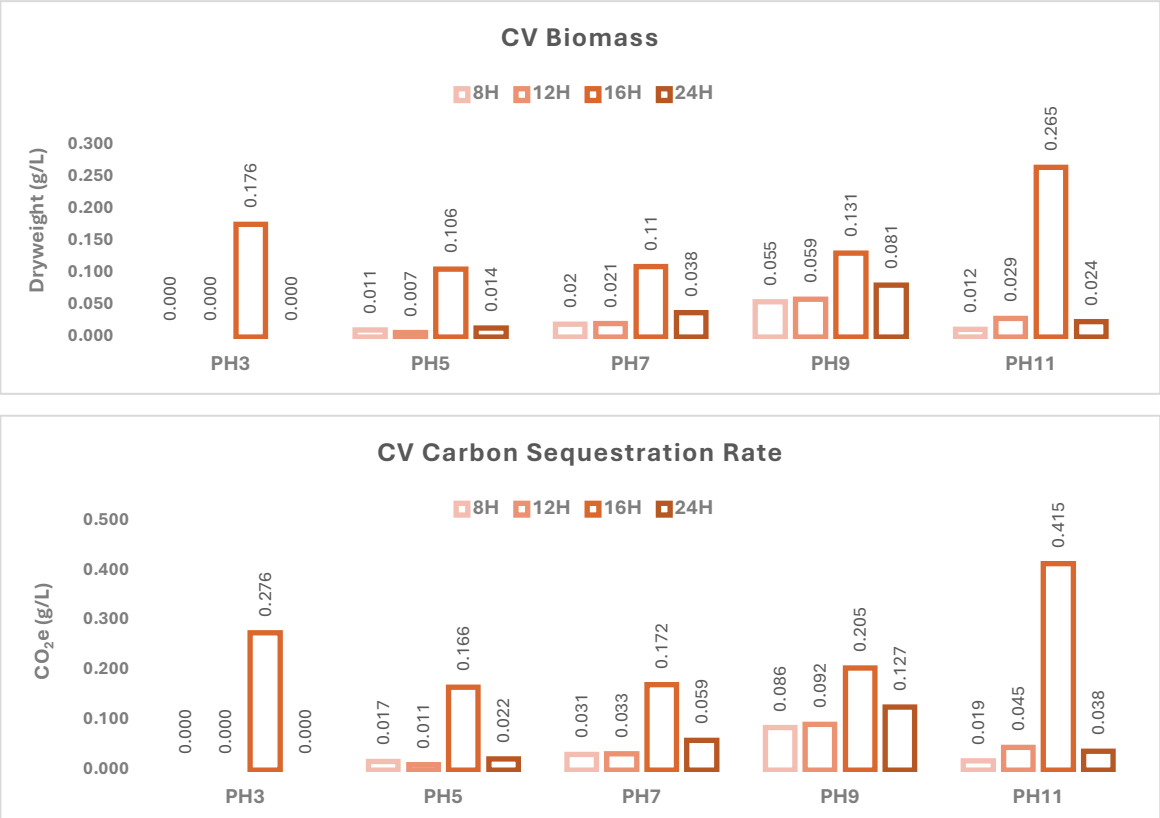


Figure 4.14 The biomass and carbon sequestration rate of *C. vulgaris*

Table 4.12 The biomass and carbon sequestration rate of *C. vulgaris* cell culture growth in BG11 medium at different pH and photoperiod.

Green Algae	**pH**	**Biomass (g/L)**	**CSR (CO2e)**
C. vulgaris	pH 3 (16H)	0.176	0.276
	pH 5 (16H)	0.106	0.166
	pH 7 (16H)	0.110	0.172
	pH 9 (16H)	0.131	0.205
	pH 11 (16H)	0.265	0.415

The highest absorbance (0.40), indicating optimal growth, was observed under a 16:8 photoperiod at pH 7. Moderate absorbance (0.21) was recorded for the 24:0 photoperiod at pH 9, while shorter photoperiods (12:12 and 8:16) resulted in lower absorbance values (0.13 and 0.10, respectively). Chlorophyll production was highest under the 16:8 photoperiod at both pH 7 and pH 9, indicating these conditions are most favorable for chlorophyll synthesis. In contrast, lower pH levels (pH 3 and 5) led to significantly reduced chlorophyll absorbance, reflecting poor growth under acidic conditions.

The 12:12 photoperiod at pH 9 produced the highest lightness value (87.43), indicating a lighter culture, while the 8:16 photoperiod at pH 3 resulted in the lowest lightness (55.36), suggesting darker pigmentation. Lightness variations are closely linked to chlorophyll content and pigment production. The greatest color difference (ΔELab = 45.92) was observed with the 8:16 photoperiod at pH 3, followed by the 16:8 photoperiod at pH 7 (ΔELab = 27.61), indicating significant pigment changes under these conditions.

Biomass and carbon sequestration rate (CSR) were maximized at pH 11 under a 16-hour photoperiod, with the highest biomass (0.265 g/L) and CSR (0.415 g/L CO2e), confirming this as the most effective condition for both growth and carbon capture. While pH 3 had relatively high CSR, its biomass production was much lower, indicating less efficient overall growth. The 16-hour photoperiod was consistently optimal for both biomass accumulation and carbon sequestration across various pH levels.

In conclusion, a 16:8 photoperiod at pH 7 or pH 9 is ideal for *C. vulgaris* growth in BG11 medium, yielding the highest cell density and chlorophyll content. Shorter photoperiods and extreme pH levels (e.g., pH 3) lead to reduced growth and pigment synthesis.

Effect of pH, Bristol medium formulation and photoperiod on C. vulgaris Cell Culture Growth, biomass, Chromaticity, and Chlorophyll content

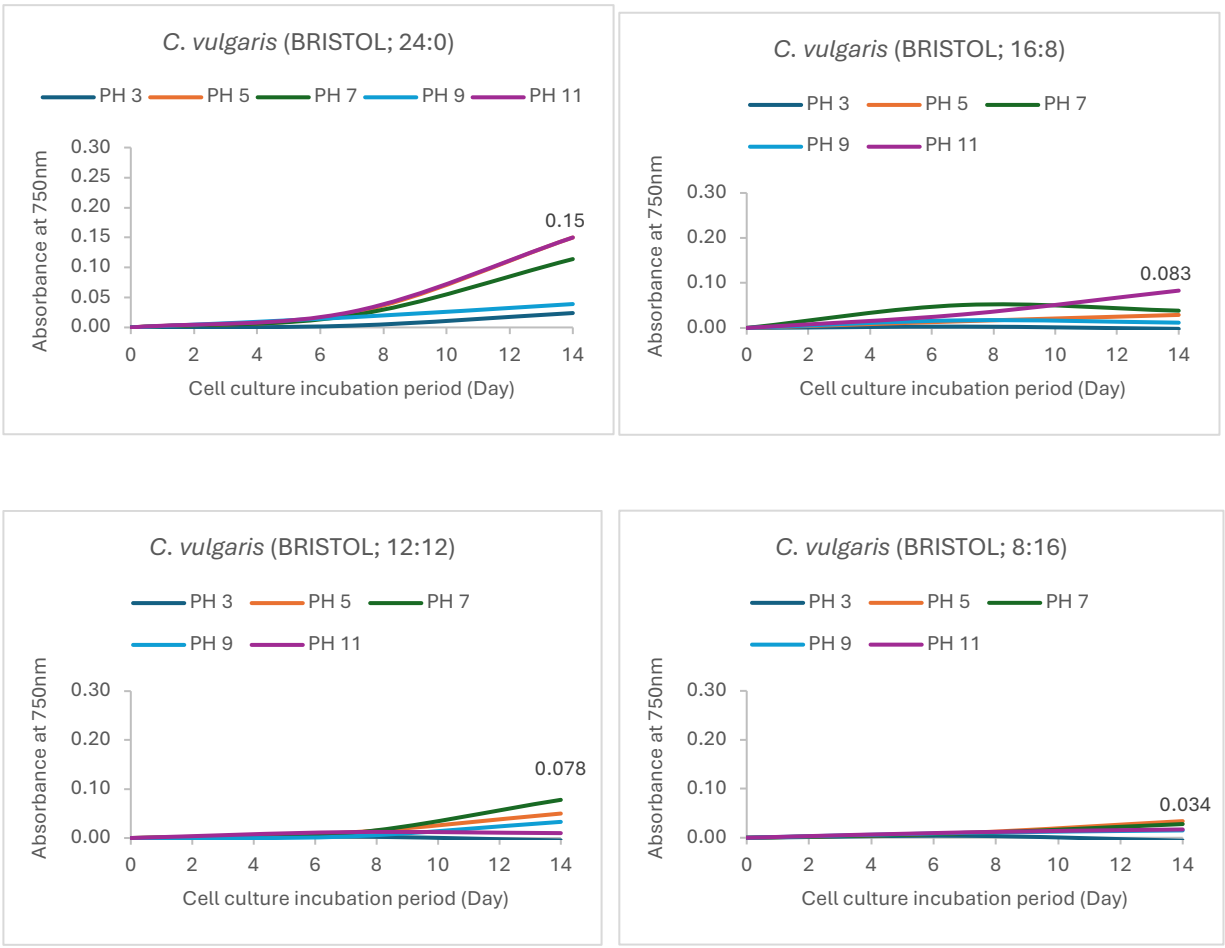


Figure 4.15 Effect of pH on cell culture growth of *C. vulgaris* in Bristol medium.

Table 4.13 The optimum cell density and day of growth of *C. vulgaris* in Bristol medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**Abs**
C. vulgaris	24:0	pH 11 (Day 14)	0.15
	16:8	pH 11 (Day 14)	0.08
	12:12	pH 7 (Day 14)	0.07
	8:16	pH 5 (Day 14)	0.03

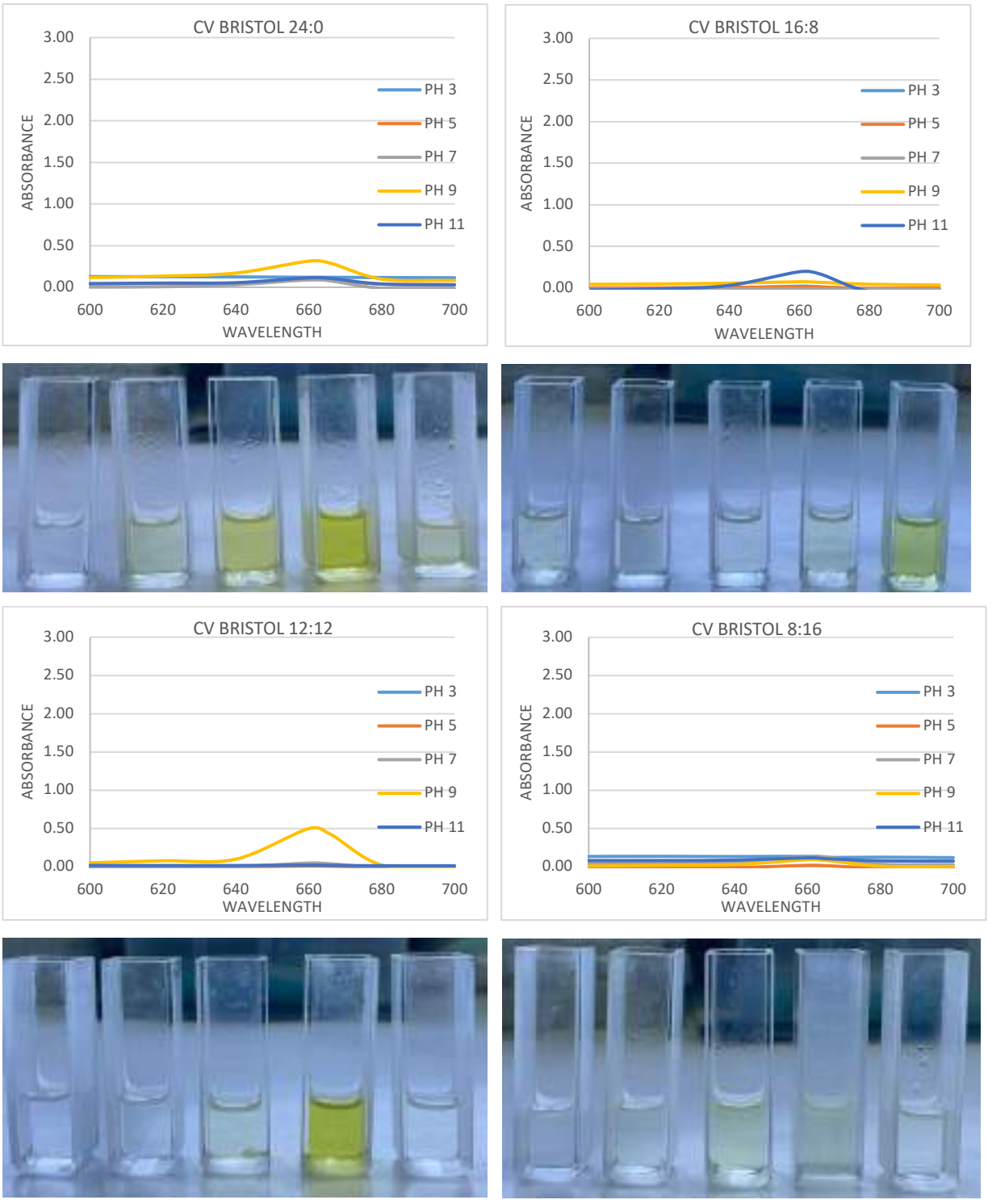


Figure 4.16 Effects of pH and photoperiod on chlorophyll content

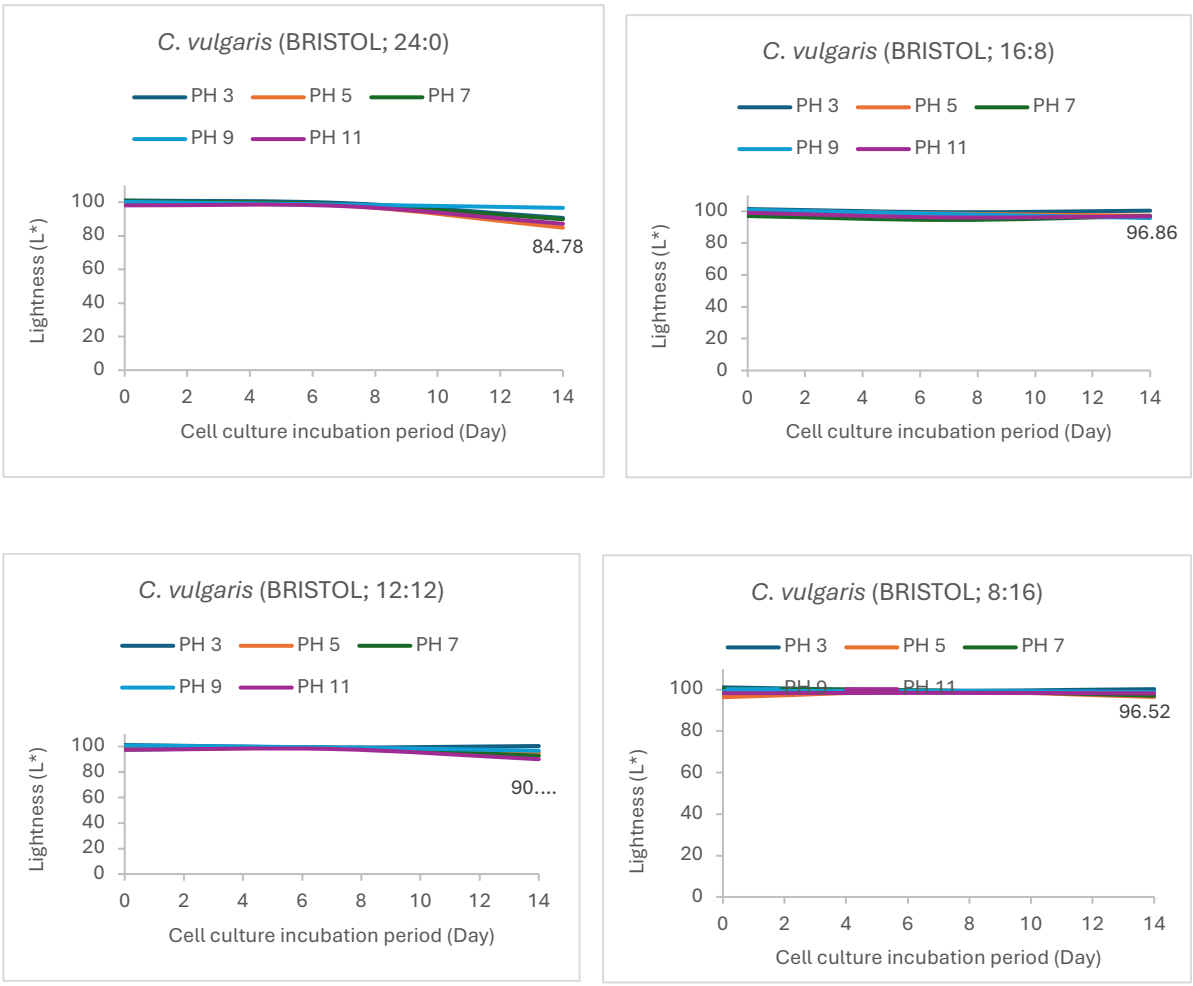


Figure 4.17 Effect of pH on lightness of *C. vulgaris* in Bristol medium.

Table 4.14 The lightness (L*) of *C. vulgaris* cell culture growth in Bristol medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**L***
C. vulgaris	24:0	pH 5 (Day 14)	84.78
	16:8	pH 11 (Day 14)	96.86
	12:12	pH 11 (Day 14)	90.09
	8:16	pH 5 (Day 14)	96.52

L, ranges from black (0) to white (100)*

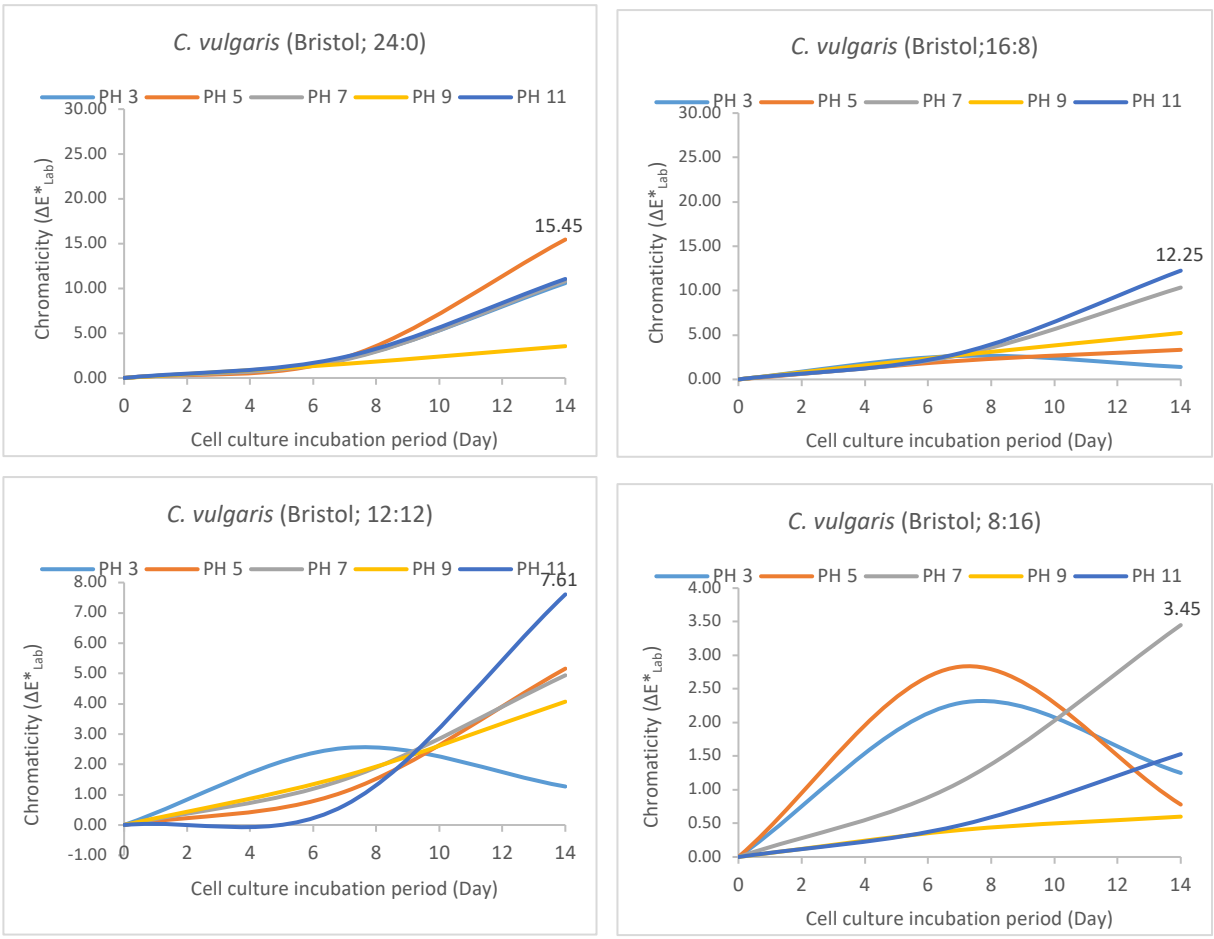


Figure 4.18 Effect of pH on l cell chromaticity of *C. vulgaris* in Bristol medium.

Table 4.15 The CIE L*a*b* colour difference or Chromaticity (ΔE^*_{Lab}) of *C. vulgaris* cell culture growth in Bristol medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**ΔE^*_{Lab}**
C. vulgaris	24:0	pH 5 (Day 14)	15.45
	16:8	pH 11 (Day 14)	12.25
	12:12	pH 11 (Day 14)	7.61
	8:16	pH 7 (Day 14)	3.45

ΔE^*_{Lab} > 5.0 - indicate clearly two different colours

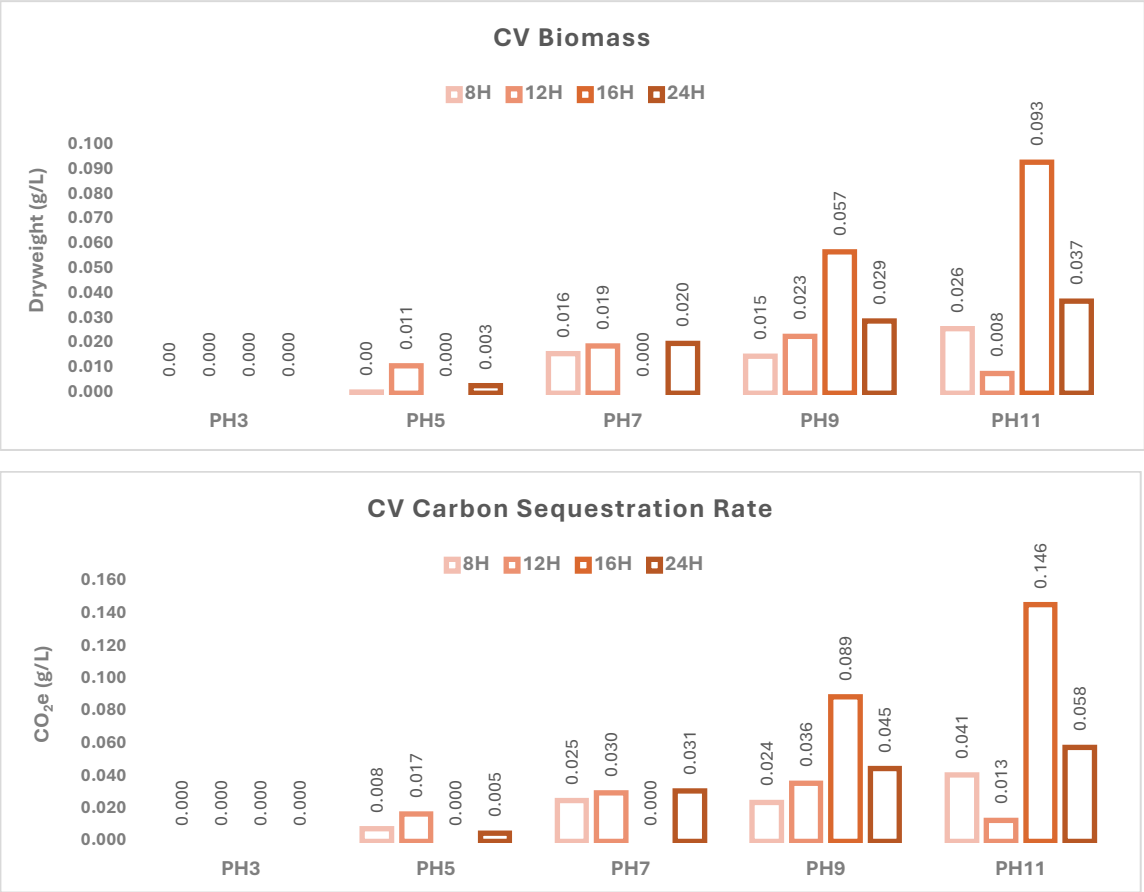


Figure 4.19 The biomass and carbon sequestration rate of *C. vulgaris*

Table 4.16 The biomass and carbon sequestration rate of *C. vulgaris* cell culture growth in Bristol medium at different pH and photoperiod.

Green Algae	**pH**	**Biomass (g/L)**	**CSR (CO2e)**
C. vulgaris	pH 3	0.000	0.000
	pH 5 (12H)	0.011	0.017
	pH 7 (24H)	0.020	0.031
	pH 9 (16H)	0.057	0.089
	pH 11 (16H)	0.093	0.146

The highest absorbance (0.15) was observed under the 24:0 photoperiod at pH 11, indicating optimal growth conditions for C. vulgaris. The 16:8 photoperiod at pH 11 exhibited moderate growth (absorbance = 0.08), while lower absorbance values were recorded for 12:12 (pH 7) and 8:16 (pH 5), measuring 0.07 and 0.03, respectively. These findings suggest that extended light exposure, particularly under alkaline conditions, promotes C. vulgaris growth.

Chlorophyll content was highest under the 12:12 photoperiod at pH 9. In contrast, lower pH levels (pH 3 and 5) and shorter photoperiods (such as 8:16) resulted in minimal chlorophyll accumulation, reflecting poor growth conditions. Lightness (L) values* were highest under the 16:8 photoperiod at pH 11 (96.86) and the 8:16 photoperiod at pH 5 (96.52), indicating lighter cultures, potentially due to lower chlorophyll content. Conversely, the 24:0 photoperiod at pH 5 exhibited a lower lightness (84.78), suggesting a darker culture and a higher pigment or chlorophyll concentration.

The 24:0 photoperiod at pH 5 also produced the greatest colour difference ($\Delta E^*Lab = 15.45$), followed by the 16:8 photoperiod at pH 11 ($\Delta E^*Lab = 12.25$). This suggests significant variations in pigment production under these conditions. Lower colour differences were observed with the 12:12 photoperiod at pH 11 and the 8:16 photoperiod at pH 7, indicating more uniform pigment distribution. Biomass production and carbon sequestration were optimised under alkaline conditions (pH 9 and pH 11) with a 16-hour photoperiod. In contrast, acidic conditions (pH 3) completely inhibited growth and carbon capture. C. vulgaris performed best in Bristol medium at pH 11 with a 16-hour photoperiod, resulting in the highest biomass and carbon sequestration rates. Acidic conditions, particularly at pH 3, did not support growth or carbon capture.

C. vulgaris exhibits optimal growth in Bristol medium under a 24:0 photoperiod at pH 11, as indicated by the highest absorbance and pigment accumulation. Conversely, shorter photoperiods and acidic conditions (e.g., pH 5 with an 8:16 photoperiod) significantly reduced growth and chlorophyll content. These findings highlight the importance of photoperiod and pH regulation in optimising *C. vulgaris* cultivation for biomass production and carbon sequestration applications.

Effect of pH, Chu medium formulation and photoperiod on C. vulgaris Cell Culture Growth, biomass, Chromaticity, and Chlorophyll content

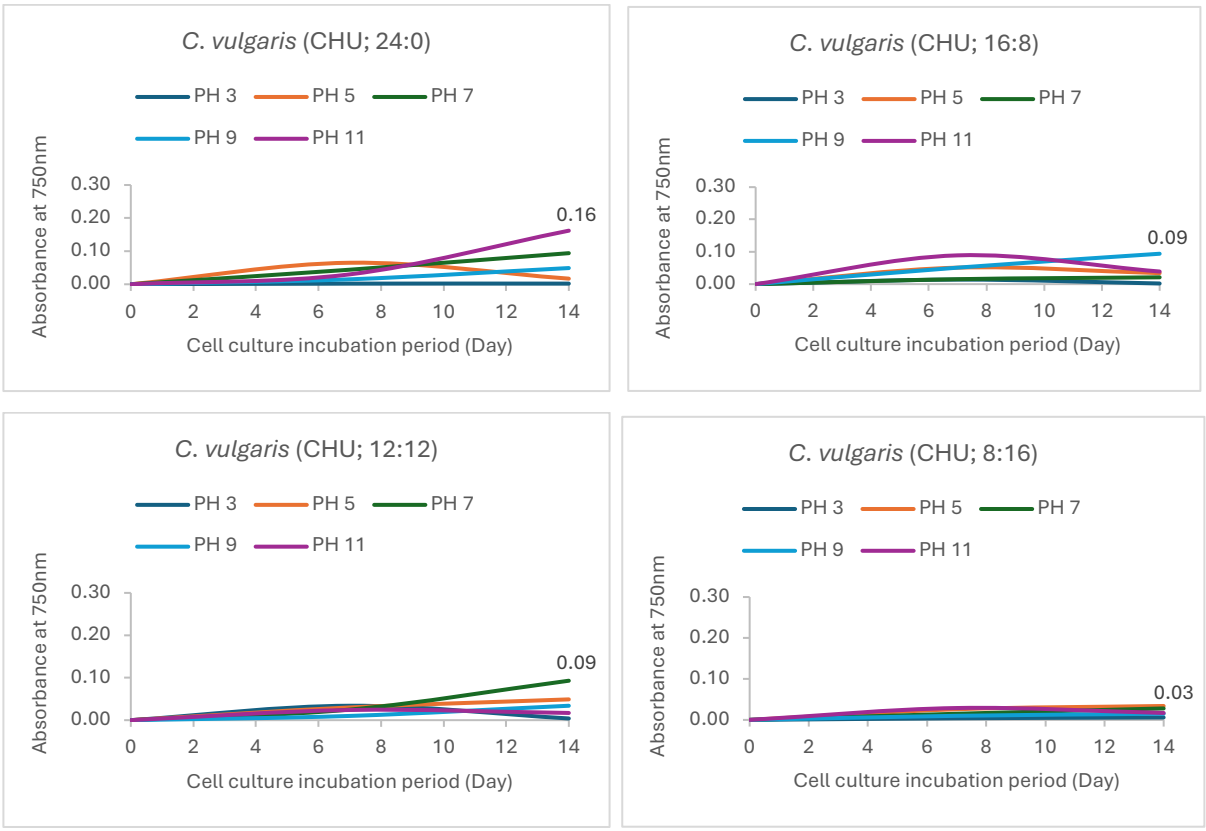


Figure 4.20 Effect of pH on cell culture growth of *C. vulgaris* in Chu medium.

Table 4.17 The optimum cell density and day of growth of *C. vulgaris* in Bristol medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**Abs**
C. vulgaris	24:0	pH 11 (Day 14)	0.16
	16:8	pH 9 (Day 14)	0.09
	12:12	pH 7 (Day 14)	0.09
	8:16	pH 5 (Day 14)	0.03

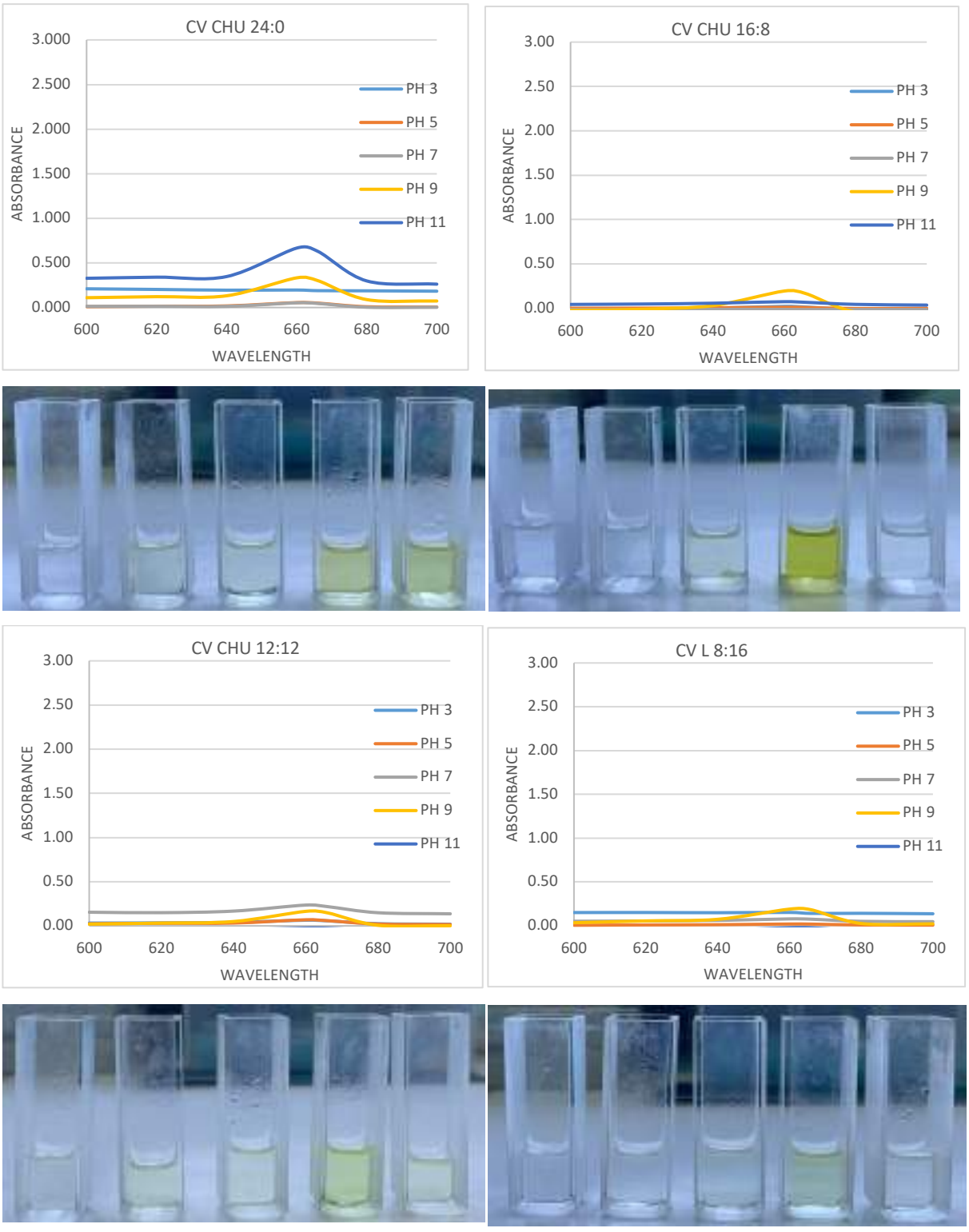


Figure 4.21 Effects of pH and photoperiod on chlorophyll content

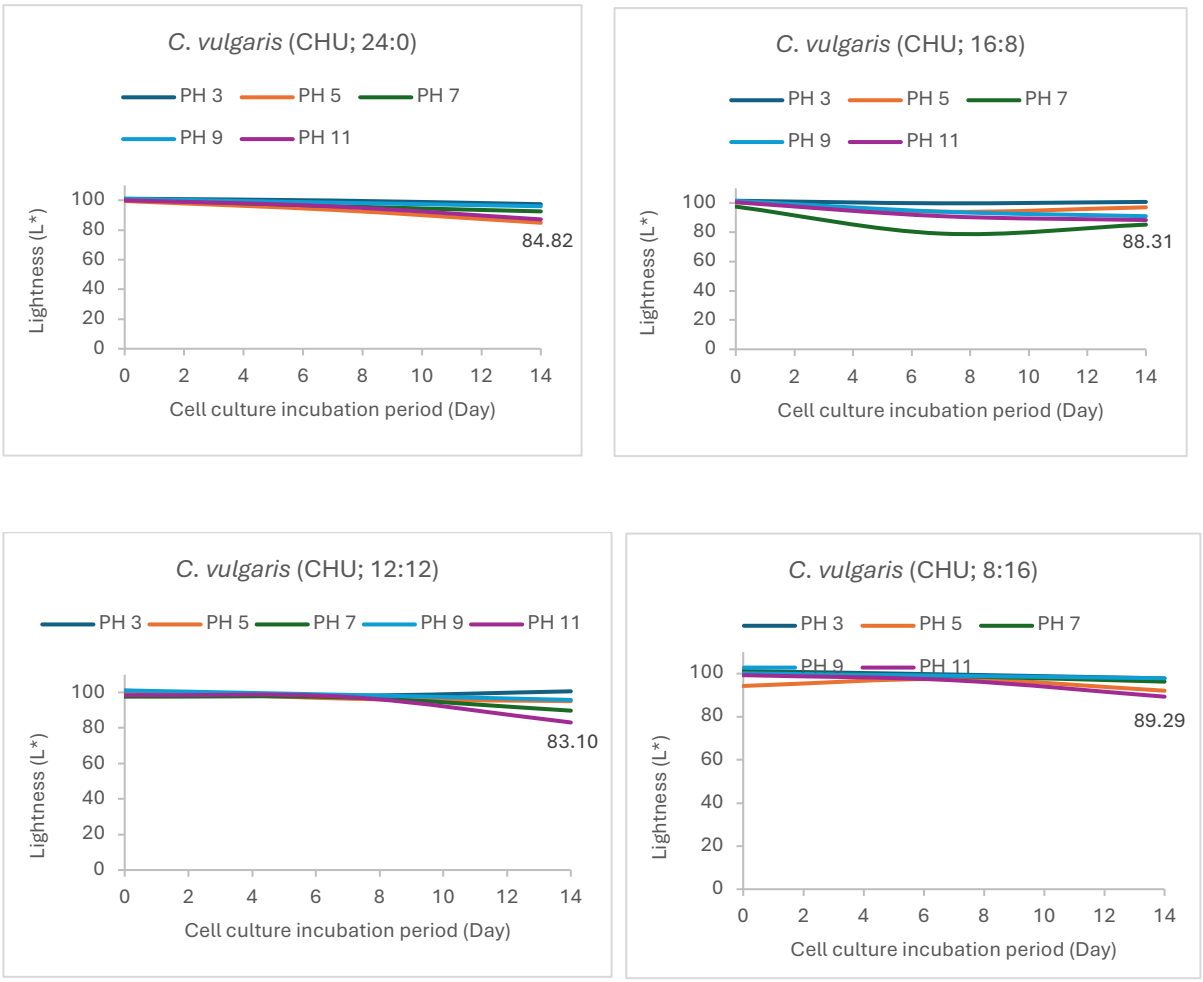


Figure 4.22 Effect of pH on lightness of *C. vulgaris* in Chu medium.

Table 4.18 The lightness (L*) of *C. vulgaris* cell culture growth in Chu medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**L***
C. vulgaris	24:0	pH 5 (Day 14)	84.82
	16:8	pH 11 (Day 14)	88.31
	12:12	pH 11 (Day 14)	83.10
	8:16	pH 11 (Day 14)	89.29

L, ranges from black (0) to white (100)*

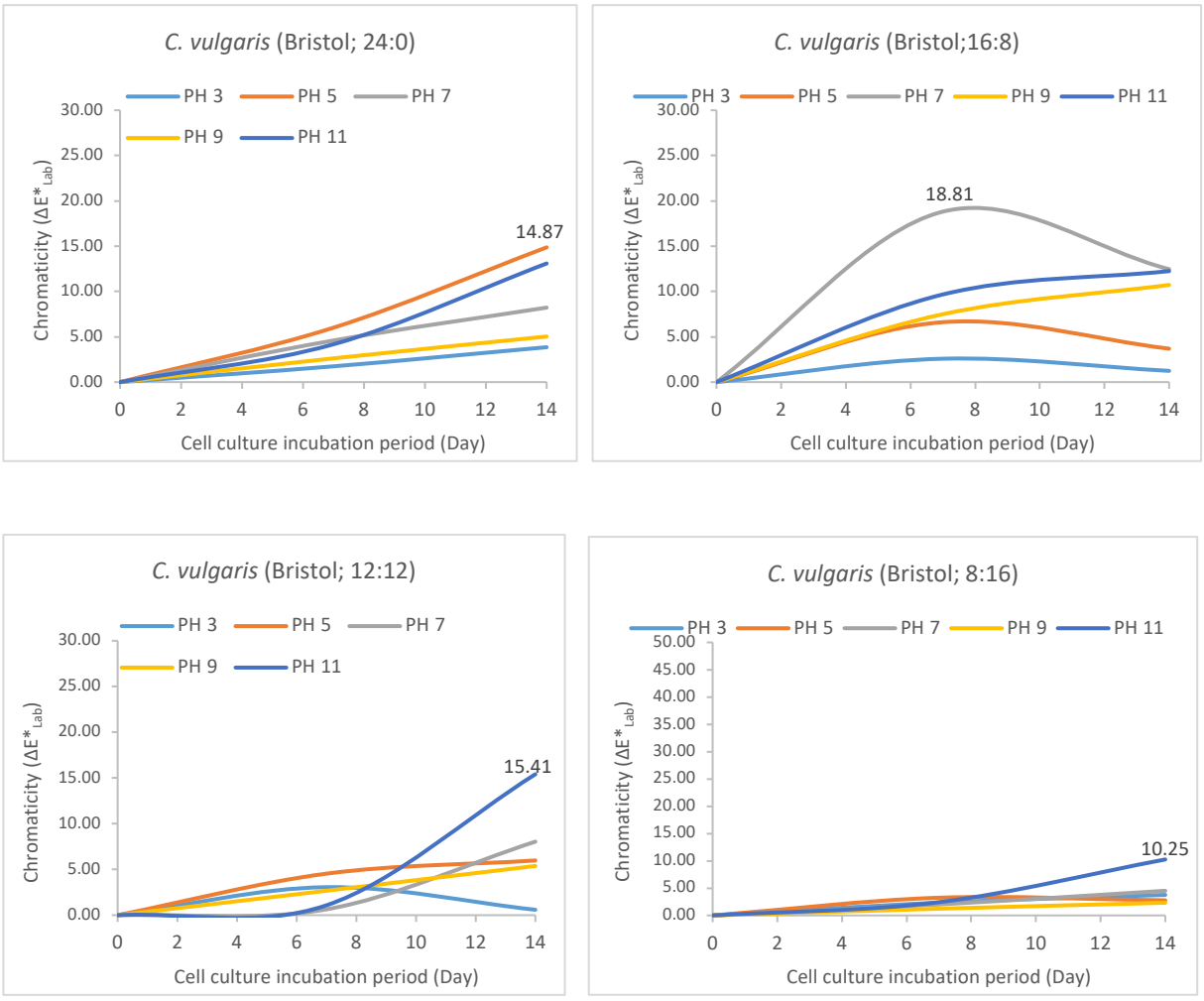


Figure 4.23 Effect of pH on l cell chromaticity of *C. vulgaris* in Chu medium

Table 4.19 The CIE L*a*b* colour difference or Chromaticity (ΔE^*_{Lab}) of *C. vulgaris* cell culture growth in Chu medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**ΔE^*_{Lab}**
C. vulgaris	24:0	pH 5 (Day 14)	14.87
	16:8	pH 7 (Day 7)	18.81
	12:12	pH 11 (Day 14)	15.41
	8:16	pH 11 (Day 14)	10.25

ΔE^*_{Lab} > 5.0 - indicate clearly two different colours

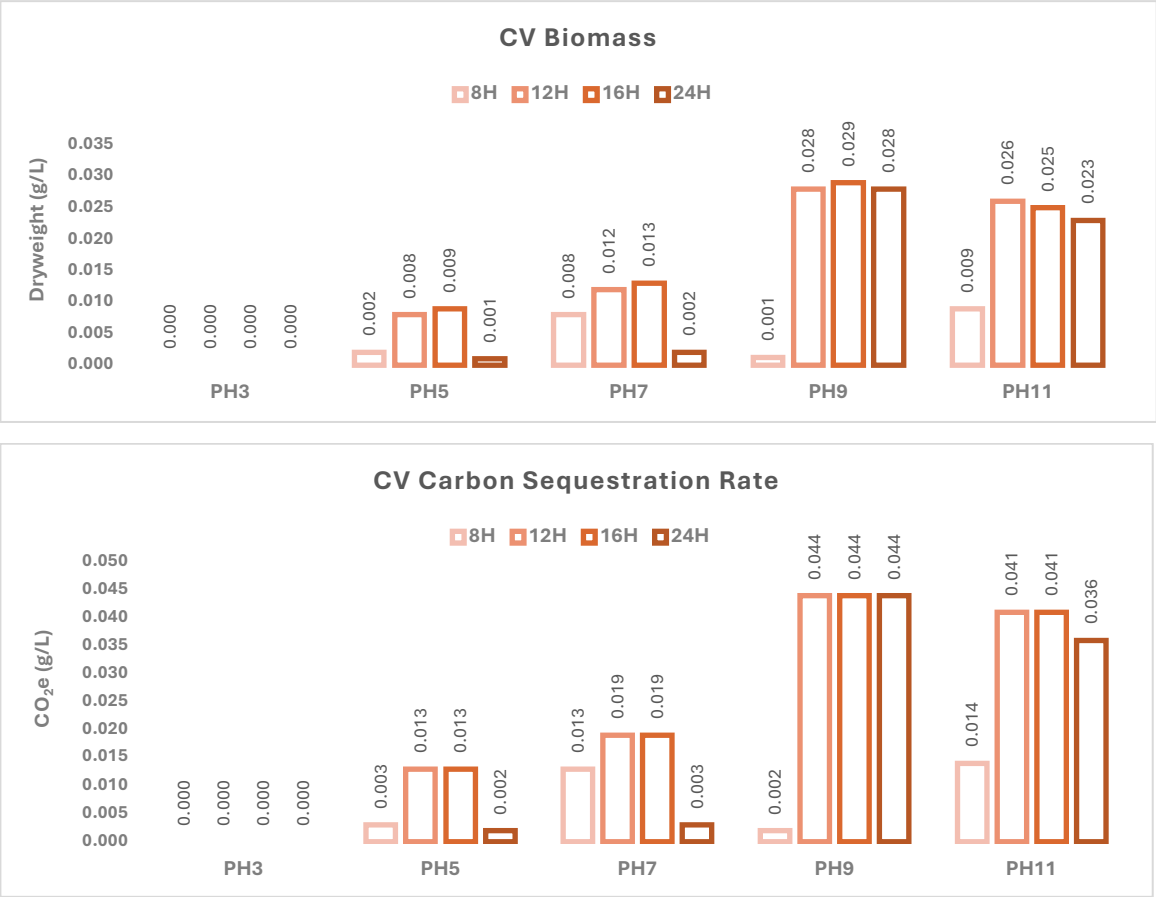


Figure 4.24 The biomass and carbon sequestration rate of *C. vulgaris*

Table 4.20 The biomass and carbon sequestration rate of *C. vulgaris* cell culture growth in Chu medium at different pH and photoperiod.

Green Algae	**pH**	**Biomass (g/L)**	**CSR (CO2e)**
C. vulgaris	pH 3	0.000	0.000
	pH 5 (16H)	0.009	0.013
	pH 7 (16H)	0.013	0.019
	pH 9 (16H)	0.029	0.044
	pH 11 (16H)	0.025	0.041

The highest absorbance (0.16) was recorded under the 24:0 photoperiod at pH 11, indicating optimal growth conditions for C. vulgaris. Moderate absorbance values (0.09) were observed under the 16:8 photoperiod at pH 9 and the 12:12 photoperiod at pH 7. The lowest absorbance (0.03) was recorded under the 8:16 photoperiod at pH 5, suggesting that acidic environments are unfavourable for growth.

Chlorophyll content was also highest under the 24:0 photoperiod at pH 11, whereas cultures grown in acidic conditions (pH 3 and pH 5) exhibited significantly lower chlorophyll production. Similarly, shorter photoperiods, such as 8:16, resulted in reduced chlorophyll content, reinforcing the importance of light exposure duration in pigment synthesis. The highest lightness (L) value (89.29)* was recorded under the 8:16 photoperiod at pH 11, indicating lighter cultures with reduced pigment accumulation. In contrast, the 24:0 photoperiod at pH 5 exhibited a lower lightness value (84.82), suggesting darker cultures with potentially higher pigment or chlorophyll concentrations.

The greatest colour difference (ΔE*Lab = 18.81) was observed under the 16:8 photoperiod at pH 7, followed by the 24:0 photoperiod at pH 5 (ΔE*Lab = 14.87), suggesting noticeable pigment variations under these conditions. Lower colour differences, such as those under the 8:16 photoperiod at pH 11 (ΔE*Lab = 10.25), indicate more uniform colour characteristics in alkaline and lower-light conditions. Biomass production and carbon sequestration rates were highest at pH 9 and pH 11 under a 16-hour photoperiod, with the highest CSR values recorded at pH 9 (0.044 g/L CO_2e) and pH 11 (0.041 g/L CO_2e). No biomass or CSR were recorded under acidic conditions (pH 3), confirming that these environments are unsuitable for growth and carbon capture.

C. vulgaris exhibits optimal growth in Chu medium under a 24:0 photoperiod at pH 11, as indicated by higher cell density, lightness, and chlorophyll content. In contrast, acidic environments (pH 5) and shorter photoperiods (8:16) result in reduced growth and pigment production. Notably, pH 3 completely inhibits both growth and carbon sequestration, reinforcing the critical role of pH and photoperiod regulation in optimising *C. vulgaris* cultivation.

4.3 DISCUSSION

The cultivation of *C. vulgaris* in different media (BBM, BG11, Bristol, and Chu) under varying photoperiods (24:0, 16:8, 12:12, and 8:16) was assessed to determine its effectiveness as a carbon sequestration agent and a potential landscape ecological indicator. Growth parameters such as absorbance at 750 nm, total chlorophyll content, and biomass production provide critical insights into its role in climate change mitigation and sustainable landscape management.

The findings demonstrate that alkaline conditions (pH 9–11) combined with continuous illumination (24:0) produced the highest biomass and carbon sequestration rates, indicating that maximising light exposure enhances *C. vulgaris's* ability to absorb CO_2 from the atmosphere. This aligns with previous studies suggesting that microalgae under optimal light conditions can act as highly efficient carbon sinks (Ratomski & Hawrot-Paw, 2021). The significant increase in biomass under extended photoperiods suggests that *C. vulgaris* could be effectively integrated into carbon sequestration strategies, particularly in controlled environments such as constructed wetlands, bioreactors, or urban green spaces where CO_2 uptake is a priority.

The chlorophyll content results further support its potential as a landscape ecological indicator. The highest pigment accumulation was observed under continuous light and alkaline conditions, suggesting that *C. vulgaris* responds sensitively to environmental changes, particularly in pH and photoperiod variations. This sensitivity makes it a valuable bio-indicator for assessing ecosystem health and the impact of climate fluctuations on aquatic environments. The lower chlorophyll content recorded under acidic conditions (pH 3–5) and shorter photoperiods (8:16) suggests that acidification and reduced light exposure can limit algal productivity, highlighting potential risks in ecosystems affected by climate change-induced acidification.

Biomass production was strongly influenced by both photoperiod and medium composition, with the highest carbon sequestration rates recorded at pH 9–11 under a 16-hour photoperiod. This suggests that *C. vulgaris* could be integrated into climate mitigation strategies where alkaline environments are maintained, such as industrial

CO_2 capture systems or wastewater treatment facilities (Guo et al., 2018). The significant reduction in biomass under acidic conditions (pH 3) confirms that low pH environments hinder carbon fixation processes, emphasising the need for pH regulation in algal-based climate mitigation systems.

Furthermore, the colour variation (ΔE*Lab) and lightness values observed in different photoperiods and pH levels reinforce the suitability of C. vulgaris as an ecological indicator. The significant pigment shifts recorded under extreme conditions suggest that algal cultures could serve as visual markers for monitoring environmental changes. High ΔE*Lab values under continuous light and alkaline pH indicate a stronger pigment response, which could be used to detect environmental stressors such as pollution, eutrophication, or pH fluctuations in water bodies.

In conclusion, *C. vulgaris* demonstrates strong potential as a carbon sequestration agent and a landscape ecological indicator, particularly in alkaline environments with optimal light exposure. Its ability to absorb atmospheric CO_2, respond to environmental changes, and visually indicate ecosystem health supports its application in climate mitigation strategies and sustainable landscape planning. Future research should explore its integration into urban green infrastructure, carbon capture technologies, and aquatic ecosystem monitoring to maximise its role in addressing climate change.

4.4 MULTIVARIATE DATA ANALYSIS

Sampling Adequacy for Multivariate analysis

The results from 12 variables were analysed using Multivariate Data Analysis to determine the significant variables contributing *C.vulgaris* cell culture growth chromaticity stability test. Data transformation is a component of the initial preparation of data prior to statistical analysis. The entire data set was normalized through the use of variable transformation. The box and whisker plot show the comparison in distributions of variables graphically to identify outliers. The boxes portrayed the 25th percentile (lower quartile), median, the 75th percentile (upper quartile) value of data and less or more than that located outside of the boxes, known as outliers. In this plot,

the outliers remained as the data is significant to show the highest values for each variable. It can be seen from the 12 different medians, the median score of H (Hue) is the highest (256.32), and b is the lowest (-0.197)

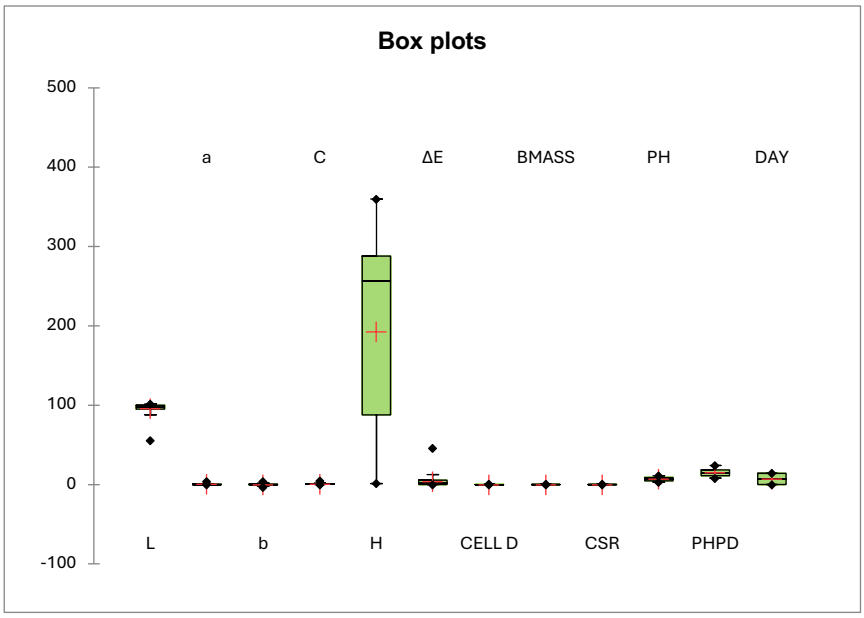


Figure 4.25: Box-and-whisker plots of *C.vulgaris* cell culture growth at different medium formulations, pH, photoperiod and cell culture growth time towards biomass, carbon sequestration rate and chromaticity

Sampling adequacy for *C.vulgaris* cell culture growth chromaticity stability test were further tested by using Kaiser-Meyer-Olkin (KMO) where the value must be greater than 0.5 followed by the Barlett's Test of Sphericity ($p < 0.05$) and Correlation Matrix Pearson where the value must be equal or greater than 0.01 (Atiqah et al., 2023). The KMO test was conducted to evaluate the dataset for adequacy at α of 0.05 to ensure the dataset is adequate for the multivariate data analysis. The average KMO value was ranked as detailed by Ismail et al. (2021)

KMO < 0.5	**inadequate**
0.5 < KMO < 0.7	mediocre
0.7 < KMO < 0.8	good
0.8 < KMO < 0.9	very good
KMO > 0.9	excellent

The KMO values for the 240 datasets produced a KMO score of 0.677 (Table 5.6) confirming the dataset is in mediocre and adequate for PCA test. Barlett's Test of Sphericity is measured to ensure that the R-matrix does not equal the identity matrix. The *p*-value for *C.vulgaris* cell culture growth chromaticity stability test exhibited highly significant differences at 0.0001.

Table 4.21 Kaiser-Meyer-Olkin and Bartlett's test for the *C.vulgaris* cell culture growth chromaticity stability test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy		**0.677**
Bartlett's Test of Sphericity	Chi-square (Observed value)	4448.64
	DF	66
	p-value (Two-tailed)	**< 0.0001**

Table 4.21 and 4.22 revealed a positive and negative correlations strength between two variables among *C.vulgaris* cell culture growth chromaticity stability test. A very strong positive correlation was observed among Biomass and CSR ($r^2 = 1.000$), whereas other variables such as ΔE versus a ($r^2 = 0.803$) as well as cell density and day versus a with day versus ΔE ($r^2 = 0.505$ to 0.540) have high and moderate positive correlations respectively. The tables also showed the relationship between 2 variables of of ΔE and a versus ΔE produced a strong significant negative correlation ($r^2 = -0.830$ to -0.965). In general, *C.vulgaris* cell culture growth chromaticity stability test variables can be grouped into four clusters with two cluster with positive correlations and another two clusters with negative correlations as indicated.

Table 4.21 Correlation coefficient matrix for the *C.vulgaris* cell culture growth chromaticity stability test.

Variables	L	a	b	C	H	ΔE	Cell Density	Biomass	CSR	pH	Photo period	Day
L	**1**											
a	**-0.830**	**1**										
b	-0.077	-0.120	**1**									
C	**-0.521**	**0.466**	**0.257**	**1**								
H	0.139	0.008	**-0.647**	-0.111	**1**							
ΔE	**-0.965**	**0.803**	0.078	**0.487**	-0.144	**1**						
Cell Density	**-0.478**	**0.505**	-0.082	**0.271**	-0.012	**0.470**	**1**					
Biomass	**-0.323**	**0.358**	**0.174**	0.072	-0.100	**0.344**	0.161	**1**				
CSR	**-0.323**	**0.358**	**0.175**	0.071	-0.100	**0.344**	0.161	**1.000**	**1**			
pH	**-0.175**	0.070	**0.372**	**0.243**	**-0.260**	0.116	0.144	0.149	0.149	**1**		
Photoperiod	-0.081	0.103	-0.007	0.062	-0.103	0.124	**0.314**	0.059	0.059	0.000	**1**	
Day	**-0.482**	**0.540**	0.153	**0.220**	-0.017	**0.523**	**0.372**	**0.331**	**0.331**	0.000	0.000	**1**

Values in bold are different from 0 with a significance level alpha=0.01

The correlation coefficient (r^2) or the strength of the correlation between two variables *C.vulgaris* cell culture growth chromaticity stability test. The correlation coefficient is measured on a scale from -1 to +1 as described by Guilford (1973).

Table 4.22 The correlation coefficient (r^2) strength between two variables among *C.vulgaris* cell culture growth chromaticity stability test

Variables	r^2	Size of Correlation	Interpretation
Biomass x CSR	**1.000**	0.90 to 1.00	Very high positive correlation
ΔE x a	**0.803**	0.70 to 0.90	High positive correlation
Cell D x a	**0.505**	0.50 to 0.70	Moderate positive correlation
Day x a	**0.540**		
Day x ΔE	**0.523**		
ΔE x C	**0.487**	0.30 to 0.50	Low positive correlation
Cell D x ΔE	**0.470**		
ΔE x L	**-0.965**	-0.70 to -0.90	High negative correlation
a x L	**-0.830**		
C x L	**-0.521**	-0.50 to -0.70	Moderate negative correlation
H x b	**-0.647**		
Cell D x L	**-0.478**	-0.30 to -0.50	Low negative correlation
Day x L	**-0.482**		
Biomass x L	**-0.323**		
CSR x L	**-0.323**		
Biomass x a	**0.358**		
CSR x a	**0.358**		
pH x b	**0.372**		
Biomass x ΔE	**0.344**		
CSR x ΔE	**0.344**		
P.Period x	**0.314**		
Cell D	**0.372**		
Day x Cell D	**0.331**		
Day x Biomas	**0.331**		
Day x CSRs			
Values in bold are different from 0 with a significance level alpha=0.01			

The next step involved analyzing the data set using PCA to identify variables with high factor loadings that explain the principal components significantly. A factor loading greater than 0.75 was considered strong, between 0.50 and 0.75 was moderate, between 0.30 and 0.49 was weak, and less than 0.30 was negligible (Rani et al., 2017). Eigenvalues were used to determine the principal components with more cumulative dataset variations. An eigenvalue of 1 or greater was considered significant for the PCA (Le et al., 2017; Zubir et al., 2016; Yang et al., 2020). PCA has been applied to the 12

variables taken from *C.vulgaris* cell culture growth chromaticity stability test to study which variables significantly contributed to CV cell culture as detailed by Siudek & Frankowski (2018) and Vuong et al. (2020). The PCA exposed two principal components (PCs) entailing 12 variables that represent cumulative variability of 51.95%. The eigenvalues (EV) were used to determine the principal components (PCs) with more cumulative dataset variations. The EV obtained for PC1 was 4.286, and for PC2 was 1.948. In principle, variables far away from the axes F1 and F2 had a strong factor loading (FL). L* and b had strong FL (FL > 0.75) whereas pH had moderate FL (0.500 < FL < 0.749) and others had a weak FL (FL < 0.499).

Table 4.23 The principal components factor loading and eigenvalues of the correlation matrix for *C.vulgaris* cell culture growth chromaticity stability test

Variables	**PC1**	**PC2**
L	0.895	0.167
a	-0.853	-0.333
b	-0.201	0.842
C	-0.558	0.040
H	0.201	-0.727
ΔE	-0.894	-0.172
CELL D	-0.590	-0.277
BMASS	-0.592	0.302
CSR	-0.592	0.303
PH	-0.257	0.517
PHPD	-0.176	-0.051
DAY	-0.647	-0.108
Eigenvalue	4.286	1.948
Variability (%)	35.721	16.234
Cumulative (%)	35.721	**51.955**

The first principal PC1 accounted for 35.72% of the total variance, which comprised strong factor loadings of L* whereas for PC2 is b comprising of 16.23% of the total variance for the data. The PCA analysis allows for identifying factors and provides insight in *C.vulgaris* cell culture growth chromaticity stability test. Therefore, factor loadings can be utilised to determine the strength of the association between variables in *C.vulgaris* cell culture growth chromaticity stability test. Figure 4.24 summarised that all variables were either positively or negatively correlated to each other which can be grouped into four.

L	**Group 1**
H	**Group 2**
PHPD, Day, Cell D, a, ΔE	**Group 3**
B, pH, C, Bmass, CSR	**Group 4**

All variables were positively correlated as long as they were grouped close together such as group 1, group 2 and group 3 variables. Group 1 and group 3 as well as group 2 and group 4 were negatively correlated as there were at the opposite positioned.

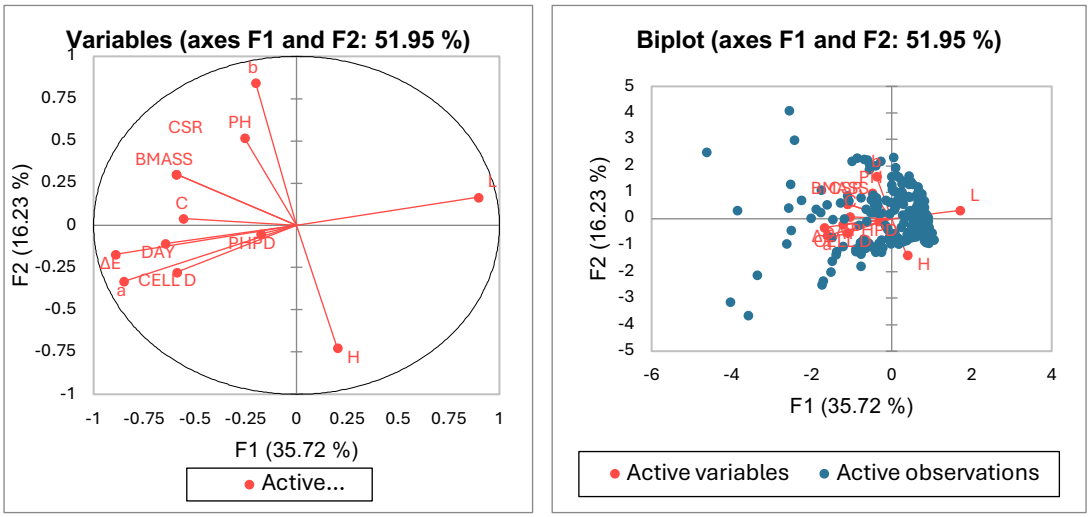


Figure 4.26 All variables were positively correlated as long as they were grouped close together as shown above

4.5 CHAPTER SUMMARY

Table below shows the comparison of *C.vulgaris* Growth Parameters Across Different Media, Photoperiods, and pH Levels

.Table 4.24 Comparison of *C.vulgaris* Growth Parameters Across Different Media, Photoperiods, and pH Levels"

Parameters	**BBM**	**BG11**	**Bristol**	**Chu**
Absorbance (750nm)	1.45 16:8 pH 9	0.40 16:8 pH 7	0.16 24:0 pH 11	0.16 24:0 pH 11
Total Chlorophyll Content (UV-Vis Peak)	pH 9 16:8	pH 9 12:12	pH 11 12:12	pH11 24:0
Lightness (L*)	12:12 pH 9	16:8 pH 11	16:8 pH 11	8:16 pH 11
Chromaticity (ΔE*Lab)	16:8 pH 9	8:16 pH 3	14:0 pH 5	16:8 pH 7
Biomass (g/L)	0.50 16:8 pH 9	0.265 16:8 pH 11	0.093 16:8 pH 11	0.029 16:8 pH 9
Carbon sequestration rate (g/L C02e)	0.783 16:8 pH 9	0.415 16:8 pH 11	0.146 16:8 pH 11	0.044 16:8 pH9

Therefore, it can be concluded that BBM performed best overall, with the highest growth (Abs 1.45), biomass (0.50 g/L), and carbon sequestration rate (0.783 g/L CO2e) under a 16:8 photoperiod at pH 9. Followed by, BG11 showed high lightness (96.86 L*) and colour variation (ΔE*Lab 45.92) at pH 11 and excelled in carbon sequestration (0.415 g/L CO2e) under a 16-hour photoperiod.

Bristol had moderate growth (Abs 0.16), with peak lightness (96.86 L*) and biomass (0.093 g/L) at pH 11. Lastly, Chu exhibited the lowest growth, with maximum biomass (0.029 g/L) and CSR (0.044 g/L CO2e) at pH 9 under a 16-hour photoperiod.

In summary, the growth of *C.vulgaris* in BBM, BG11, Chu, and Bristol media under varying photoperiods demonstrates that continuous light maximizes growth parameters, while shorter photoperiods lead to reduced biomass and chlorophyll content. These findings underscore the importance of optimizing light conditions and media composition to enhance the biomass production of *C.vulgaris* as a viable carbon sequestration agent.

CHAPTER FIVE

SYNECHOCOCCUS

5.1 INTRODUCTION

Synechococcus is a genus of unicellular cyanobacteria that plays a critical role in aquatic ecosystems, particularly in marine and freshwater environments. These organisms are significant contributors to primary production and are essential components of the microbial food web. Their abundance and diversity can be influenced by various environmental factors, including nutrient availability, light conditions, and interactions with viruses, particularly cyanophages.

5.2 RESULTS AND DISCUSSIONS

5.2.1 Cell Culture Growth of *Synechococcus* in different medium formulation, PEG and SA concentrations

Effect of Different Medium Formulation on Synechococcus Cell Culture Growth

In this experiment, four types of medium formulation which are BBM, BG11, Bristol, and Chu10 were used. The cell culture growth performance of *Synechococcus* across the various media formulations were measured through their absorbance at the wavelength of 750 nm each day for 2 weeks. By optimizing the medium composition, it is possible to enhance cell density and potentially improve the overall productivity and bioactivity of *Synechococcus* in various applications.

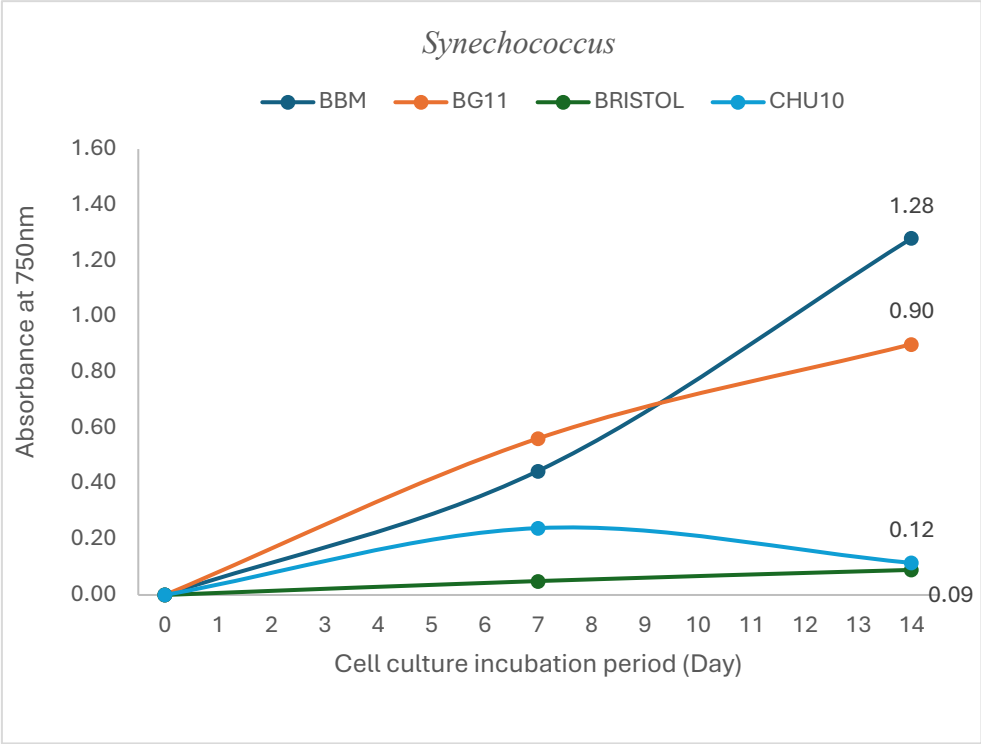


Figure 5.1 Distribution of cell culture growth of *Synechococcus* in different medium formulations

Table 5.1 The optimum absorbance value *Synechococcus* cell culture growth in different media formulation

Blue-Green Algae	**Medium**	**Abs**
Synechococcus	BBM	1.28
	BG11	0.90
	BRISTOL	0.12
	CHU10	0.09

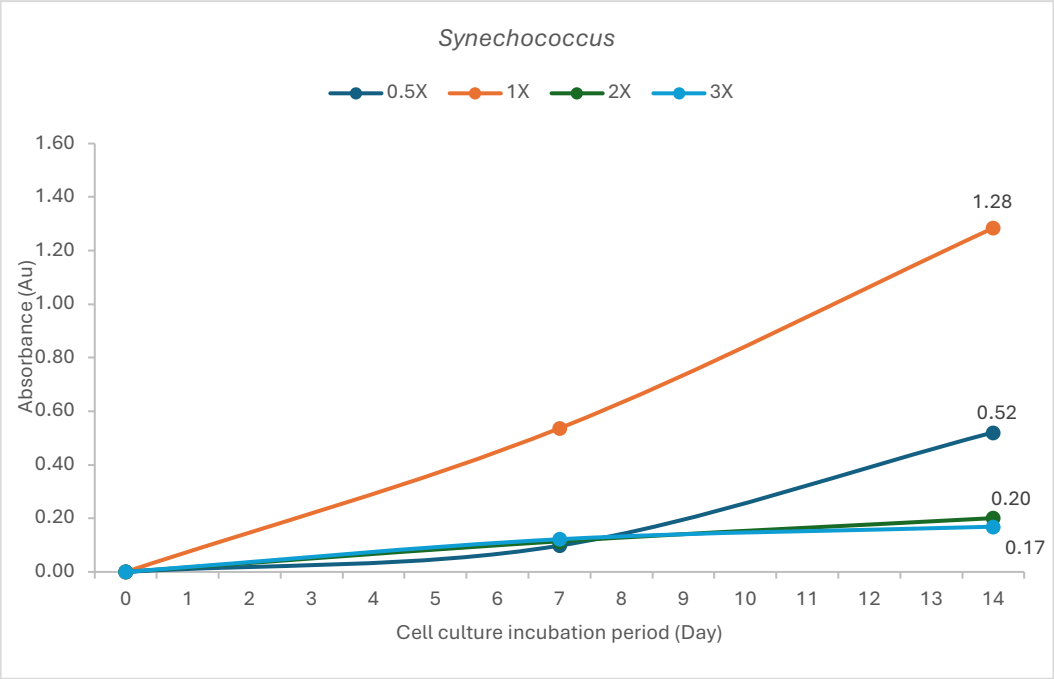


Figure 5.2 Distribution of cell culture growth of *Synechococcus* in different nutrient strength.

Table 5.2 The highest absorbance value of *Synechococcus* cell culture growth in different BBM medium formulation strength

Blue-Green Algae	**Medium strength**	**Abs**
Synechococcus	0.5	0.52
	1x	1.28
	2x	0.20
	3x	0.17

Firstly, the BBM medium resulted in the highest absorbance value of 1.28, indicating optimal growth in this medium. Other media, such as BG11, showed moderate growth with an absorbance of 0.69, while Bristol and CHU10 media had much lower absorbance values of 0.12 and 0.09, respectively, suggesting that these media are

less effective for *Synechococcus* growth. The highest absorbance of 1.28 was achieved at a 1x BBM medium strength, indicating that this nutrient concentration is the most suitable for *Synechococcus* growth. A reduced medium strength of 0.5x yielded a moderate absorbance value of 0.52. However, increasing the BBM concentration to 2x and 3x resulted in lower absorbance values of 0.20 and 0.17, respectively, implying that higher nutrient concentrations do not enhance growth and may even hinder it. These findings highlight BBM as the most effective medium for the growth of *Synechococcus* with a standard 1x BBM concentration providing optimal conditions for *C. vulgaris* growth.

BBM is particularly useful for specific experimental setups where particular nutrient conditions are required, but it generally yields equal or lower biomass compared to BG-11 (Prihantini, 2020). The choice of BBM may depend on the specific research goals, such as studying the effects of varying nutrient concentrations on growth dynamics.. Research indicates that BG-11 can also supports robust growth and high biomass productivity due to its balanced nutrient composition, which includes nitrogen, phosphorus, and trace metals (Parthiban & Ranjitha, 2023). Bristol medium is less commonly used for *Synechococcus* but serves as a viable alternative in certain contexts. Its nutrient composition can support the growth of various cyanobacterial species; however, it may not provide the same level of growth efficiency as BBM and BG-11 for *Synechococcus* (Prihantini, 2020). Lastly, Chu's medium is primarily designed for the cultivation of freshwater cyanobacteria and can be adapted for *Synechococcus*. While it has been utilized in some studies, its effectiveness compared to BBM and BG-11 is often questioned. Research indicates that while Chu's medium can support growth, it may not achieve the same biomass yields as BG-11 under optimal conditions (Watanabe et al., 2015). The choice to use Chu's medium may be influenced by the specific environmental conditions being simulated or the particular strain of *Synechococcus* being studied.

Next experiment, *Synechococcus* was measured by absorbance at 750nm over a 14-day incubation period, in two separate studies: one with different PEG concentrations and the other with varying salicylic acid concentrations.

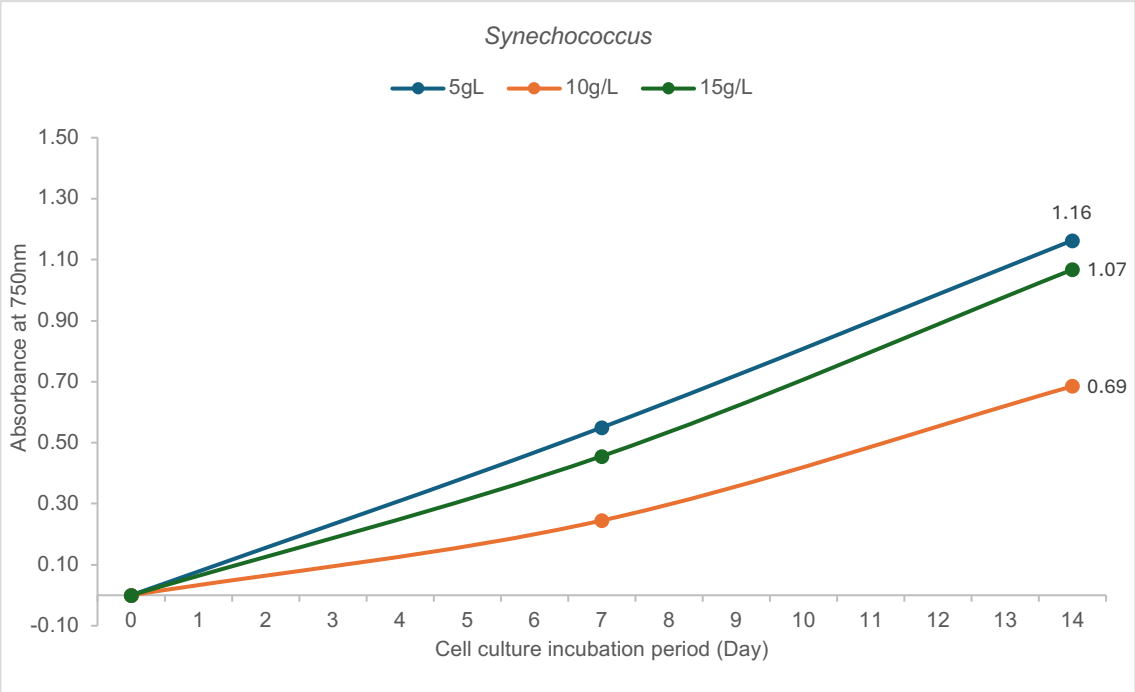


Figure 5.3 Distribution of cell culture growth of *Synechococcus* in different PEG concentrations.

Table 5.3 The highest absorbance value of *Synechococcus* cell culture growth in different PEG concentrations

Blue-Green Algae	**PEG Conc. (g/L)**	**Peak (Day)**	**Abs**
Synechococcus	5	D14	1.16
	10	D14	0.68
	15	D14	1.07

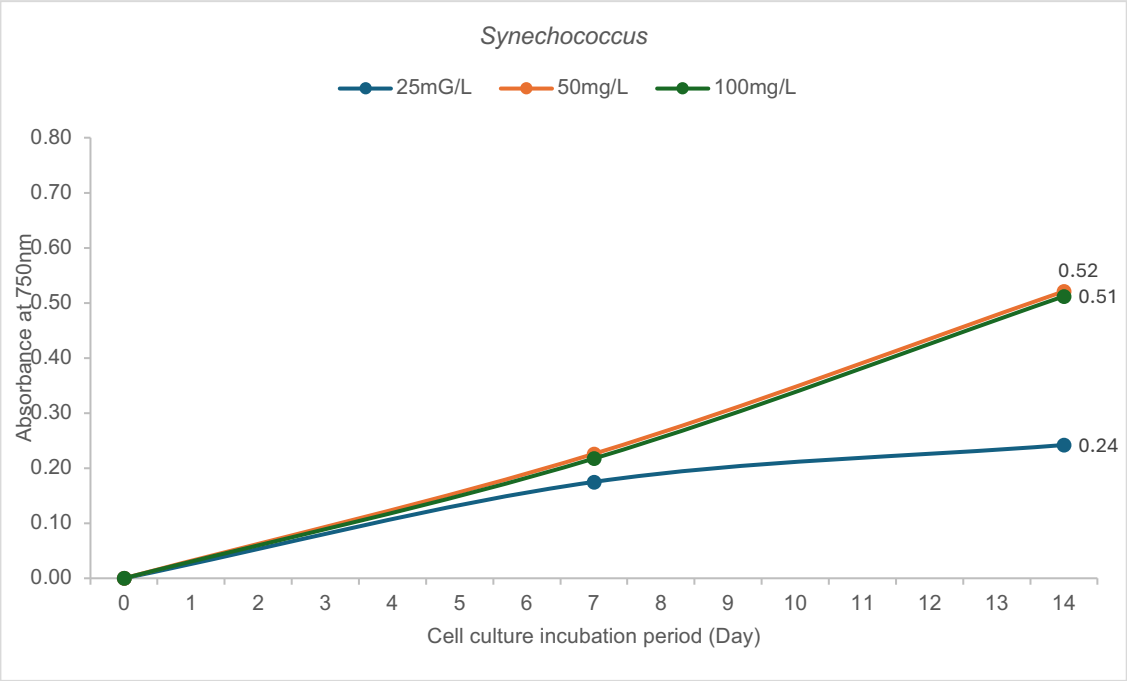


Figure 5.4 Distribution of cell culture growth of Synechococcus in different SA concentrations.

Table 5.4 The highest absorbance value of *Synechococcus* cell culture growth in different SA concentrations

Blue-Green Algae	**SA Conc. (mg/L)**	**Peak (Day)**	**Abs**
Synechococcus	25	D14	0.24
	50	D14	0.52
	100	D14	0.51

For PEG concentrations, the highest absorbance value (1.16) was recorded at a PEG concentration of 5 g/L, with peak growth observed on Day 14. At a higher concentration of 10 g/L, the absorbance dropped to 0.68, while at 15 g/L, the absorbance reached 1.07 but peaked earlier, on Day 7. This indicates that a PEG concentration of 5 g/L is optimal for *Synechococcus* growth, with higher concentrations either delaying or reducing the growth. For SA concentrations, the highest absorbance (0.52) was recorded at a concentration of 50 mg/L, with peak growth occurring on Day 14. At concentrations of 25 mg/L and 100 mg/L, the absorbance values were lower, at 0.24 and 0.51, respectively, with peak growth observed on Day 7 for both concentrations. This suggests that 50 mg/L of SA is the most favorable concentration for *Synechococcus* growth, while both lower and higher concentrations result in slightly reduced growth. In summary, the optimum conditions for *Synechococcus* growth are a PEG concentration of 5 g/L and an SA concentration of 50 mg/L, with peak growth typically occurring around Day 14. Higher concentrations of PEG or SA tend to decrease growth or alter the growth pattern.

Studies have demonstrated that PEG can induce physiological changes in cyanobacteria, leading to alterations in growth rates and cellular responses (Kearney et al., 2020). The osmotic stress caused by PEG can trigger adaptive mechanisms in Synechococcus, potentially influencing its growth dynamics and metabolic pathways. For example, the presence of PEG may lead to increased production of osmoprotectants or alterations in pigment composition, which could be critical for maintaining photosynthetic efficiency under stress. Meanwhile, research indicates that low concentrations of salicylic acid can promote growth and physiological responses in plants, suggesting potential benefits for cyanobacterial cultures as well (X et al., 2023).

5.2.2 Effect of pH, and medium formulation and photoperiod on *Synechococcus* Cell Culture Growth, biomass, Chromaticity, and Chlorophyll content

This section revealed the behaviour of blue-green algae *Synechococcus* cell culture growth in four different mediums of BBM, BG11, Chu-10, and Bristol under different pH (3.0, 5.0, 7.0, 9.0 and 11.0) and photoperiod (24:0, 16:8, 12:12 and 8:16). The parameter will include:

1. Absorbance (750nm) of *Synechococcus*
2. Effects of pH and photoperiod on UV-vis spectra absorbance values for chlorophyll content
3. The lightness (L*) of *Synechococcus* cell culture growth
4. The CIE L*a*b* colour difference or Chromaticity (ΔE^*_{Lab}) of *Synechococcus* cell culture growth
5. Assessment of *Synechococcus* Biomass as Carbon Sequestration Agent

Effect of pH, BBM medium formulation and photoperiod on Synechococcus Cell Culture Growth, biomass, Chromaticity, and Chlorophyll content

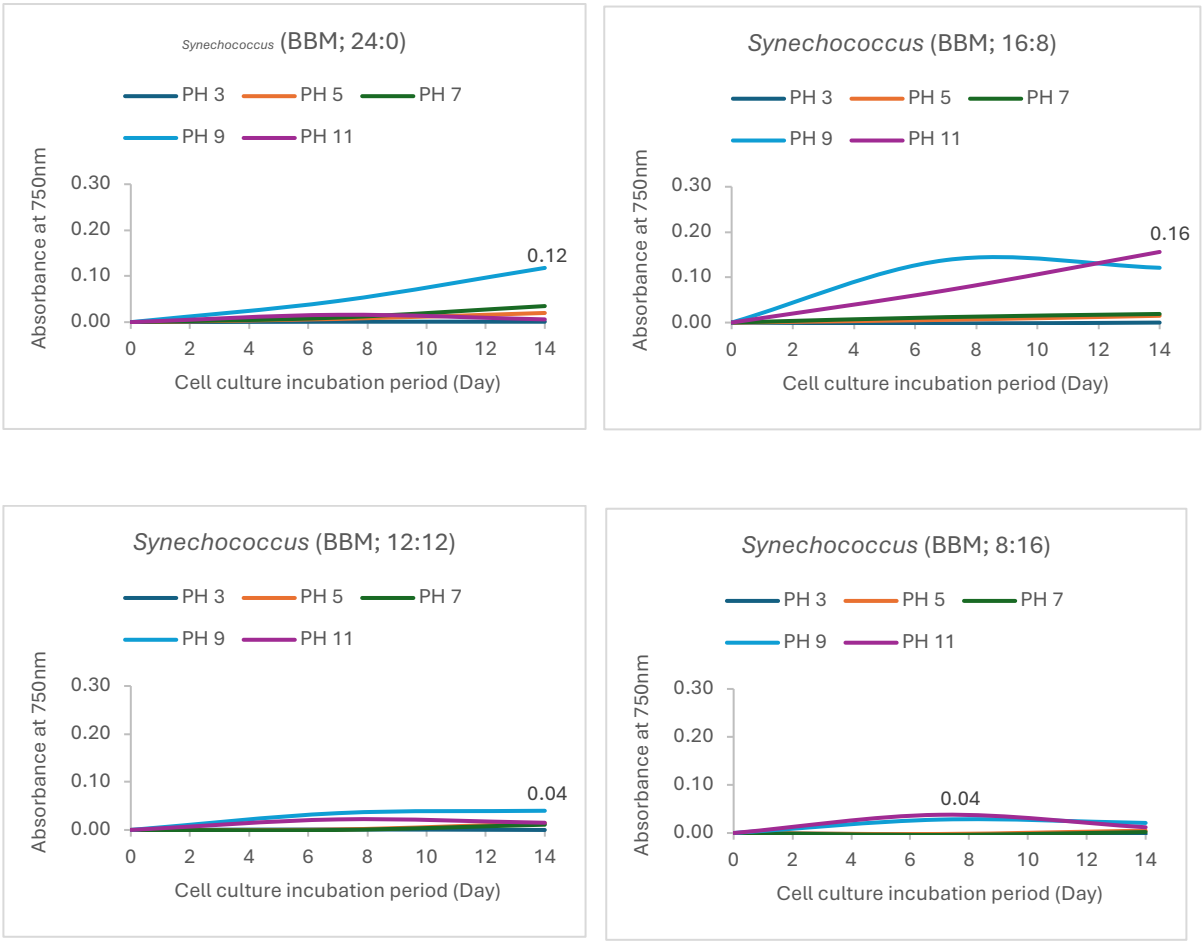


Figure 5.5 Effect of pH on cell culture growth of *Synechococcus* in BBM medium.

Table 5.5 The optimum cell density and day of growth of *Synechococcus* in BBM medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**Abs**
Synechococcus	24:0	pH 11 (Day 14)	0.12
	16:8	pH 11 (Day 14)	0.16
	12:12	pH 9 (Day 14)	0.04
	8:16	pH 11 (Day 7)	0.04

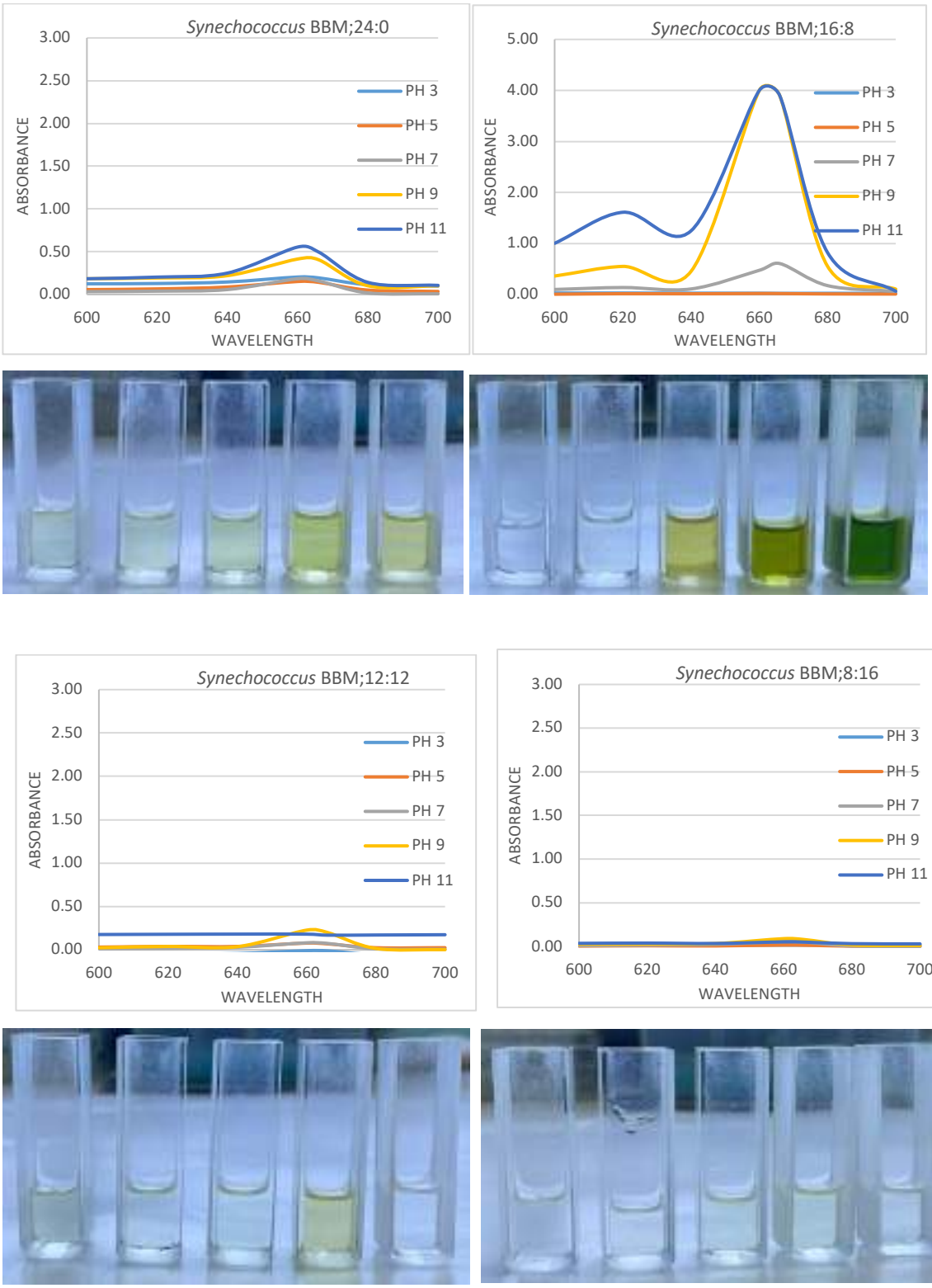


Figure 5.6 Effects of pH and photoperiod on chlorophyll content

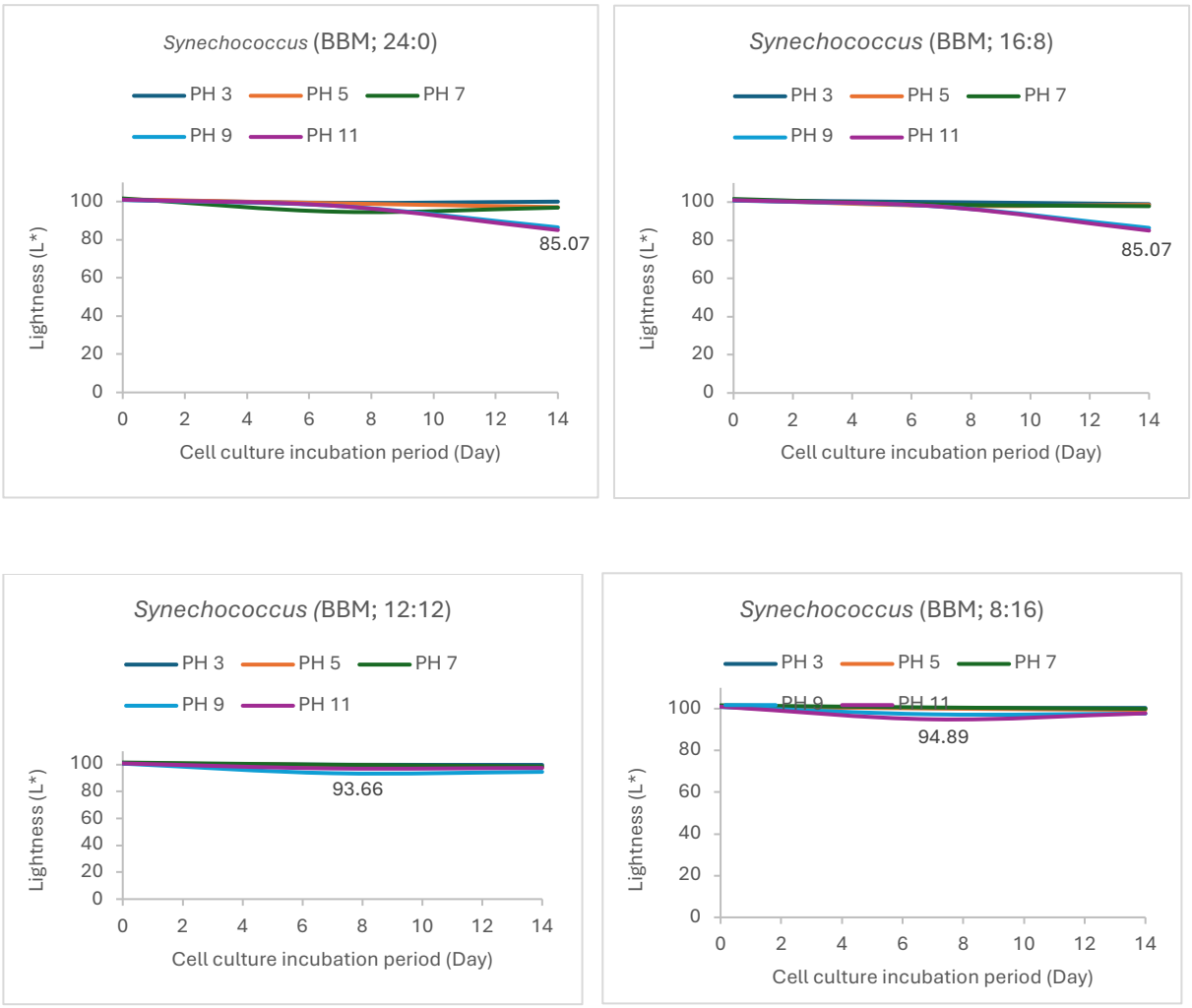


Figure 5.7 Effect of pH on lightness of *Synechococcus* in BBM medium.

Table 5.6 The lightness (L*) of *Synechococcus* cell culture growth in BBM medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**L***
Synechococcus	24:0	pH 11 (Day 14)	85.07
	16:8	pH 11 (Day 14)	85.07
	12:12	pH 9 (Day 7)	93.66
	8:16	pH 11 (Day 7)	94.89

L, ranges from black (0) to white (100)*

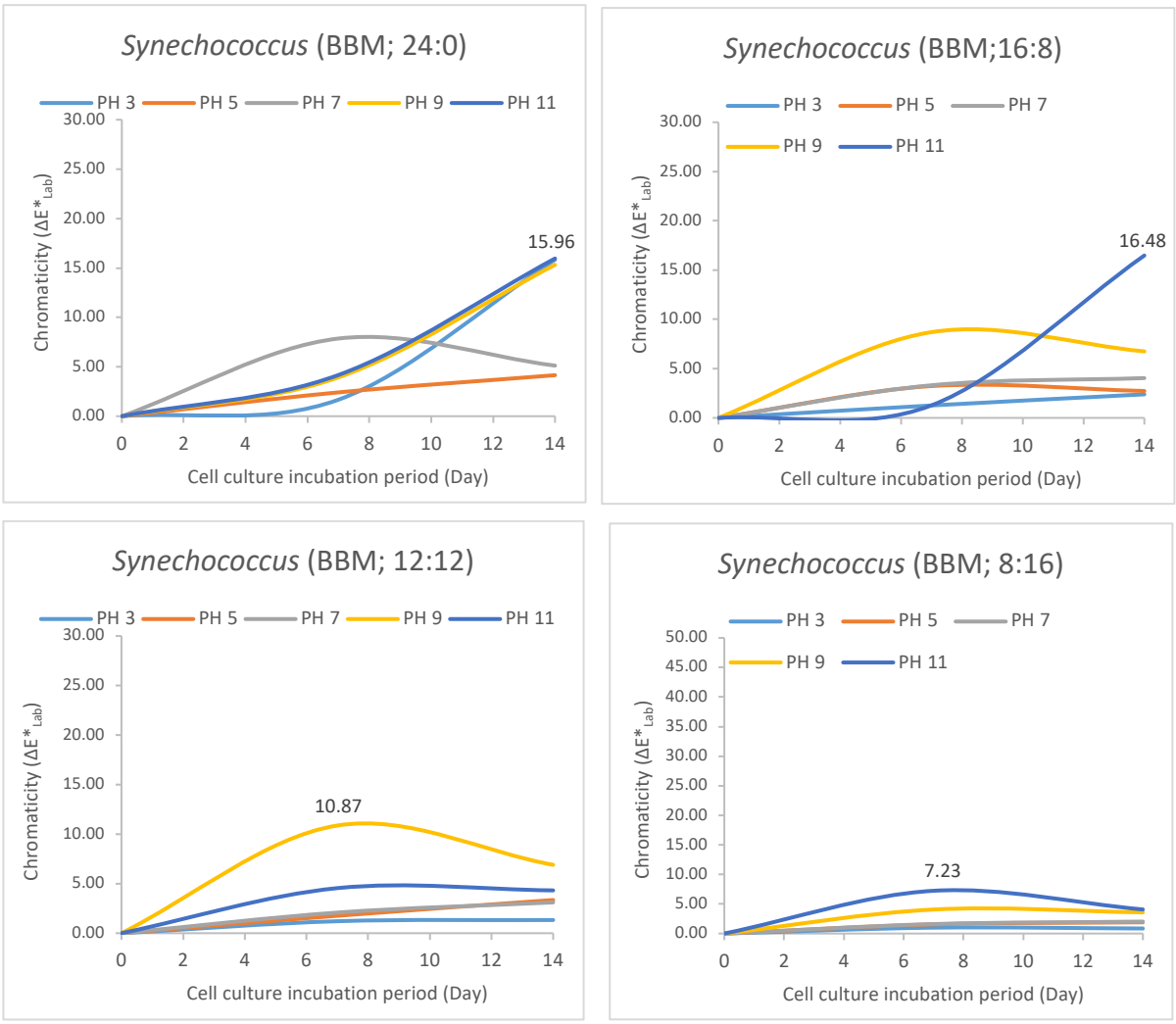


Figure 5.8 Effect of pH on Chromaticity of *Synechococcus* in BBM medium.

Table 5.7 The CIE L*a*b* colour difference or Chromaticity (ΔE^*_{Lab}) of *Synechococcus* cell culture growth in BBM medium at different photoperiod.

Blue Green Algae	**Photoperiod**	**Peak pH (day)**	**ΔE^*_{Lab}**
Synechococcus	24:0	pH 11 (Day 14)	15.96
	16:8	pH 11 (Day 14)	16.48
	12:12	pH 9 (Day 7)	10.87
	8:16	pH 11 (Day 7)	7.23

ΔE^*_{Lab} > 5.0 - indicate clearly two different colours

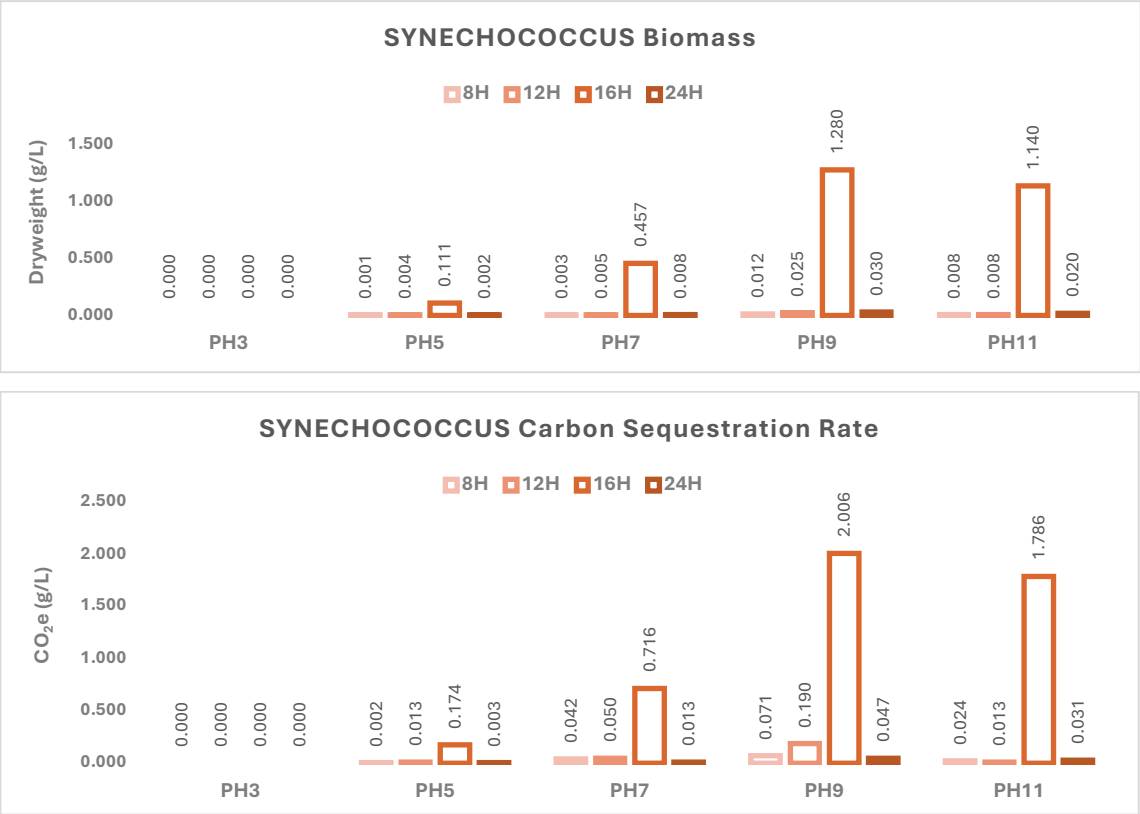


Figure 5.9 The biomass and carbon sequestration rate of *Synechococcus*

Table 5.8 The biomass and carbon sequestration rate of *Synechococcus* cell culture growth in BBM medium at different pH and photoperiod.

Blue-Green Algae	**pH**	**Biomass (g/L)**	**CSR (CO2e)**
Synechococcus	pH 3	0.000	0.000
	pH 5 (16H)	0.111	0.174
	pH 7 (16H)	0.457	0.716
	pH 9 (16H)	1.280	2.006
	pH 11 (16H)	1.140	1.786

Firstly, the highest absorbance value (0.16) was observed under the 16:8 photoperiod at pH 11. Absorbance was significantly lower in all other combinations of photoperiods and pH levels. The 24:0 photoperiod at pH 11 recorded an absorbance value of 0.12, indicating slightly reduced growth compared to the 16:8 condition. Lower pH levels, particularly pH 3, resulted in poor growth across all mediums, with the lowest absorbance values recorded. Cultures grown under the 8:16 and 12:12 photoperiods exhibited weaker chlorophyll peaks across all pH levels, indicating reduced chlorophyll accumulation. Notably, cultures grown at lower pH (3 and 5) across all photoperiods showed the lowest absorbance, suggesting poor chlorophyll production in acidic environments.

The highest lightness (94.89) was recorded under the 8:16 photoperiod at pH 11. Similarly, the 12:12 photoperiod at pH 9 showed a high lightness value of 93.66, indicating lighter cultures. In contrast, the 24:0 and 16:8 photoperiods at pH 11 exhibited lower lightness values (85.07), suggesting darker pigmentation under these conditions. Furthermore, the 16:8 photoperiod at pH 11 produced the highest chromaticity value of 16.48, indicating significant pigment variation. The 24:0 photoperiod at pH 11 resulted in a chromaticity of 15.96. Lower photoperiod and pH combinations, such as the 12:12 at pH 9 and the 8:16 at pH 11, showed lower chromaticity values (10.87 and 7.23), indicating more stable pigmentation under these conditions. The biomass and carbon sequestration rate (CSR) results indicated that *Synechococcus* achieves optimal growth and carbon sequestration in alkaline conditions (pH 9 and pH 11). The highest biomass (1.28 g/L) and CSR (2.006 g/L CO_2e) were observed under pH 9 with a 16-hour photoperiod. A similar performance was recorded at pH 11, with biomass reaching 1.14 g/L and CSR at 1.786 g/L CO_2e. In contrast, at pH 3, no measurable biomass or carbon sequestration was detected, underscoring the detrimental effects of acidic conditions on growth and CO_2 capture.

Synechococcus growth in BBM medium is optimal under a 16:8 photoperiod at pH 9, as evidenced by the highest absorbance, chlorophyll content, biomass, and carbon sequestration rates. Alkaline pH levels (9–11) consistently produced the best results for growth and pigment stability, while acidic conditions (pH 3–5) significantly inhibited growth and carbon sequestration.

Effect of pH, BG11 medium formulation and photoperiod on Synechococcus Cell Culture Growth, biomass, Chromaticity, and Chlorophyll content

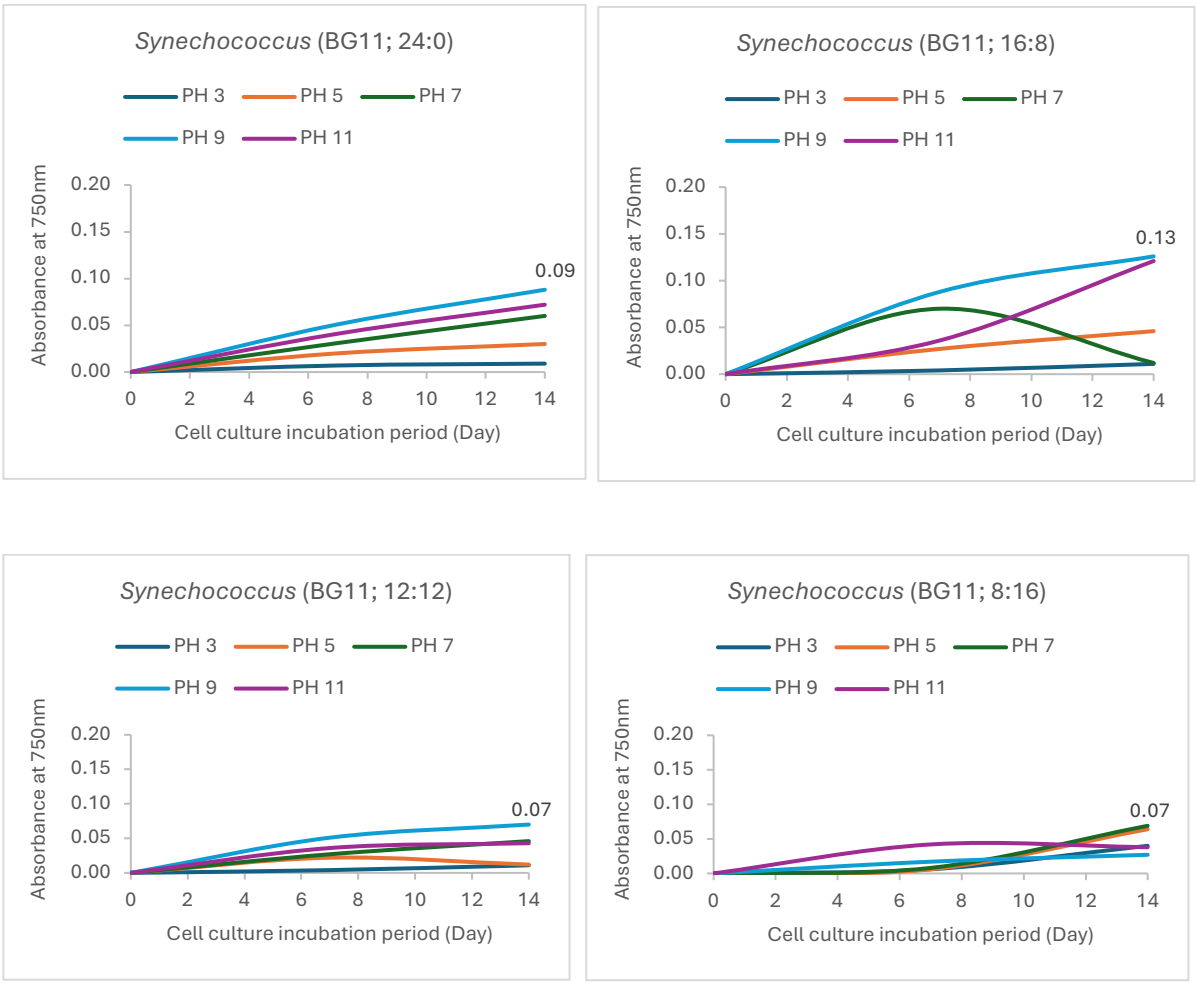


Figure 5.10 Effect of pH on cell culture growth of *Synechococcus* in BG11 medium.

Table 5.9 The optimum cell density and day of growth of *Synechococcus* in BG11 medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**Abs**
Synechococcus	24:0	pH 9 (Day 14)	0.09
	16:8	pH 9 (Day 14)	0.13
	12:12	pH 9 (Day 14)	0.07
	8:16	pH 11 (Day 7)	0.07

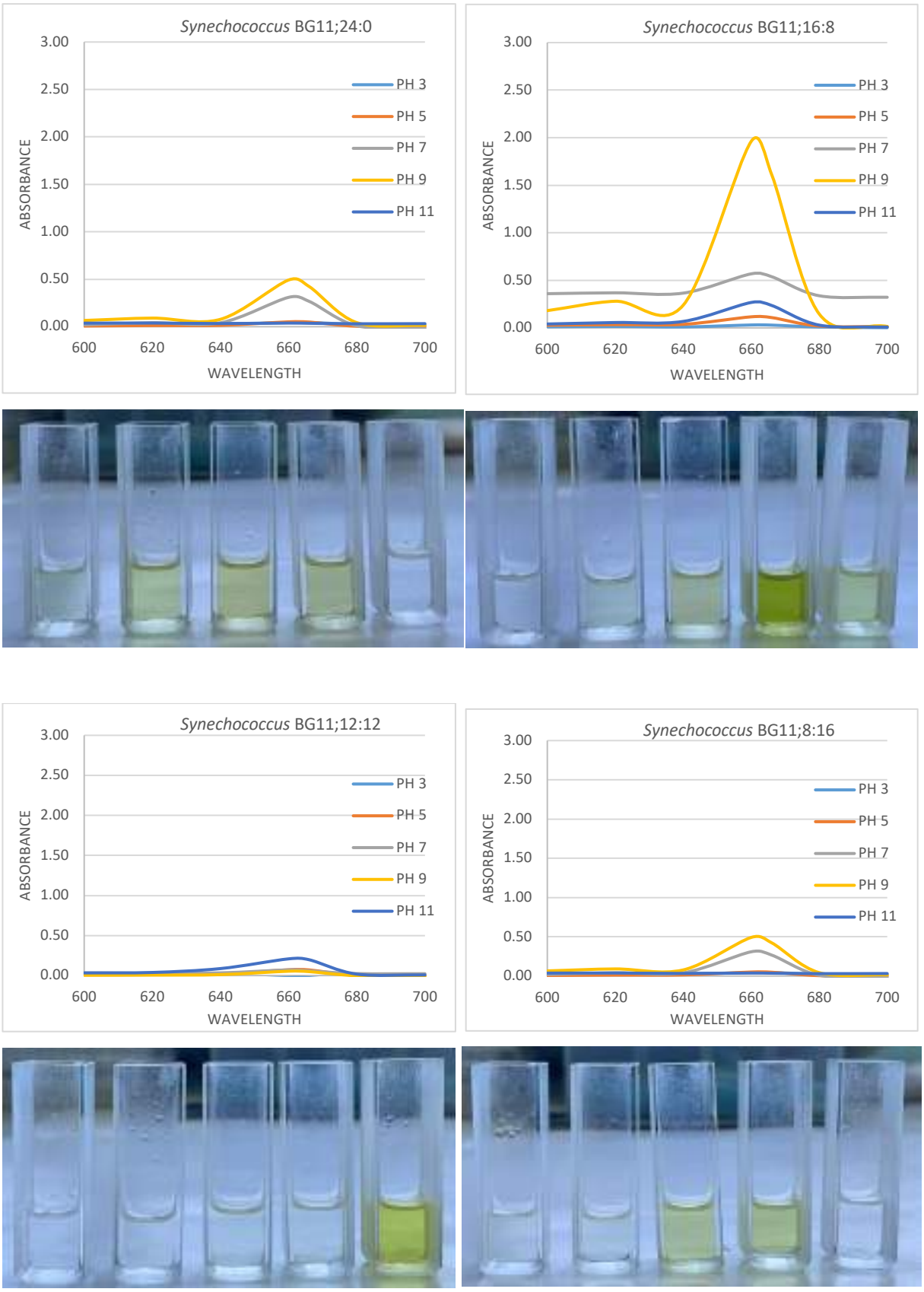


Figure 5.11 Effects of pH and photoperiod on for chlorophyll content

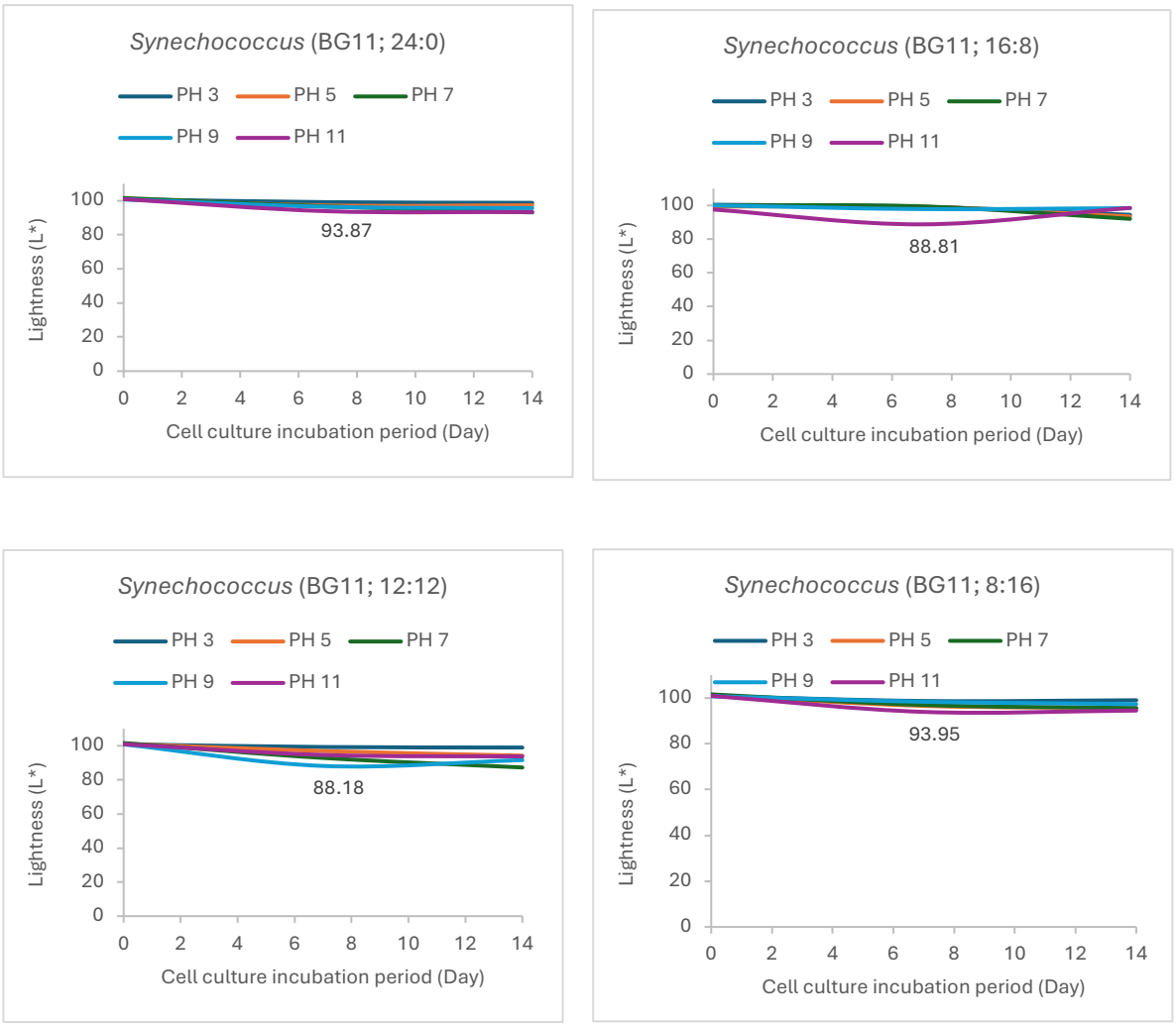


Figure 5.12 Effect of pH on lightness of *Synechococcus* in BG11 medium.

Table 5.10 The lightness (L*) of *Synechococcus* cell culture growth in BG11 medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**L***
Synechococcus	24:0	pH 11 (Day 7)	93.87
	16:8	pH 11 (Day7)	88.81
	12:12	pH 9 (Day 7)	88.18
	8:16	pH 11 (Day 7)	93.95

L, ranges from black (0) to white (100)*

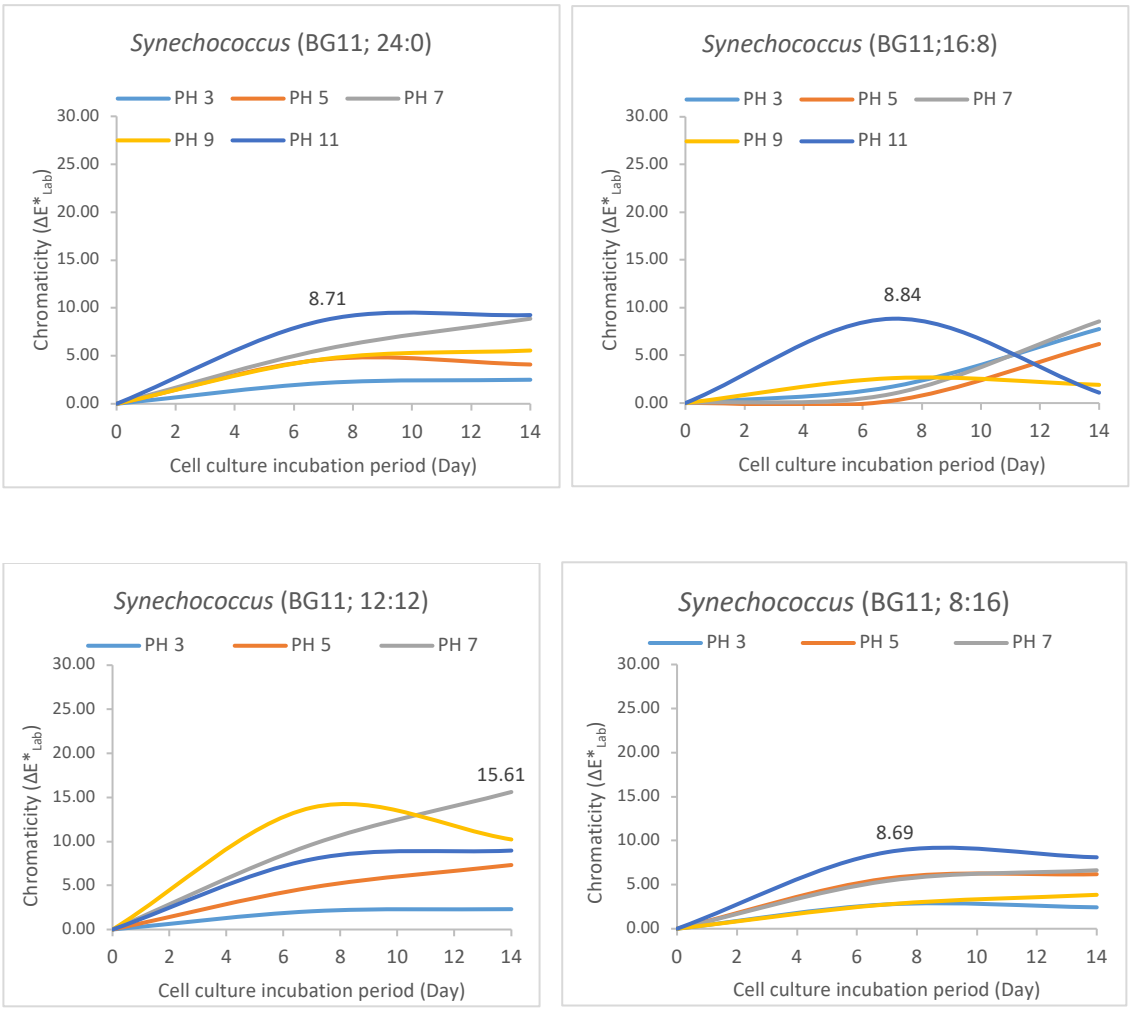


Figure 5.13 Effect of pH on Chromaticity of *Synechococcus* in BG11 medium.

Table 5.11 The CIE L*a*b* colour difference or Chromaticity (ΔE^*_{Lab}) of *Synechococcus* cell culture growth in BG11 medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**ΔE^*_{Lab}**
C. vulgaris	24:0	pH 11 (Day 7)	8.71
	16:8	pH 11 (Day7)	8.84
	12:12	pH 7 (Day 14)	15.61
	8:16	pH 11 (Day 14)	8.69

ΔE^*_{Lab} > 5.0 - indicate clearly two different colours

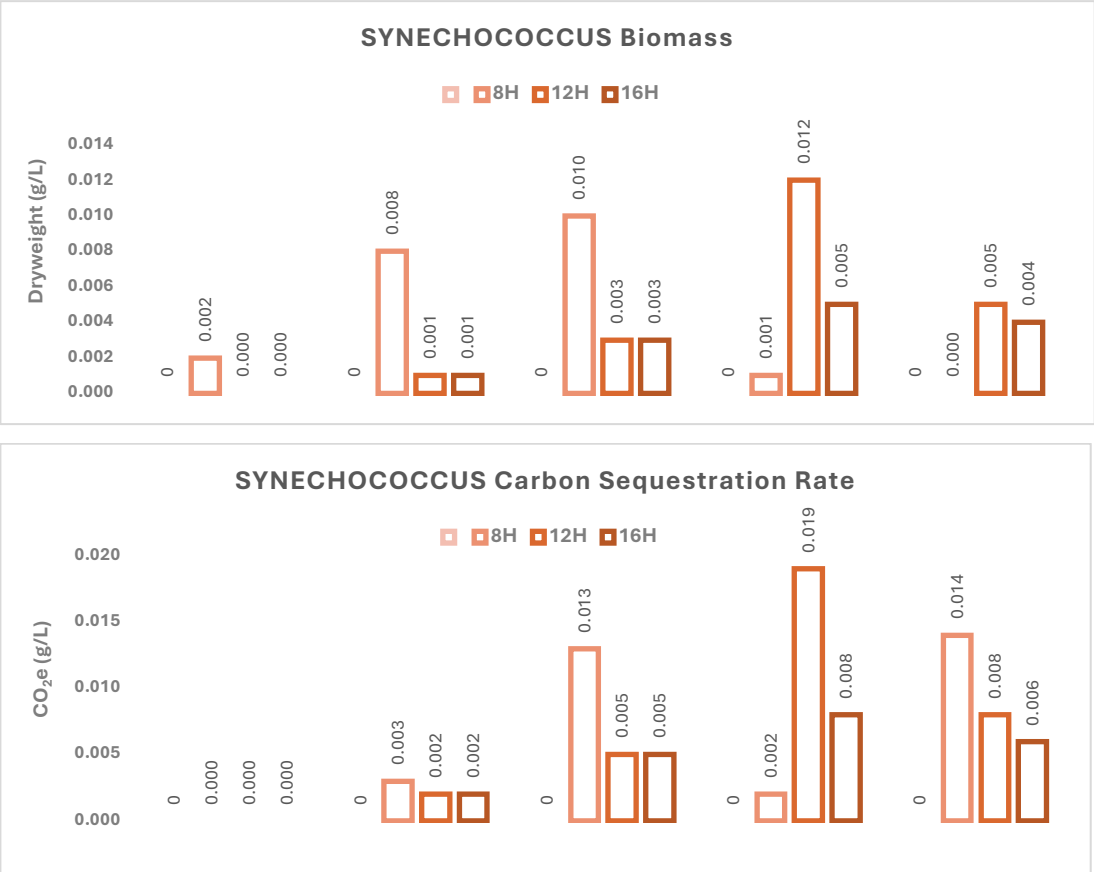


Figure 5.14 The biomass and carbon sequestration rate of *Synechococcus*

Table 5.12 The biomass and carbon sequestration rate of *Synechococcus* cell culture growth in BG11 medium at different pH and photoperiod.

Blue-Green Algae	**pH**	**Biomass (g/L)**	**CSR (CO2e)**
Synechococcus	pH 3 (16H)	0.117	0.183
	pH 5 (16H)	0.165	0.259
	pH 7 (16H)	0.190	0.298
	pH 9 (16H)	0.239	0.375
	pH 11 (16H)	0.412	0.646

The highest cell density (0.13) was observed under the 16:8 photoperiod at pH 9, with slightly lower absorbance (0.09) under a 24:0 photoperiod. Lower pH levels (pH 3 and 5) resulted in minimal growth, with the lowest absorbance at pH 3. These results suggest optimal growth for *Synechococcus* at pH 9 under a 16:8 photoperiod. UV-Vis spectra showed the highest chlorophyll content under the 16:8 photoperiod at pH 9, with strong peaks around 660 nm. In contrast, cultures at pH 3 and 5 had significantly reduced chlorophyll production, confirming that alkaline conditions improve chlorophyll accumulation.

The highest lightness (L* = 93.95) was recorded under an 8:16 photoperiod at pH 11, while longer photoperiods in alkaline conditions resulted in lighter cultures. Cultures under 24:0 and 16:8 photoperiods at pH 11 exhibited lower chromaticity values (8.71 and 8.84), indicating more stable pigmentation. The highest biomass (0.412 g/L) and CSR (0.646 g/L CO_2e) were recorded at pH 11 with a 16-hour photoperiod. pH 9 showed similar results (0.239 g/L biomass, 0.375 g/L CO_2e), while acidic conditions (pH 3 and 5) resulted in minimal growth and carbon capture.

Synechococcus growth, biomass, and carbon sequestration were optimal under alkaline conditions (pH 9-11) with a 16-hour photoperiod. Acidic conditions (pH 3-5) inhibited growth, and continuous light (24:0) reduced performance slightly compared to the 16-hour photoperiod.

Effect of pH, Bristol medium formulation and photoperiod on Synechococcus Cell Culture Growth, biomass, Chromaticity, and Chlorophyll content

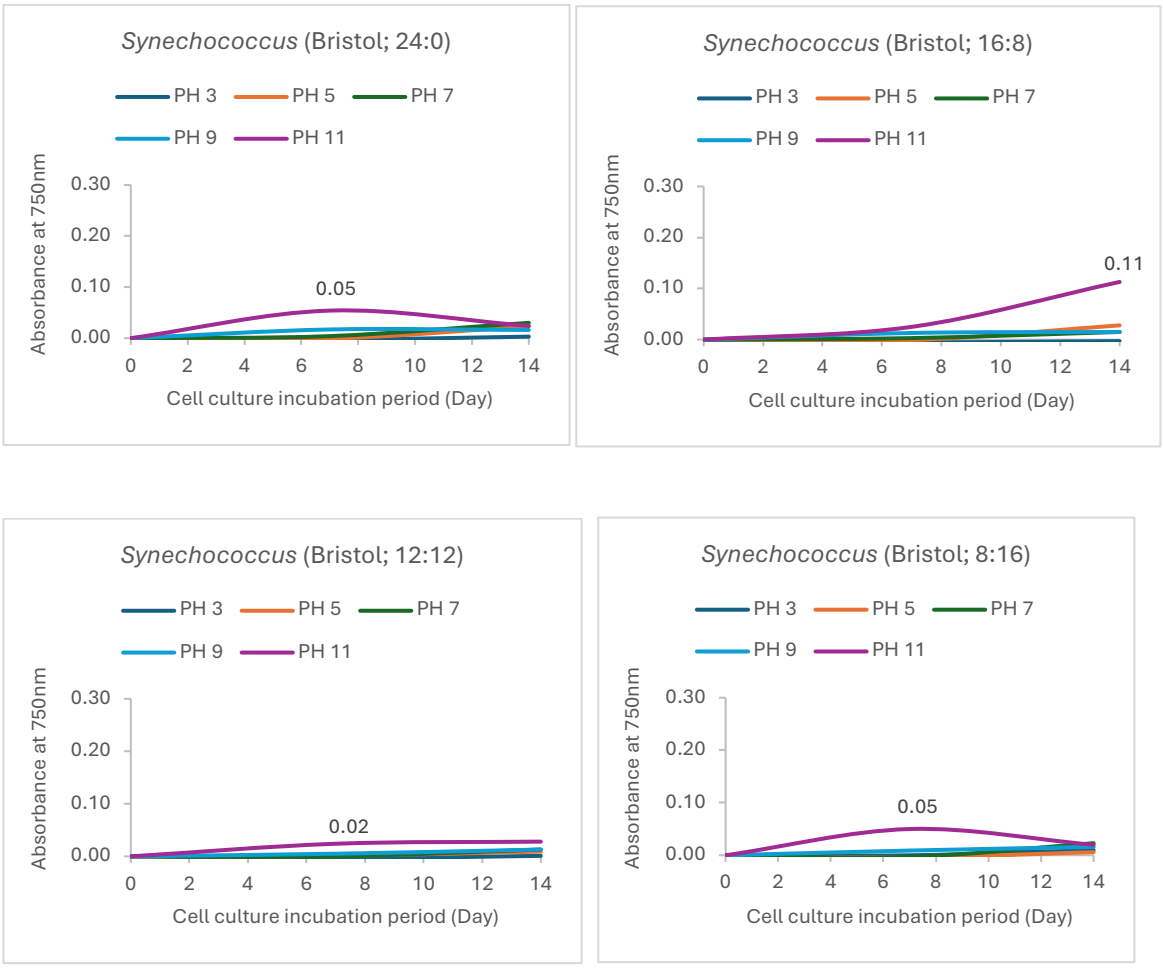


Figure 5.15 Effect of pH on cell culture growth of *Synechococcus* in Bristol medium.

Table 5.13 The optimum cell density and day of growth of *Synechococcus* in Bristol medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**Abs**
Synechococcus	24:0	pH 11 (Day 7)	0.05
	16:8	pH 11 (Day 14)	0.11
	12:12	pH 11 (Day 7)	0.02
	8:16	pH 11 (Day 7)	0.05

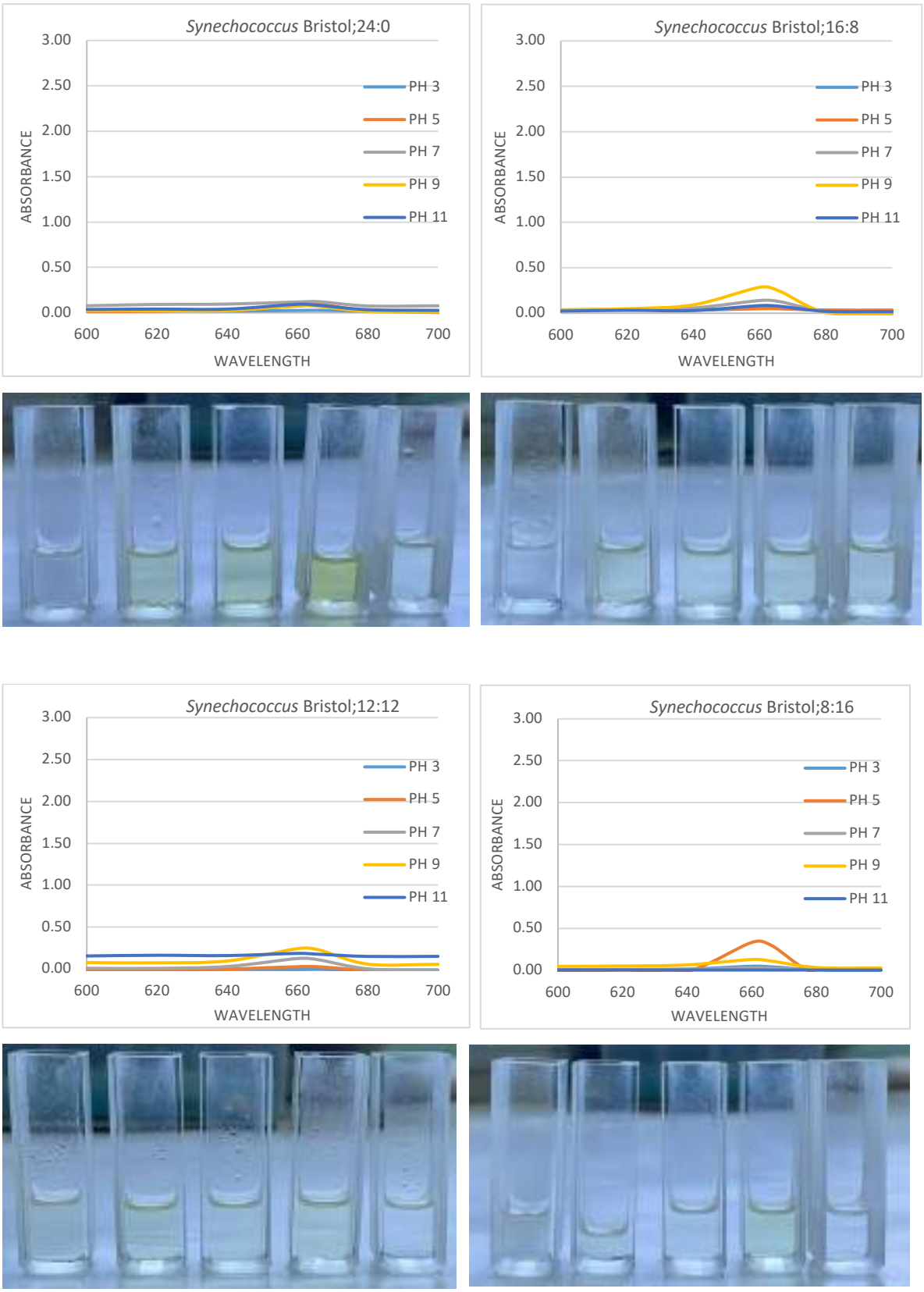


Figure 5.16: Effects of pH and photoperiod on chlorophyll content

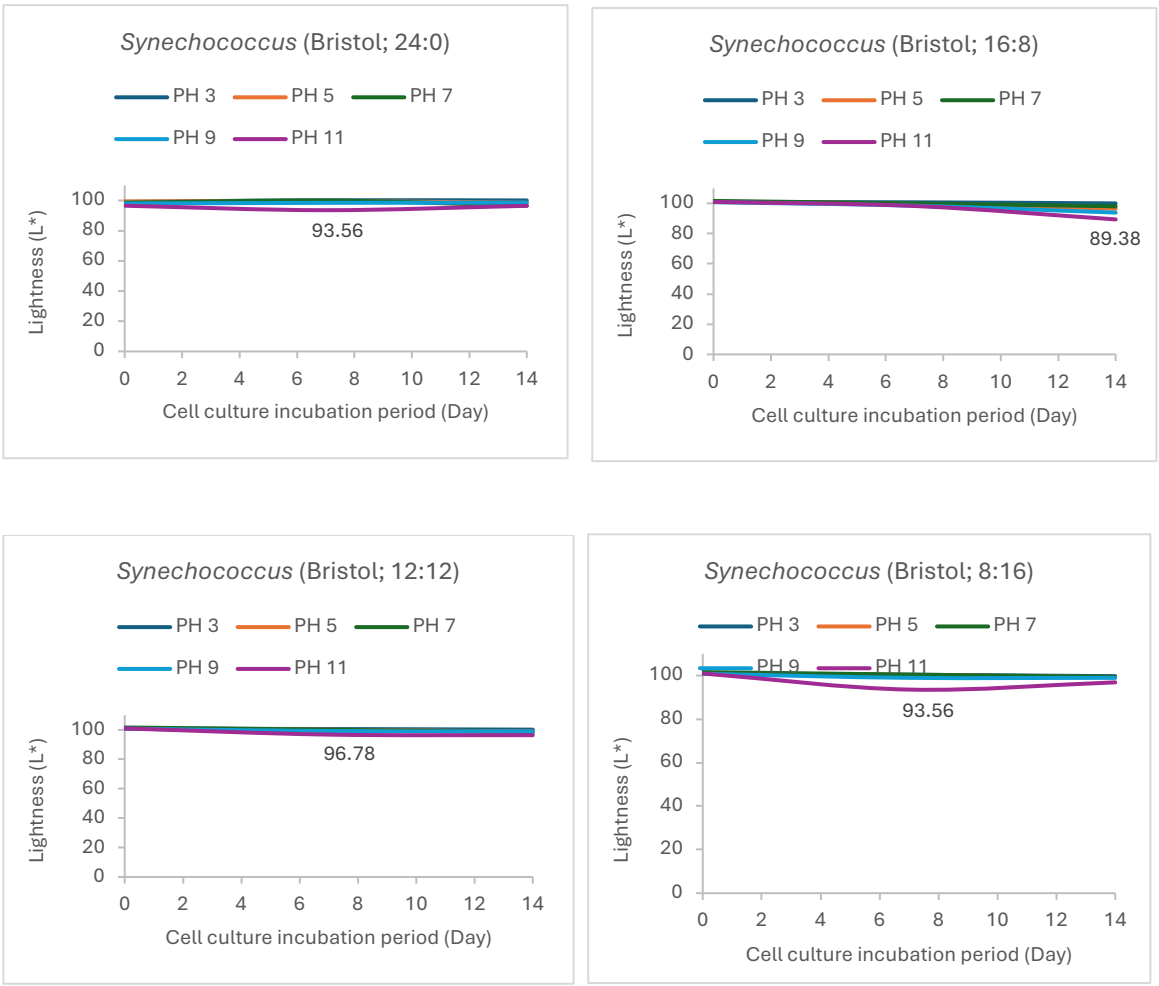


Figure 5.17 Effect of pH on lightness of *Synechococcus* in Bristol medium

Table 5.14 The lightness (L*) of *Synechococcus* cell culture growth in Bristol medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**L***
Synechococcus	24:0	pH 11 (Day 7)	93.56
	16:8	pH 11 (Day14)	89.38
	12:12	pH 11 (Day 7)	96.78
	8:16	pH 11 (Day 7)	93.56

L, ranges from black (0) to white (100)*

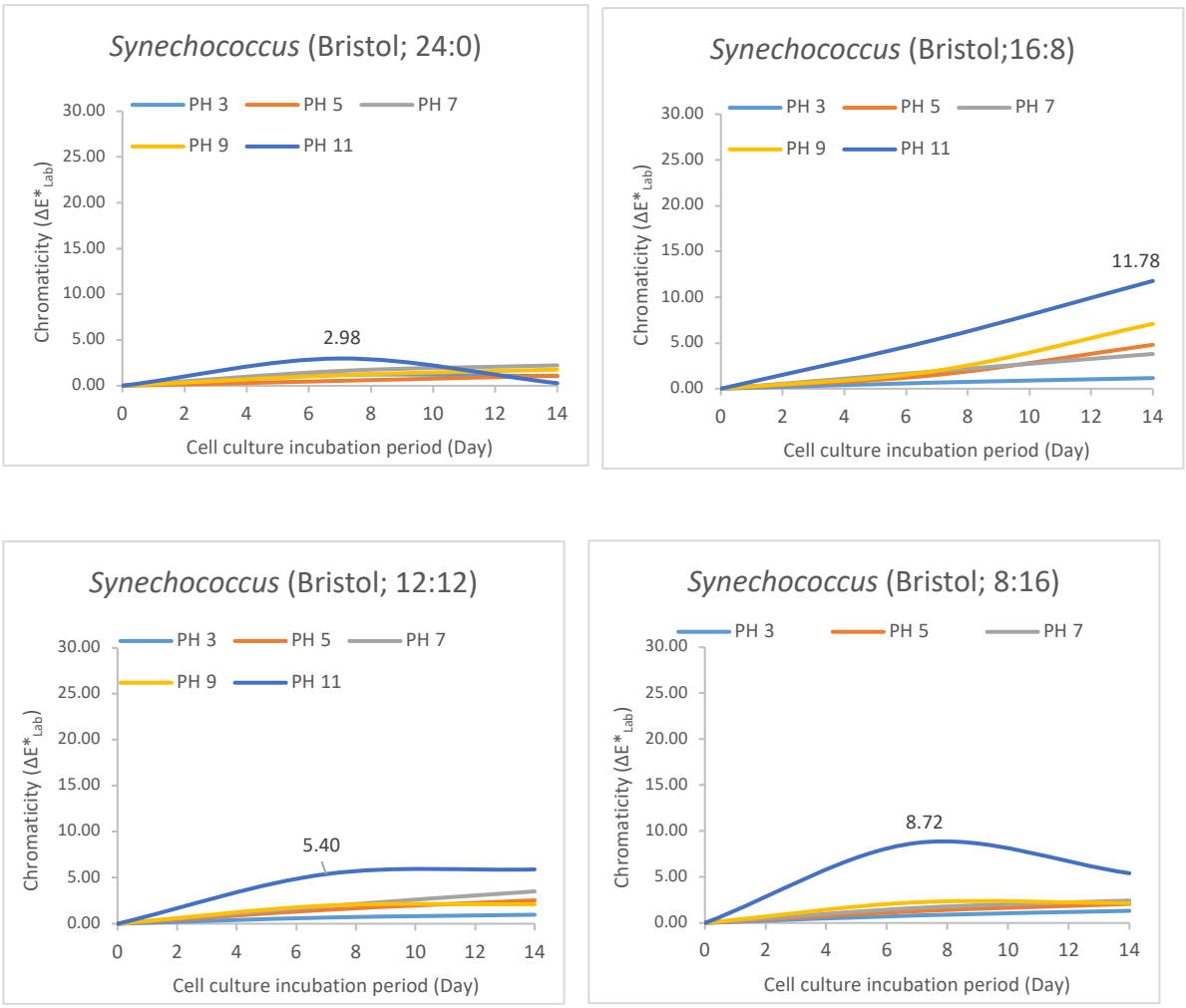


Figure 5.18 Effect of pH on Chromaticity of *Synechococcus* in Bristol medium

Table 5.15 CIE L*a*b* colour difference or Chromaticity (ΔE^*_{Lab}) of *Synechococcus* cell culture growth in Bristol medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**ΔE^*_{Lab}**
Synechococcus	24:0	pH 11 (Day 7)	2.98
	16:8	pH 11 (Day14)	11.78
	12:12	pH 11 (Day 7)	5.40
	8:16	pH 11 (Day7)	8.72

ΔE^*_{Lab} > 5.0 - indicate clearly two different colours

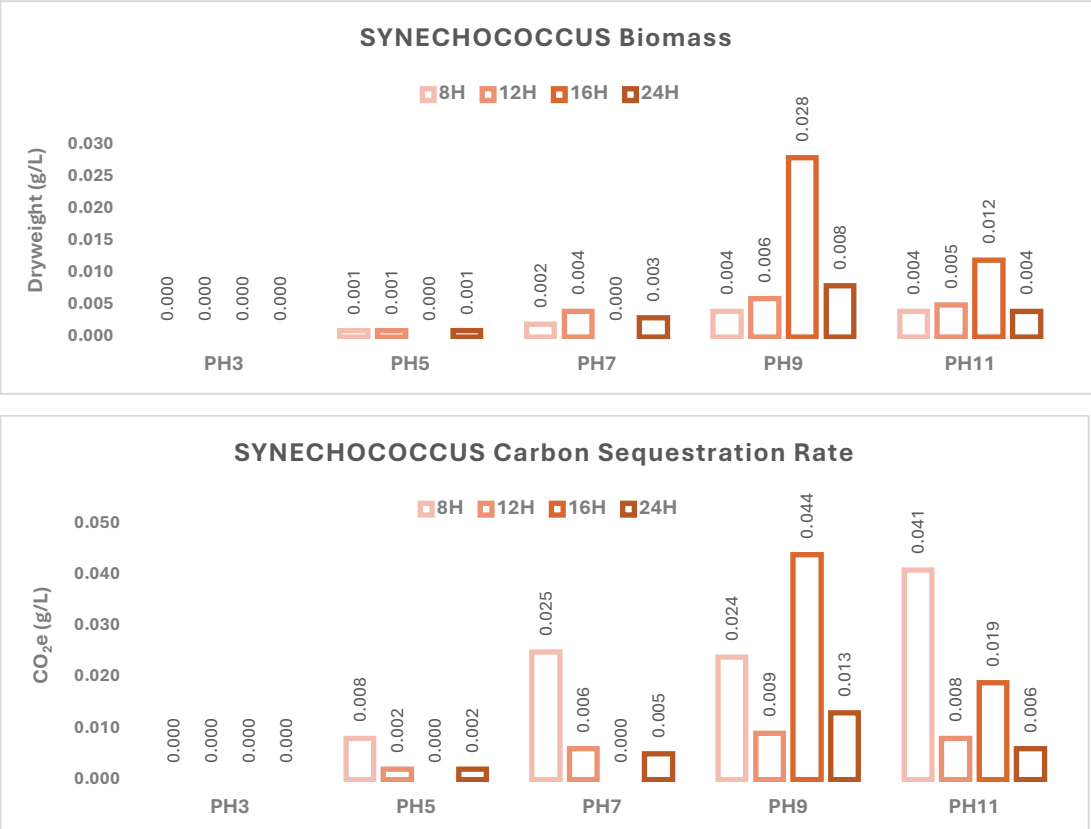


Figure 5.19 The biomass and carbon sequestration rate of *Synechococcus*

Table 5.16 The biomass and carbon sequestration rate of *Synechococcus* cell culture growth in Bristol medium at different pH and photoperiod.

Blue-Green Algae	**pH**	**Biomass (g/L)**	**CSR (CO2e)**
Synechococcus	pH 3(12H)	0.001	0.000
	pH 5 (8H)	0.001	0.003
	pH 7 (8H)	0.004	0.013
	pH 9 (16H)	0.028	0.044
	pH 11 (16H)	0.012	0.019

The highest absorbance (0.11) was recorded at pH 11 under a 16:8 photoperiod, while other photoperiods and pH levels, particularly pH 7 and 9, showed significantly lower growth. Cultures grown at pH 11 under a 24:0 photoperiod exhibited a reduced absorbance value of 0.05. Chlorophyll content peaked under a 16:8 photoperiod at pH 9, with strong absorbance around 660 nm. In contrast, minimal chlorophyll accumulation was observed at lower pH levels (3 and 5), confirming that chlorophyll is optimized in alkaline conditions (pH 9-11) with moderate light exposure.

The highest lightness (96.78) was recorded under a 12:12 photoperiod at pH 11, with slightly lower lightness values (93.56) under the 24:0 and 8:16 photoperiods. The lowest lightness (89.38) was observed under the 16:8 photoperiod at pH 11. This suggests that moderate light-dark cycles (12:12) promote lighter pigmentation, while prolonged light exposure results in slightly darker cultures. The highest chromaticity (11.78) was observed under the 16:8 photoperiod at pH 11, indicating greater pigment variation. In contrast, the lowest chromaticity (2.98) occurred under a 24:0 photoperiod, suggesting more uniform pigmentation with continuous light exposure.

Biomass and carbon sequestration rates (CSR) were highest at pH 9 under a 16-hour photoperiod, with biomass at 0.028 g/L and CSR at 0.044 g/L CO_2e. pH 11 showed similar but slightly lower results, while minimal growth and CSR were observed at lower pH levels (3 and 5). These findings confirm that alkaline conditions (pH 9-11) and a 16-hour photoperiod are ideal for *Synechococcus* growth and CO_2 capture.

Synechococcus growth in Bristol medium is optimized under alkaline conditions (pH 9-11) with a 16-hour photoperiod. Continuous light exposure (24:0) led to lower growth and less pigment variation compared to shorter photoperiods (16:8). Acidic conditions (pH 3-5) resulted in poor growth and minimal carbon sequestration, while moderate light exposure and alkaline pH supported higher biomass and CSR.

Effect of pH, Chul medium formulation and photoperiod on Synechococcus Cell Culture Growth, biomass, Chromaticity, and Chlorophyll content

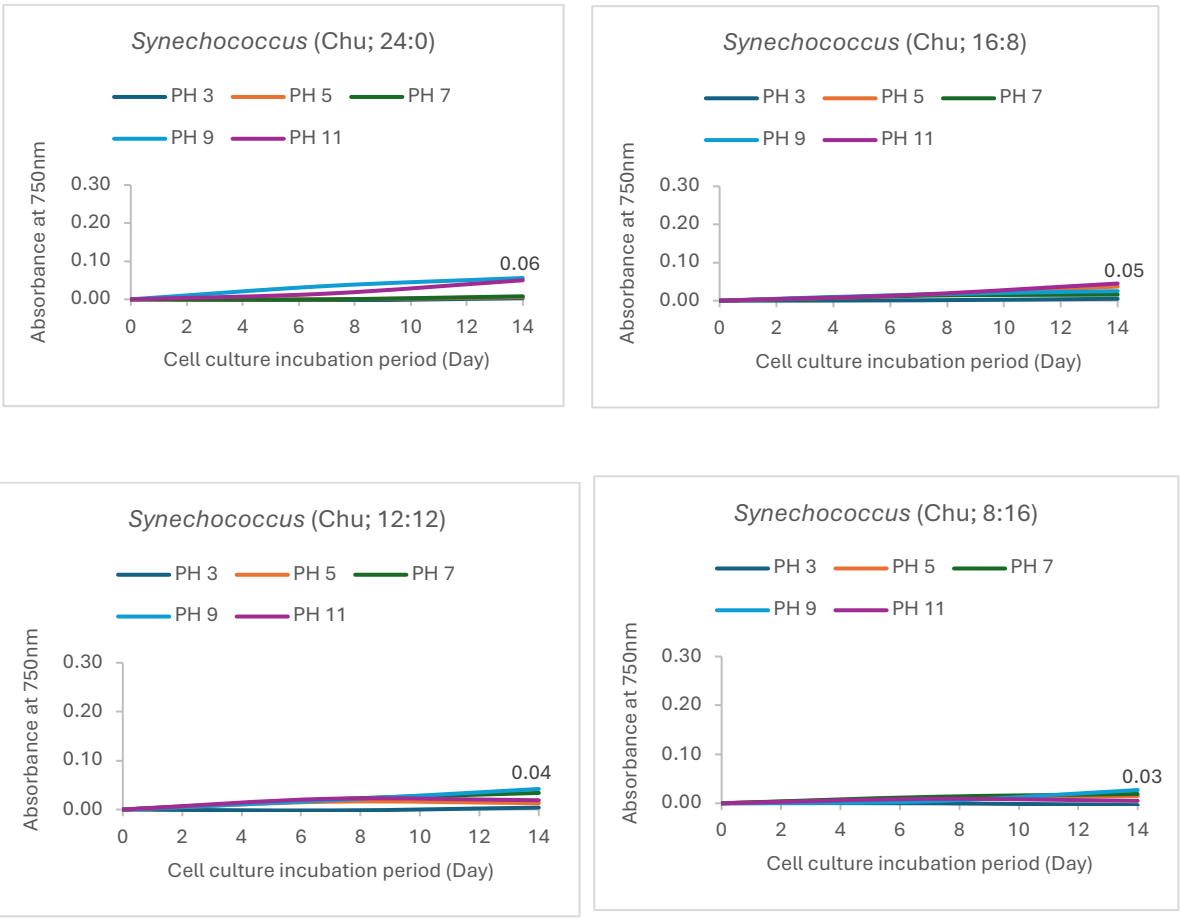


Figure 5.20 Effect of pH on cell culture growth of *Synechococcus* in Chu medium.

Table 5.17 The optimum cell density and day of growth of *Synechococcus* in Chu medium at different photoperiod

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**Abs**
Synechococcus	24:0	pH 9 (Day 14)	0.06
	16:8	pH 11 (Day 14)	0.05
	12:12	pH 9 (Day 7)	0.04
	8:16	pH 9 (Day 7)	0.03

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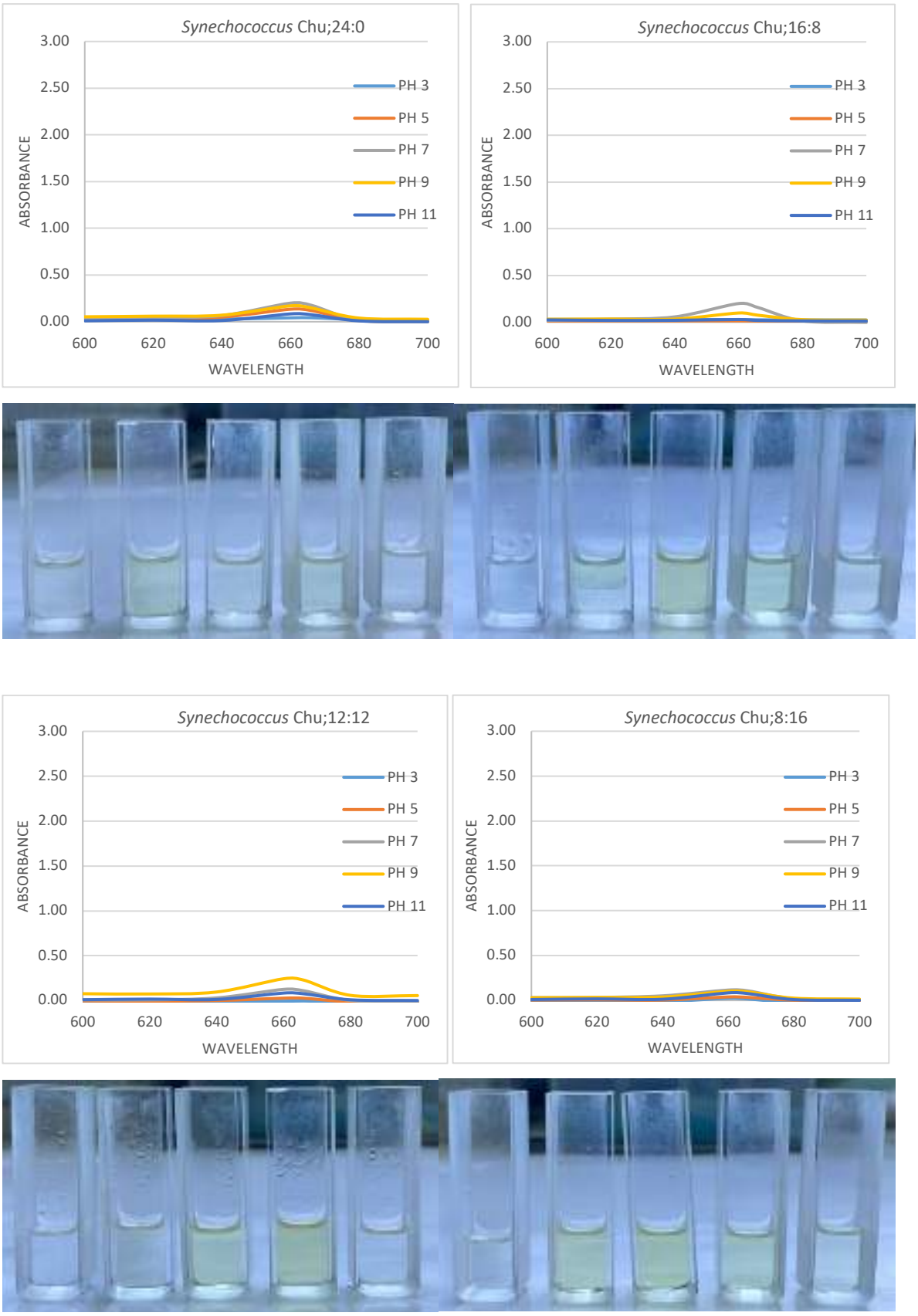


Figure 5.21: Effects of pH and photoperiod on for chlorophyll content

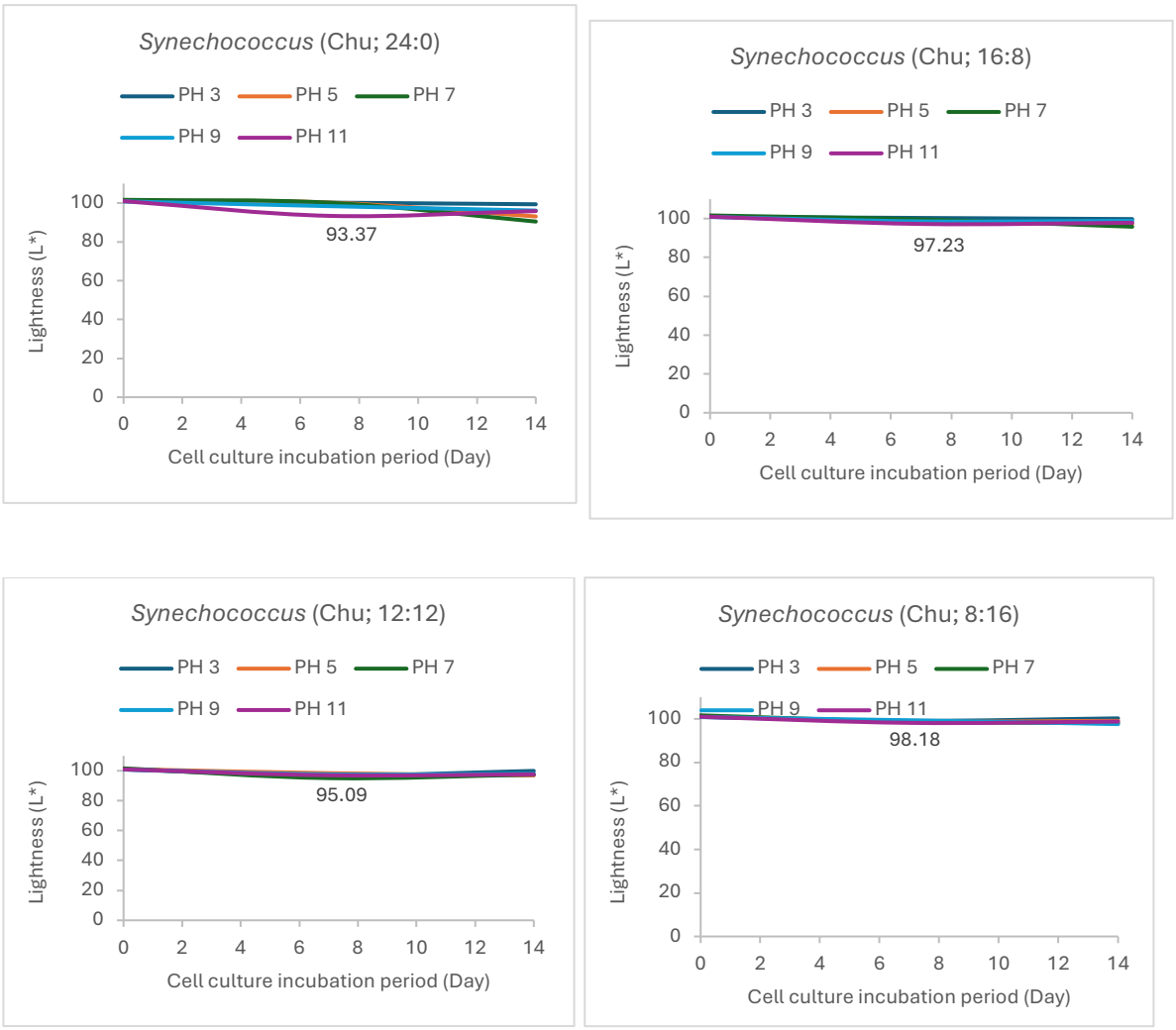


Figure 5.22 Effect of pH on lightness of *Synechococcus* in Chu medium

Table 5.18 The lightness (L*) of *Synechococcus* cell culture growth in Chu medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**L***
Synechococcus	24:0	pH 11 (Day 7)	93.37
	16:8	pH 11 (Day 7)	97.23
	12:12	pH 7 (Day 7)	95.09
	8:16	pH 11 (Day 7)	98.81

L, ranges from black (0) to white (100)*

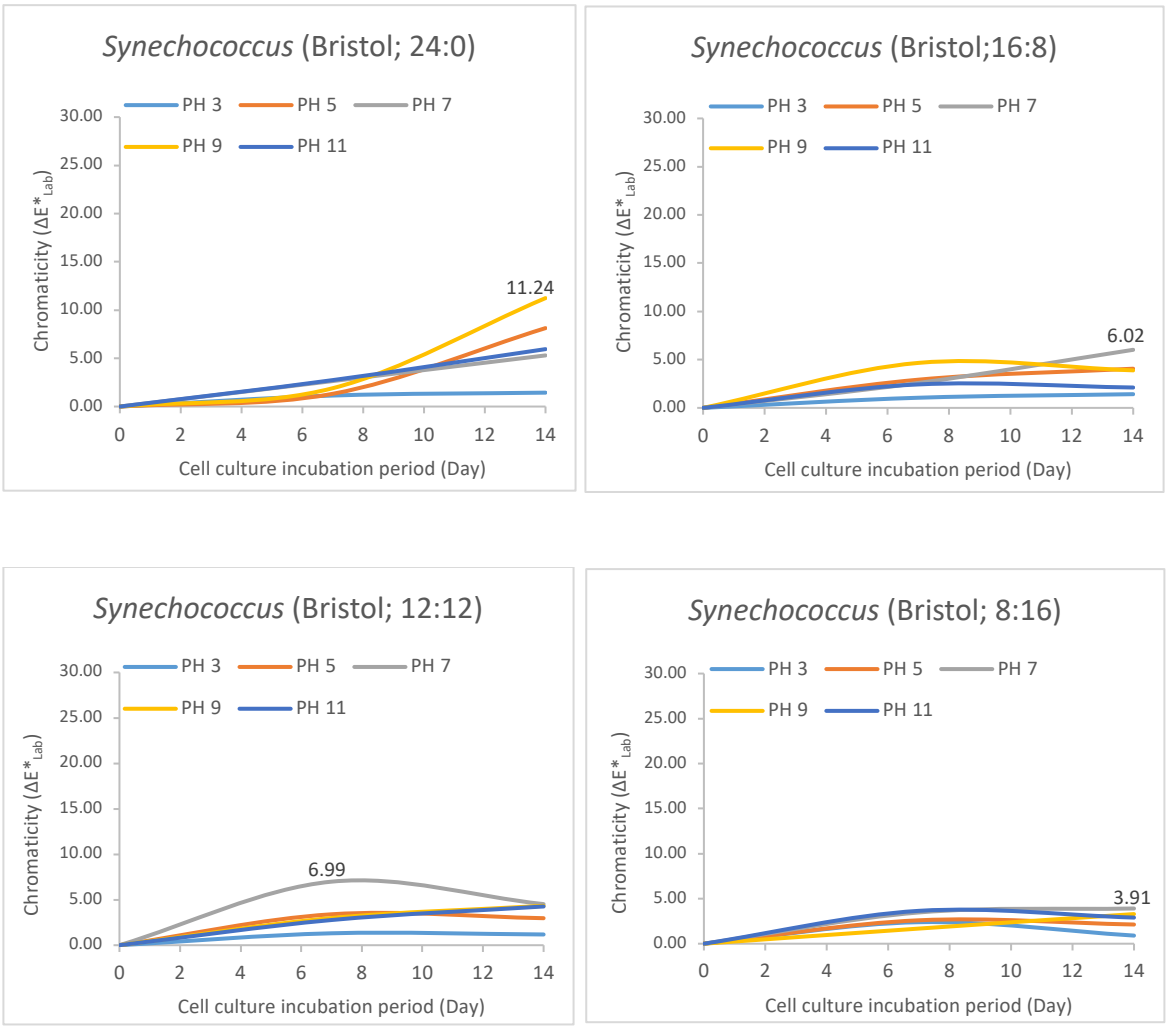
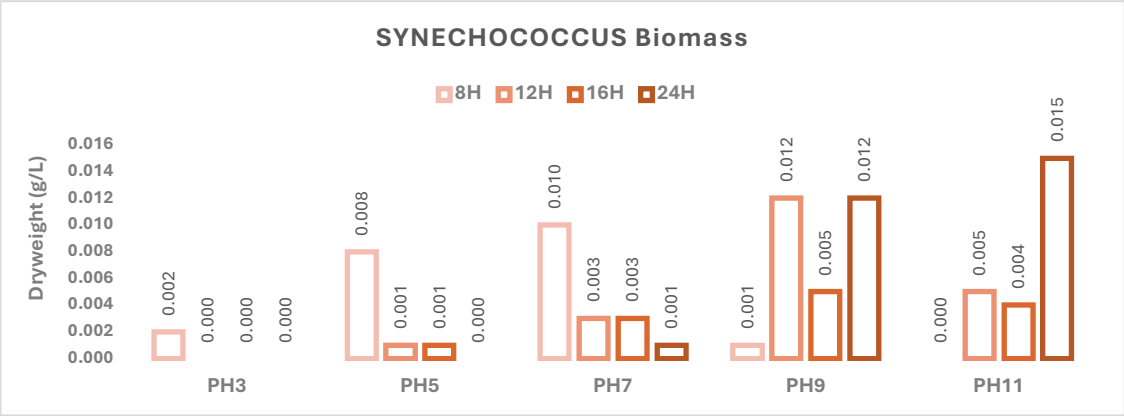


Figure 5.23 Effect of pH on Chromaticity of *Synechococcus* in Chu medium.

Table 5.19 The CIE L*a*b* colour difference or Chromaticity (ΔE^*_{Lab}) of *Synechococcus* cell culture growth in Chu medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**ΔE^*_{Lab}**
Synechococcus	24:0	pH 9 (Day 14)	11.24
	16:8	pH 7 (Day14)	6.02
	12:12	pH 7 (Day 7)	6.99
	8:16	pH 7 (Day 14)	3.91

ΔE^*_{Lab} > 5.0 - indicate clearly two different colours



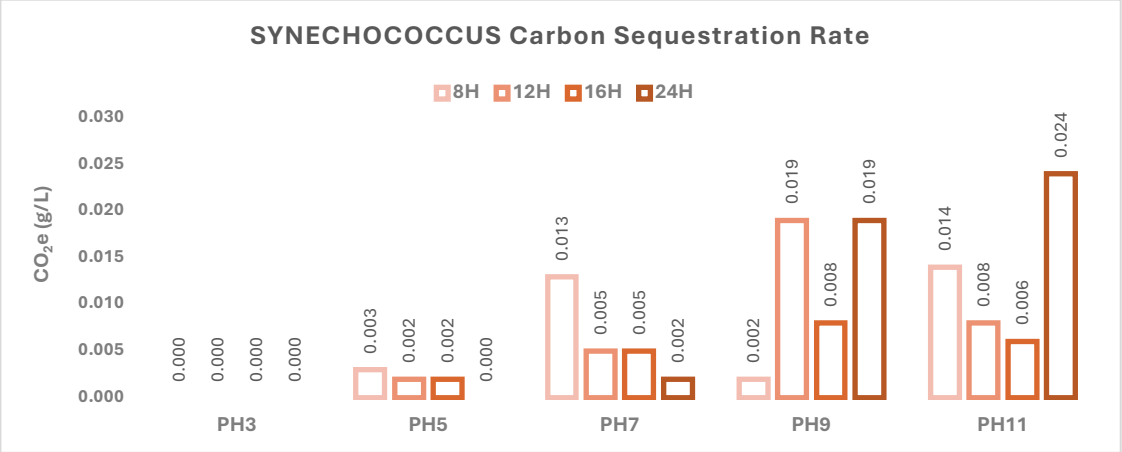


Figure 5.24 The biomass and carbon sequestration rate of *Synechococcus*

Table 5.20 The biomass and carbon sequestration rate of *Synechococcus* cell culture growth in Chu medium at different pH and photoperiod.

Blue-Green Algae	**pH**	**Biomass (g/L)**	**CSR (CO2e)**
Synechococcus	pH 3(12H)	0.002	0.000
	pH 5 (8H)	0.008	0.003
	pH 7 (8H)	0.010	0.013
	pH 9 (12H and 24H)	0.012	0.019
	pH 11 (24H)	0.015	0.024

The highest absorbance (0.06) was recorded at pH 9 on Day 14 under continuous light (24:0), indicating optimal growth for *Synechococcus* in Chu medium. The 16:8 photoperiod at pH 11 also showed good growth (absorbance 0.05), highlighting those alkaline conditions, especially with extended light, favor growth. Lower absorbance values were observed under shorter light periods (12:12 and 8:16), indicating less efficient photosynthesis. Chlorophyll content was highest at pH 9 and pH 11 under continuous light and 16:8 photoperiods, while shorter photoperiods (8:16) had reduced chlorophyll production, reflecting lower photosynthetic activity.

The highest lightness (L* = 98.81) was observed at pH 11 under an 8:16 photoperiod, with similarly high lightness (L* = 97.23) under the 16:8 photoperiod. This suggests that longer light periods in alkaline conditions lead to lighter pigmentation due to increased pigment production. The highest chromaticity (ΔE*Lab = 11.24) was recorded at pH 9 under continuous light, indicating significant color variation. Lower chromaticity was found in shorter light periods, suggesting reduced pigment changes.

Biomass production (0.015 g/L) and CSR (0.024 g/L CO_2e) were highest at pH 11 under continuous light, confirming that alkaline conditions and extended light exposure maximize growth and carbon capture. Moderate growth and CSR were observed under pH 9 with 12:12 and 16:8 photoperiods. Lower values were seen at acidic pH and shorter light periods.

Synechococcus growth in Chu medium is optimal under alkaline conditions (pH 9-11) and extended light exposure (24:0 and 16:8). These conditions enhance photosynthesis, biomass, and carbon sequestration, while shorter light periods and acidic pH reduce growth and metabolic activity.

5.3 DISCUSSION

The growth of *Synechococcus* under different culture media, pH levels, and photoperiods plays a crucial role in determining its effectiveness as a carbon sequestration agent and a landscape ecological indicator. These parameters directly impact biomass production, chlorophyll content, and photosynthetic efficiency, all of which contribute to its role in carbon cycling and climate mitigation.

Among the various media, BG11 is consistently cited as the most effective for *Synechococcus* growth, with studies reporting rapid doubling times and higher biomass production compared to BBM and Bristol (Yu et al., 2015; Watanabe et al., 2015). The superior performance of BG11 is attributed to its optimized nutrient composition, which supports efficient photosynthesis and carbon fixation. In contrast, BBM and Bristol media, while supporting growth, often result in lower biomass yields, limiting their effectiveness in large-scale carbon sequestration applications. The ability of *Synechococcus* to thrive in BG11 suggests its potential for CO_2 capture in engineered aquatic systems, such as algal biofilters, photobioreactors, and climate-resilient water bodies.

The pH of the culture medium is a critical determinant of *Synechococcus* growth and carbon fixation efficiency. A neutral pH (around 7) has been shown to support optimal growth, with the highest absorbance values at 750 nm correlating with increased cell density and photosynthetic activity (Ma et al., 2022). Extreme pH conditions (pH 3 and 11) negatively impact cell viability, pigment stability, and carbon sequestration potential, likely due to metabolic stress and altered enzymatic activity. These findings highlight the importance of pH regulation in sustainable algal cultivation systems, ensuring maximum CO_2 absorption in climate mitigation strategies.

Photoperiod also plays a significant role in regulating *Synechococcus's* growth and carbon sequestration capacity. Continuous light (24:0) and a 16:8 light/dark cycle promote higher biomass accumulation and chlorophyll content, which in turn enhances carbon fixation (Li et al., 2017). Shorter photoperiods limit light availability, reducing photosynthetic efficiency and carbon sequestration rates. Additionally, variations in photoperiod influence chromaticity and lightness values, which can serve as indicators

of environmental stress or optimal conditions. The ability of *Synechococcus* to adjust its pigment composition in response to light regimes reinforces its potential as a bio-indicator for monitoring ecosystem health and climate resilience.

The ability of *Synechococcus* to fix atmospheric CO_2 is directly influenced by its growth conditions. Optimal cultivation in BG11 at neutral pH under appropriate photoperiods enhances its carbon sequestration capacity, contributing to carbon cycling in aquatic ecosystems (Naaz et al., 2021). Biomass quantification under these conditions provides valuable insights into its role as a natural carbon sink, supporting its application in sustainable landscape planning, aquatic ecosystem management, and climate mitigation strategies.

Overall, *Synechococcus* demonstrates strong potential as a carbon sequestration agent and ecological indicator, particularly when cultivated in BG11 under neutral pH and extended photoperiods. These conditions optimize CO_2 fixation, biomass production, and pigment synthesis, making *Synechococcus* a viable candidate for sustainable carbon capture technologies and ecosystem monitoring. Future research should explore scaling its applications in urban blue-green infrastructure, bioengineered wetlands, and CO_2 capture systems to maximise its role in climate change mitigation.

5.4 MULTIVARIATE DATA ANALYSIS

Sampling Adequacy for Multivariate analysis

Figure 5.23 revealed the box and whisker plot to show the comparison in distributions of variables graphically to identify outliers. The boxes portrayed the 25th percentile (lower quartile), median, the 75th percentile (upper quartile) value of data and less or more than that located outside of the boxes, known as outliers. In this plot, the outliers remained as the data is significant to show the highest values for each variable. Among the 12 different medians, it is evident that the median value of H (Hue) stands out as the highest at 260.829, while the lowest median is observed for b, with a value of -

0.090. This distinction between the variables emphasizes the range and variability present within the dataset.

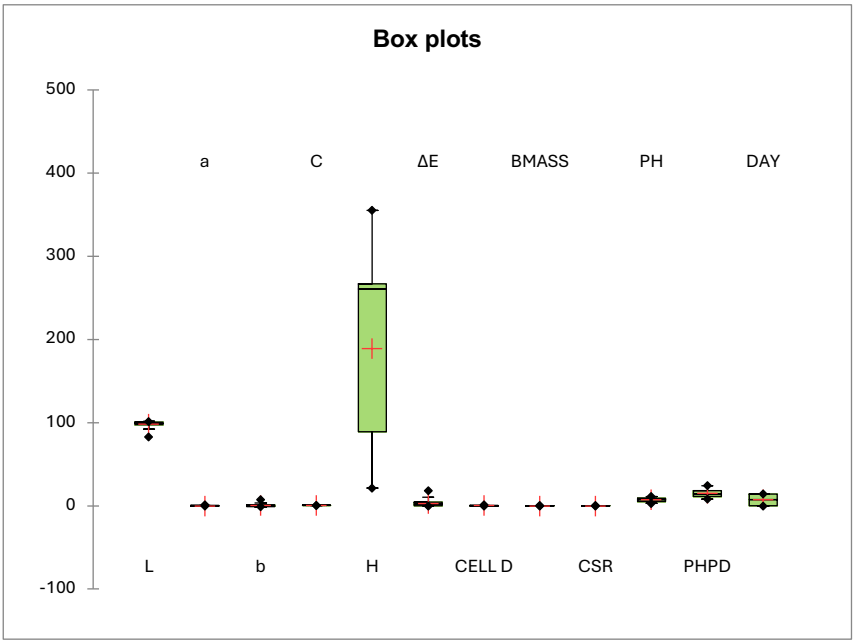


Figure 5.25: Box-and-whisker plots of *Synechococcus* cell culture growth at different medium formulations, pH, photoperiod and cell culture growth time towards biomass, carbon sequestration rate and chromaticity

The KMO values for the 240 datasets produced a KMO score of 0.817 (Table 5.21) confirming the dataset is very good for PCA test. Barlett's Test of Sphericity is measured to ensure that the R-matrix does not equal the identity matrix. The *p*-value for *Synechococcus* cell culture growth chromaticity stability test exhibited highly significant differences at 0.0001.

Table 5.21 Kaiser-Meyer-Olkin and Bartlett's test for the *Synechococcus* cell culture growth chromaticity stability test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy		**0.817**
Bartlett's Test of Sphericity	Chi-square (Observed value)	4687.795
	DF	66
	p-value (Two-tailed)	**< 0.0001**

A positive and negative correlations strength between two variables among *Synechococcus* cell culture growth chromaticity stability test. A very strong positive correlation was observed among CSR and Biomass (r^2 = 1.000), whereas other variables such as C and ΔE versus b (r^2 = 0.722 to 0.855) as well as H and L with b, C, ΔE, Biomass, CSR, Day versus a and Day versus ΔE (r^2 = 0.523 to 0.686) have high and moderate positive correlations respectively. The tables also showed the relationship between 2 groups of a , b, c and ΔE versus L* and H and b produced a strong significant negative correlation (r^2 = -0.853 to 0.703). In general, *C.vulgaris* cell culture growth chromaticity stability test variables can be grouped into four clusters with two cluster with positive correlations and another two clusters with negative correlations.

Table 5.22 Correlation coefficient matrix for the *Synechococcus* cell culture growth chromaticity stability test.

Variables	**L**	**a**	**b**	**C**	**H**	**ΔE**	**Cell Density**	**Biomass**	**CSR**	**pH**	**Photo period**	**Day**
L	**1**											
a	**-0.826**	1										
b	**-0.816**	-0.120	1									
C	**-0.795**	0.466	0.257	1								
H	**0.596**	0.008	-0.647	-0.111	1							
ΔE	**-0.853**	0.803	0.078	0.487	-0.144	1						
Cell Density	**-0.345**	0.505	-0.082	0.271	-0.012	0.470	1					
Biomass	**-0.486**	0.358	0.174	0.072	-0.100	0.344	0.161	1				
CSR	**-0.486**	0.358	0.175	0.071	-0.100	0.344	0.161	1.000	1			
pH	**-0.325**	0.070	0.372	0.243	-0.260	0.116	0.144	0.149	0.149	1		

Photoperiod	-0.156	0.103	-0.007	0.062	-0.103	0.124	0.314	0.059	0.059	0.000	1
Day	**-0.550**	0.540	0.153	0.220	-0.017	0.523	0.372	0.331	0.331	0.000	0.000	1

Values in bold are different from 0 with a significance level alpha=0.01

The correlation coefficient (r^2) or the strength of the correlation between two variables *Synechococcus* cell culture growth chromaticity stability test. The correlation coefficient is measured on a scale from -1 to +1 as described by Guilford (1973).

Table 5.23 The correlation coefficient (r^2) strength between two variables among *Synechococcus* cell culture growth chromaticity stability test

Variables	r^2	Size of Correlation	Interpretation
CSR x Biomass	**1.000**	0.90 to 1.00	Very high positive correlation
C x b	**0.855**	0.70 to 0.90	High positive correlation
ΔE x b	**0.722**		
H x L	**0.596**	0.50 to 0.70	Moderate positive correlation
b x a	**0.541**		
C x a	**0.560**		
ΔE x a	**0.686**		
Biomass x a	**0.575**		
CSR x a	**0.575**		
Day x a	**0.523**		
Day x ΔE	**0.582**		
Cell D x a	**0.327**	0.30 to 0.50	Low positive correlation
Biomass x b	**0.378**		
CSR x b	**0.378**		
pH x a	**0.365**		
Day x b	**0.461**		
Day x C	**0.497**		
Cell D x ΔE	**0.375**		
Biomass x ΔE	**0.345**		
CSR x ΔE	**0.345**		
pH x Cell D	**0.314**		
Day x Cell D	**0.372**		
Day x Biomass	**0.331**		
Day x Biomass	**0.331**		

A x L	**-0.826**	-0.70 to -0.90	High negative correlation
B x L	**-0.816**		
C x L	**-0.795**		
ΔE x L	**-0.853**		
H x b	**-0.703**		
Day x L	**-0.550**	-0.50 to -0.70	Moderate negative correlation
H x C	**-0.644**		
ΔE x H	**-0.539**		
Cell D x L	**-0.345**	-0.30 to -0.50	Low negative correlation
Biomass x L	**-0.486**		
CSR x L	**-0.486**		
pH x L	**-0.325**		
H x a	**-0.339**		
pH x H	**-0.336**		
Day x H	**-0.399**		
Values in bold are different from 0 with a significance level alpha=0.01			

The next step involved analyzing the data set using PCA to identify variables with high factor loadings that explain the principal components significantly. A factor loading greater than 0.75 was considered strong, between 0.50 and 0.75 was moderate, between 0.30 and 0.49 was weak, and less than 0.30 was negligible (Rani et al., 2017). Eigenvalues were used to determine the principal components with more cumulative dataset variations. An eigenvalue of 1 or greater was considered significant for the PCA (Le et al., 2017; Zubir et al., 2016; Yang et al., 2020). PCA has been applied to the 12 variables taken from *Synechococcus* cell culture growth chromaticity stability test to study which variables significantly contributed to CV cell culture as detailed by Siudek & Frankowski (2018) and Vuong et al. (2020). The PCA exposed two principal components (PCs) entailing 12 variables that represent a cumulative variability of 61.028%. The eigenvalues (EV) were used to determine the principal components (PCs) with more cumulative dataset variations. The EV obtained for PC1 was 5.68, and for PC2 was 1.644 as shown in Table 5.24. In principle, variables far away from the axes F1 and F2 had a strong factor loading (FL). Referring to Table 5.24, L* had strong FL (FL > 0.75) whereas H, Biomass and CSR had moderate FL (0.500 < FL < 0.749) and others had a weak FL (FL < 0.499).

Table 5.24 The principal components factor loading and eigenvalues of the correlation matrix for *Synechococcus* cell culture growth chromaticity stability test

Variable	PC1	PC2
L	0.942	0.073
a	-0.812	0.253
b	-0.855	-0.279
C	-0.834	-0.350
H	0.674	0.431
ΔE	-0.858	-0.170
CELL D	-0.406	0.034
BMASS	-0.613	0.731
CSR	-0.613	0.731
PH	-0.371	-0.289
PHPD	-0.163	0.071
DAY	-0.658	0.023
Eigenvalue	5.680	1.644
Variability (%)	47.331	13.697
Cumulative (%)	47.331	**61.028**

The first principal PC1 accounted for 47.331% of the total variance, which comprised strong factor loadings of L* and s whereas for PC2, no single strong variables were identified, comprising of 13.697% of the total variance for the data. The PCA analysis allows for identifying factors and provides insight in *Synechococcus* cell culture growth chromaticity stability test. Therefore, factor loadings can be utilised to determine the strength of the association between variables in *C.vulgaris* cell culture growth chromaticity stability test.

Table 5.25 Summarised that all variables were either positively or negatively correlated to each other which can be grouped into four as below.

H, L	**Group 1**
-	**Group 2**
ΔE, b, C, pH	**Group 3**
Phpd, Cell D, Day, a, Biomass, CSR	**Group 4**

All variables were positively correlated as long as they were grouped close together such as group 1, group 2 and group 3 variables. Group 1 and group 3 as well as group 2 and group 4 were negatively correlated as there were at the opposite positioned.

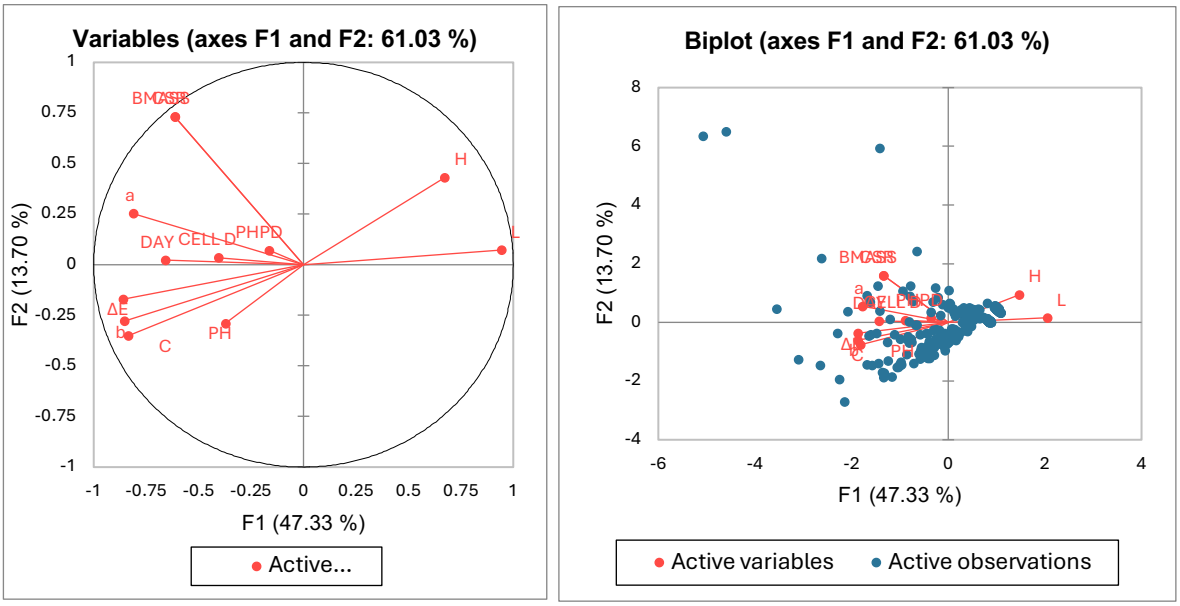


Figure 5.26 All variables were positively correlated as long as they were grouped close together as shown above

5.5 CHAPTER SUMMARY

Table below shows the comparison of *Synechococcus* Growth Parameters Across Different Media, Photoperiods, and pH Levels

Table 5.26 Comparison of *Synechococcus* Growth Parameters Across Different Media, Photoperiods, and pH Levels"

Parameters	**BBM**	**BG11**	**Bristol**	**Chu**
Absorbance	0.16	0.13	0.11	0.006
(750nm)	16:8	16:8	16:8	24:0
	pH 11	pH 9	pH 11	pH 9
Total Chlorophyll Content	pH 9	pH 9	pH 11	pH 9
(UV-Vis Peak)	16:8	16:8	16:8	24:0
Lightness	8:16	8:16	12:12	8:16
(L*)	pH 11	pH 11	pH 11	pH 11
Chromaticity	16:8	12:12	16:8	24:0
(ΔE*Lab)	pH 11	pH 7	pH 11	pH 9
Biomass	1.28	0.412	0.028	0.015
(g/L)	16:8	16:8	16:8	24:0
	pH 9	pH 11	pH 9	pH 11
Carbon sequestration rate	2.006	0.646	0.044	0.024
(g/L C02e)	16:8	16:8	16:8	24:0
	pH 9	pH 11	pH 9	pH 11

BBM showed the best overall performance for *Synechococcus* growth, achieving the highest absorbance (0.16 at 750 nm), biomass (1.28 g/L), and carbon sequestration rate (2.006 g/L CO2e) under a 16:8 photoperiod at pH 9. BG11 also performed well, with an absorbance of 0.13, biomass of 0.412 g/L, and a CSR of 0.646 g/L CO2e at pH 9 under a 16:8 photoperiod.

Bristol exhibited moderate performance, with an absorbance of 0.11, peak lightness under a 12:12 photoperiod at pH 11, and biomass of 0.028 g/L at pH 9. Chu displayed the lowest growth parameters, with a maximum biomass of 0.015 g/L and CSR of 0.024 g/L CO2e under a 24:0 photoperiod at pH 11.

The growth of *Synechococcus* across BBM, BG11, Chu, and Bristol media under different photoperiods and pH levels shows that continuous light (24:0) and alkaline conditions (pH 9-11) maximize growth parameters. BBM under a 16:8 photoperiod and pH 9 provided the highest biomass and carbon sequestration rates. In contrast, shorter photoperiods and acidic conditions, particularly in Chu medium, led to reduced growth and chlorophyll content.

CHAPTER SIX

PHORMIDIUM

6.1 INTRODUCTION

Phormidium sp. is a filamentous cyanobacterium belonging to the family Phormidiaceae, recognized for its ecological versatility and significant role in various environments, including freshwater, hypersaline, and terrestrial habitats. This genus is particularly notable for its ability to thrive in extreme conditions, such as deserts and saline environments, where it contributes to the formation of microbial mats and soil stabilization processes. Furthermore, *Phormidium* Also known to be a resilient cyanobacterium that plays a multifaceted role in various ecosystems, contributing to soil health, water quality, and ecological stability. Its adaptability to extreme environments and its potential for biotechnological applications make it a subject of interest for further research in environmental science and biotechnology.

6.2 RESULTS AND DISCUSSIONS

6.2.1 Cell Culture Growth of *Phormidium* in different medium formulation, PEG and SA Concentrations

Effect of Different Medium Formulation on Phormidium Cell Culture Growth

In this experiment, four types of medium formulations which are BBM, BG11, Bristol, and Chu10 were used. The cell culture growth performance of *Syne.* across the various media formulations were measured through their absorbance at the wavelength of 750 nm each day for 2 weeks. By optimizing the medium composition, it is possible to enhance cell density and potentially improve the overall productivity and bioactivity of *Phormidium* in various applications.

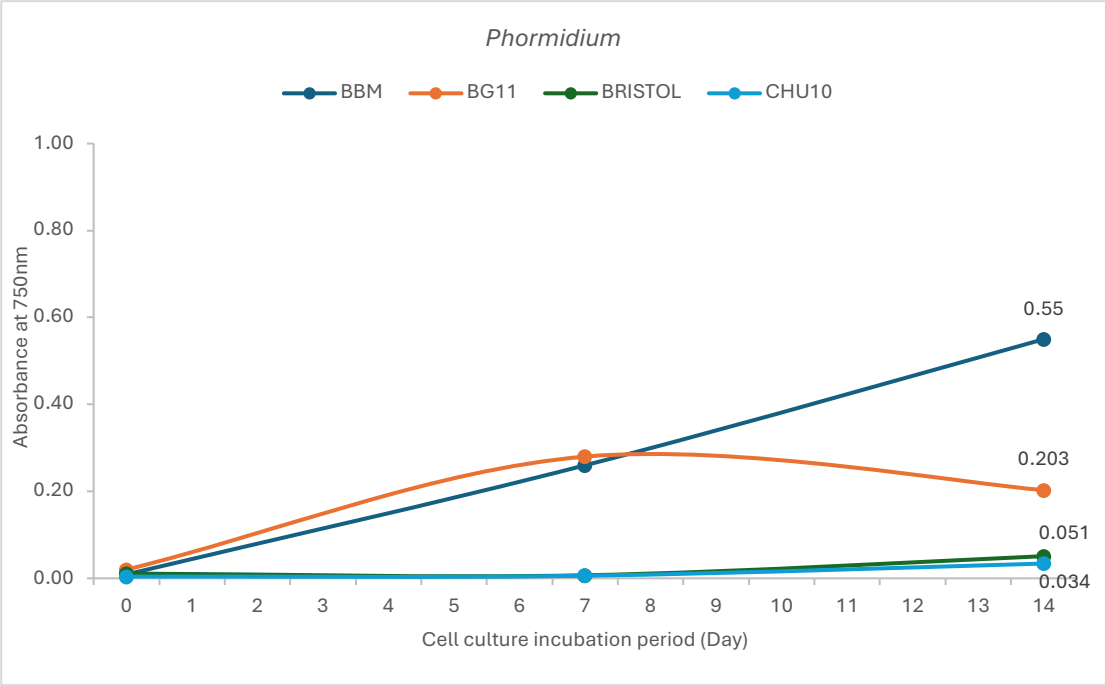


Figure 6.1 Distribution of cell culture growth of *Phormidium* in different medium formulations

Table 6.1 The optimum absorbance value *Phormidium* cell culture growth in different media formulation

Blue-Green Algae	**Medium**	**Abs**
Phormidium	BBM	0.55
	BG11	0.20
	BRISTOL	0.05
	CHU10	0.03

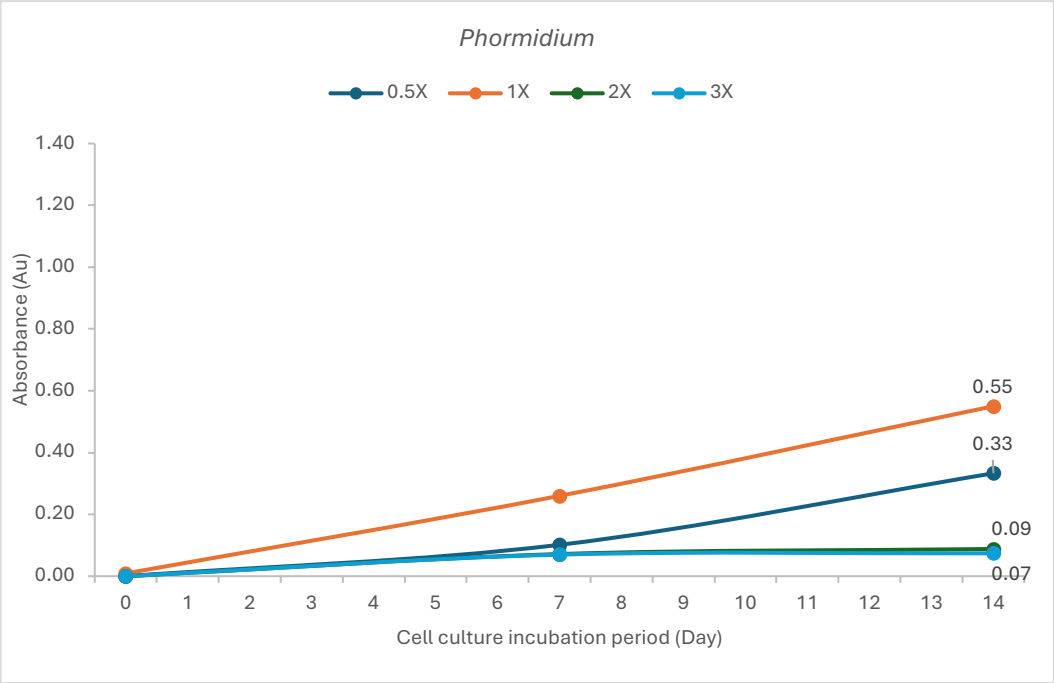


Figure 6.2 Distribution of cell culture growth of *Phormidium* in different medium strength

Table 6.2 The highest absorbance value of *Phormidium* cell culture growth in different BBM medium formulation strength

Blue-Green Algae	**Medium strength**	**Abs**
Phormidium	0.5	0.33
	1x	0.55
	2x	0.09
	3x	0.07

The highest absorbance value of 0.55 was recorded in BBM medium, suggesting that this medium formulation is the most favorable for its growth. BG11 medium also supported *Phormidium* growth to a lesser extent, with an absorbance value of 0.20. However, Bristol and Chu10 media exhibited significantly lower absorbance values of 0.05 and 0.03, respectively, indicating suboptimal conditions for *Phormidium* growth in these media. The disparity in growth across these media suggests that *Phormidium*

thrives better in nutrient-rich environments such as BBM, compared to other formulations.

The optimal absorbance value of 0.55 was observed at a standard 1x BBM medium strength, highlighting that this concentration provides the most suitable conditions for growth. In contrast, varying the BBM medium strength resulted in decreased absorbance values, with 0.33 for 0.5x strength, 0.09 for 2x strength, and 0.07 for 3x strength. This pattern indicates that both dilution and concentration of the medium beyond the standard 1x formulation hinder the growth of *C. vulgaris*, emphasizing the importance of maintaining an optimal nutrient balance for maximum cell density.

The optimal absorbance value for *Phormidium* cultured in Bold Basal Medium (BBM) can be influenced by several factors, including light conditions, growth phase, and specific wavelengths used for measurement. While specific absorbance values for *Phormidium* in BBM are not extensively documented in the literature, general trends in cyanobacterial absorbance can provide insights. In BBM, the absorbance values can vary depending on the growth conditions. For example, under optimal light conditions, *Phormidium* can exhibit absorbance values that indicate high biomass production. Research has indicated that the absorbance of cyanobacteria can reach significant levels when cultured under favorable light spectra, which enhances the synthesis of chlorophyll and phycobiliproteins (Alfredo et al., 2011). This suggests that monitoring absorbance at specific wavelengths can provide a reliable estimate of biomass in BBM.

Next experiment, *Phormidium* was measured by absorbance at 750nm over a 14-day incubation period, in two separate studies: one with different PEG concentrations and the other with varying salicylic acid concentrations.

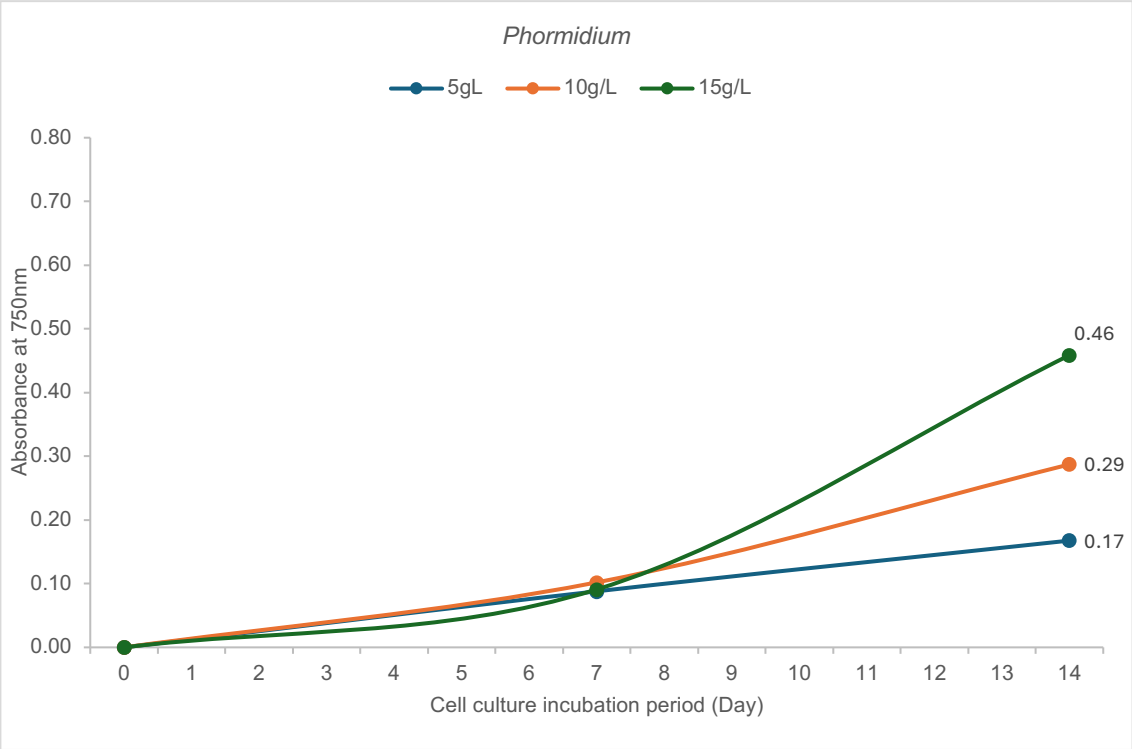


Figure 6.3 Distribution of cell culture growth of *Phormidium* in different PEG concentrations.

Table 6.3 The highest absorbance value of *Phormidium*. cell culture growth in different PEG concentrations

Blue-Green Algae	**PEG Conc. (g/L)**	**Peak (Day)**	**Abs**
Phormidium	5	D14	0.17
	10	D14	0.29
	15	D14	0.46

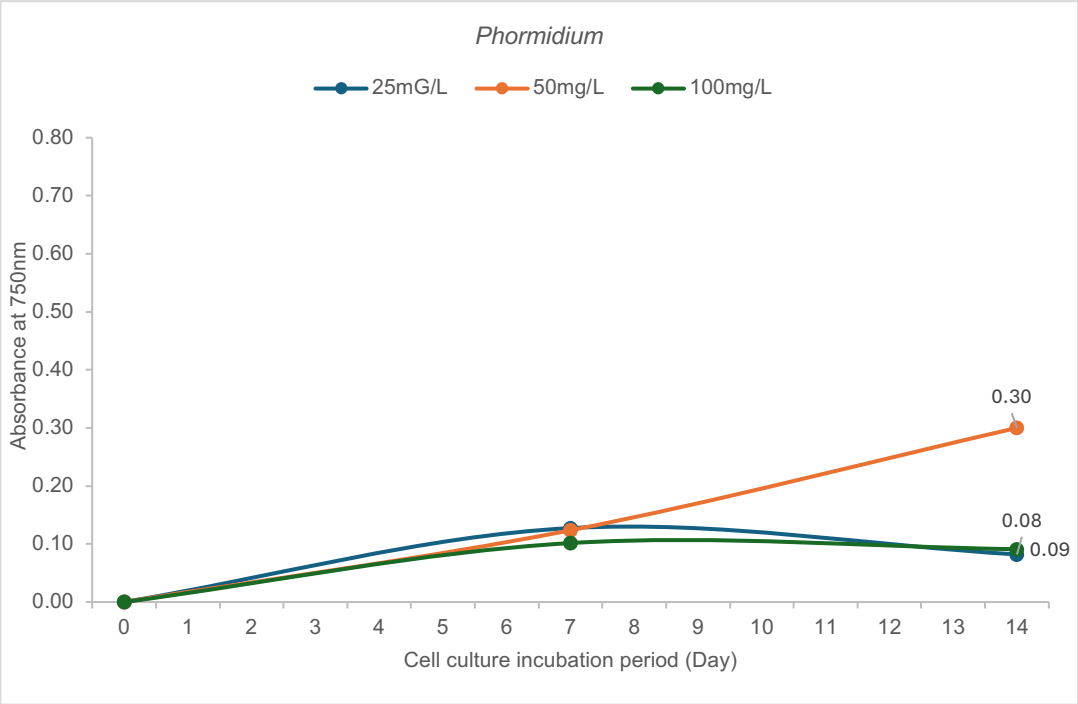


Figure 6.4 Distribution of cell culture growth of *Phormidium* in different SA concentrations.

Table 6.4 The highest absorbance value *Phormidium* cell culture growth in different SA concentrations

Blue-Green Algae	**SA Conc. (mg/L)**	**Peak (Day)**	**Abs**
Phormidium	25	D7	0.13
	50	D14	0.30
	100	D7	0.10

For PEG concentrations, the absorbance values increase as the concentration of PEG rises. At 5 g/L PEG, the absorbance is recorded as 0.17 on day 14, indicating moderate growth. As the PEG concentration increases to 10 g/L and 15 g/L, the absorbance values increase to 0.29 and 0.46, respectively, also on day 14. This trend suggests that higher PEG concentrations positively influence *Phormidium* growth under these conditions, with 15 g/L PEG yielding the highest absorbance. The medium

concentration of SA (50 mg/L) resulted in the highest absorbance, suggesting it is the most effective at promoting growth in *Syne*. cultures over time. Lower concentrations (25 mg/L) showed a moderate effect, peaking earlier, while higher concentrations (100 mg/L) may have caused a slight inhibitory effect, leading to lower growth levels.

Studies have shown that PEG can stimulate the accumulation of various metabolites in plant cell cultures, suggesting a similar potential in cyanobacterial cultures (Deshmukh et al., 2023). The application of PEG has been linked to increased production of phenolic compounds and other secondary metabolites, which are crucial for the survival and adaptation of organisms under stress conditions (Deshmukh et al., 2023). Salicylic acid, on the other hand, has been shown to enhance the production of secondary metabolites in various plant species. For example, reported that the application of SA in cell suspension cultures led to a significant increase in the accumulation of phenolic compounds (Açıkgöz et al., 2019). This suggests that SA could similarly enhance the production of valuable metabolites in *Phormidium* cultures.

The combination of PEG and SA as elicitors may synergistically enhance the growth and metabolite production in *Phormidium* The stress induced by PEG could prime the cells for a more robust response to SA, potentially leading to higher yields of desired metabolites. This dual elicitation strategy has been explored in other plant systems, where the combined effect of different elicitors resulted in enhanced production of secondary metabolites (Du et al., 2014).

Effect of pH, BBM medium formulation and photoperiod on Phormidium Cell Culture Growth, biomass, Chromaticity, and Chlorophyll content

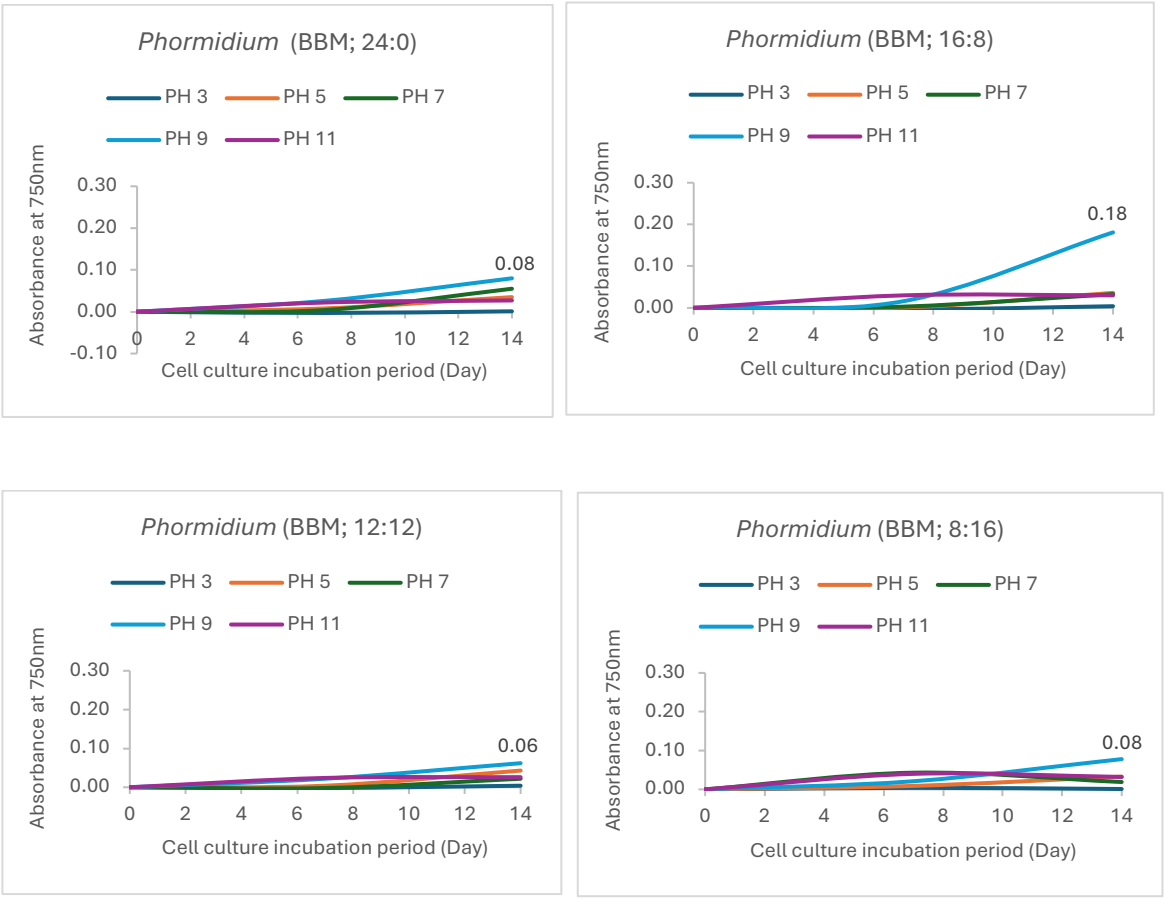


Figure 6.5 Effect of pH on cell culture growth of *Phormidium* in BBM medium.

Table 6.5 The optimum cell density and day of growth of *Phormidium* in BBM medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**Abs**
Phormidium	24:0	pH 9 (Day 14)	0.08
	16:8	pH 9 (Day 14)	0.18
	12:12	pH 9 (Day 14)	0.06
	8:16	pH 9 (Day 14)	0.08

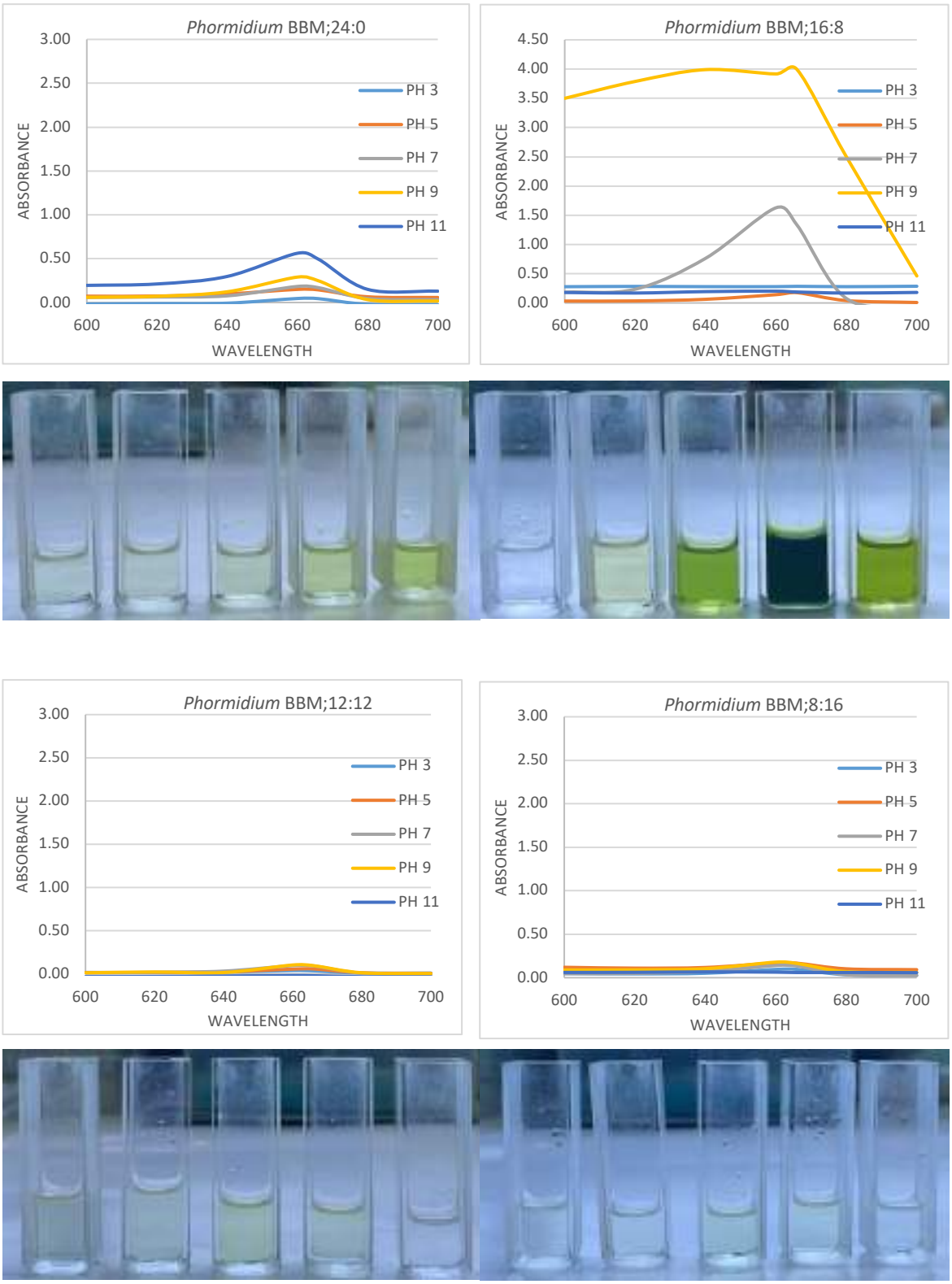


Figure 6.6 Effects of pH and photoperiod on chlorophyll content

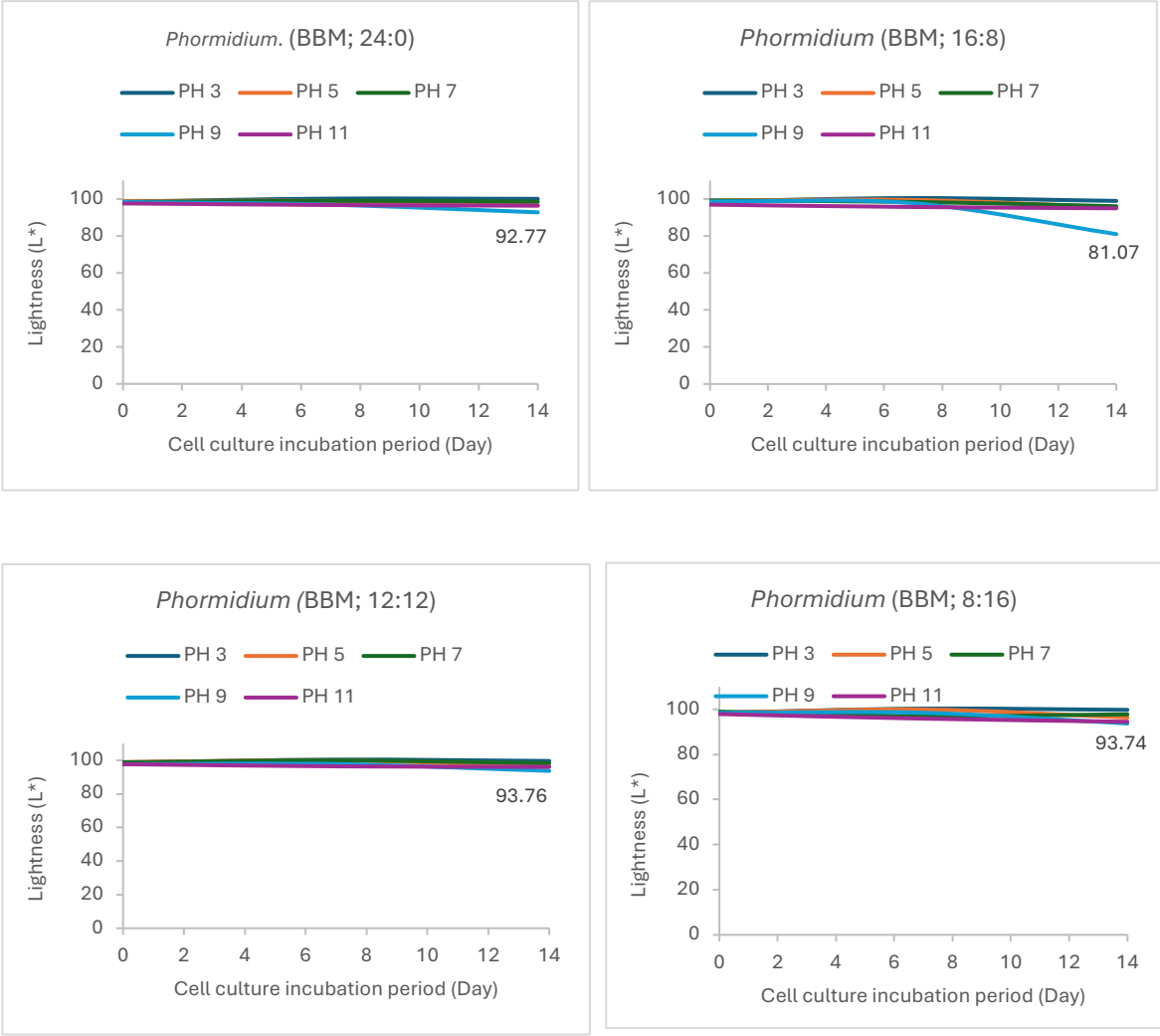


Figure 6.7 Effect of pH on lightness of *Phormidium* in BBM medium

Table 6.6 The lightness (L*) of *Phormidium* cell culture growth in BBM medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**L***
Phormidium	24:0	pH 9 (Day 14)	92.77
	16:8	pH 9 (Day 14)	81.07
	12:12	pH 9 (Day 14)	93.76
	8:16	pH 11 (Day 14)	93.74

L, ranges from black (0) to white (100)*

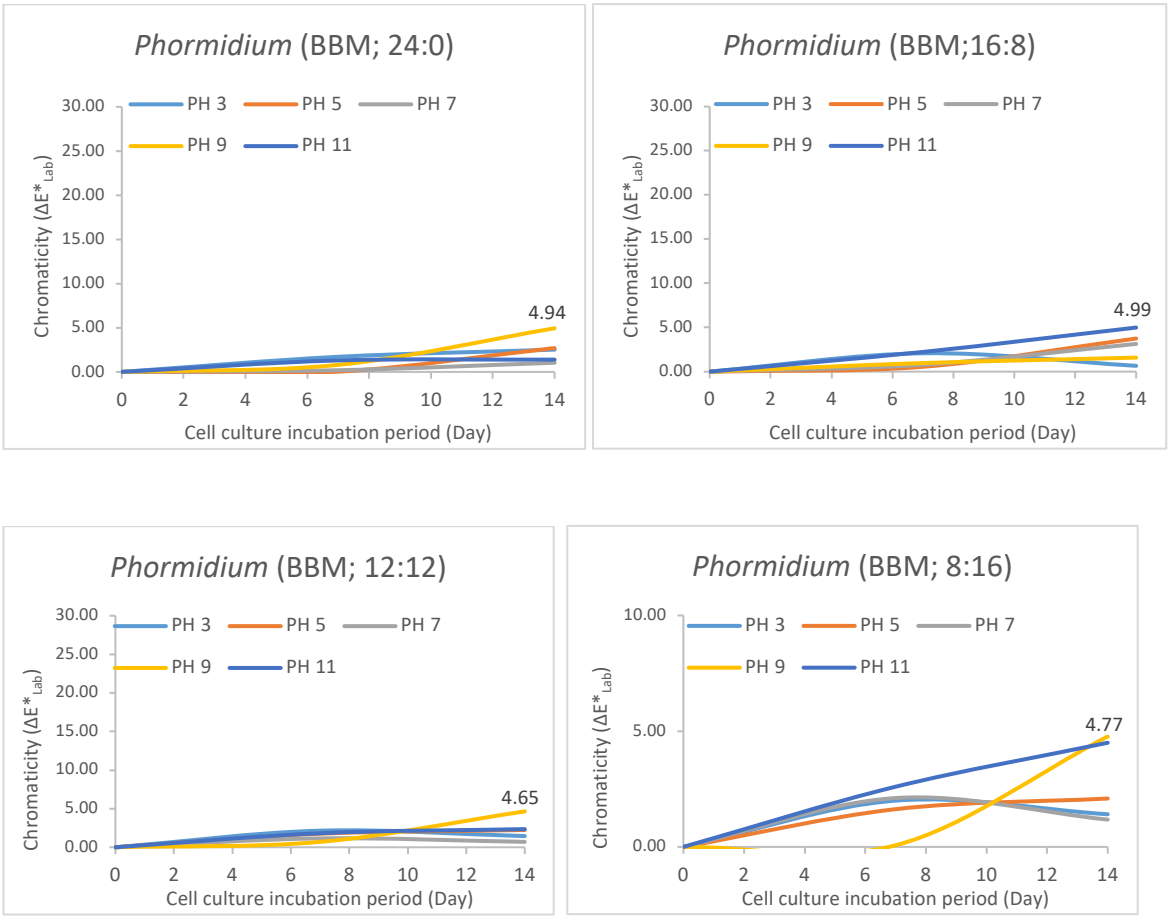
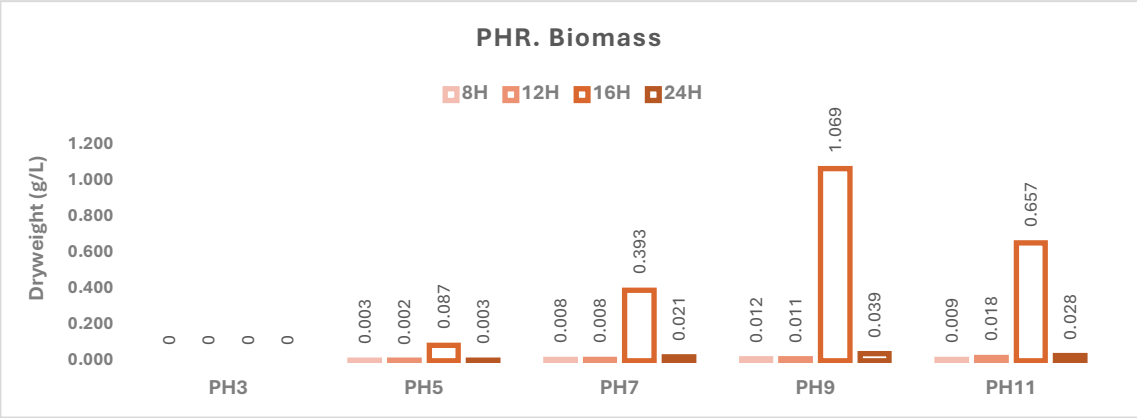


Figure 6.8 Effect of pH on Chromaticity of *Phormidium* in BBM medium

Table 6.7 The CIE L*a*b* colour difference or Chromaticity (ΔE^*_{Lab}) of *Phormidium* cell culture growth in BBM medium at different photoperiod.

Blue Green Algae	**Photoperiod**	**Peak pH (day)**	**ΔE^*_{Lab}**
Phormidium	24:0	pH 9 (Day 14)	4.94
	16:8	pH 11 (Day 14)	4.99
	12:12	pH 9 (Day 7)	4.65
	8:16	pH 11 (Day 7)	4.77

ΔE^*_{Lab} > 5.0 - indicate clearly two different colours



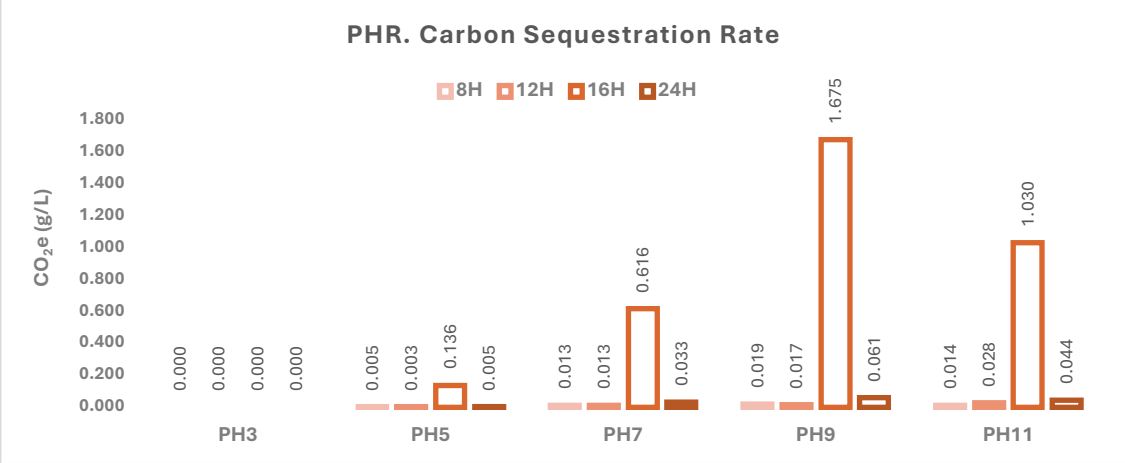


Figure 6.9 The biomass and carbon sequestration rate of *Phormidium*

Table 6.8 The biomass and carbon sequestration rate of *Phormidium* cell culture growth in BBM medium at different pH and photoperiod.

Blue-Green Algae	**pH**	**Biomass (g/L)**	**CSR (CO2e)**
Phormidium	pH 3	0.000	0.000
	pH 5 (16H)	0.087	0.136
	pH 7 (16H)	0.393	0.616
	pH 9 (16H)	1.069	1.675
	pH 11 (16H)	0.657	1.030

The highest absorbance value of 0.18 was recorded in the 16:8 photoperiod at pH 9 on Day 14, suggesting optimal growth conditions for *Phormidium* The corresponding cell density also peaked under this photoperiod and pH level, indicating a favorable environment for the organism's growth. Furthermore, the chlorophyll content, measured between 600-700 nm, exhibited a peak absorbance of 4.0 at 660 nm under the same 16:8 photoperiod and pH 9, signifying enhanced chlorophyll production. This result confirms that the combination of this photoperiod and pH is conducive to maximizing photosynthetic activity in *Phormidium*

Lightness (*L*), a measure of the brightness or color intensity of the cultures, varied across photoperiods. The highest *L* value of 93.74 was recorded under the 8:16 photoperiod at pH 11, which implies a lighter color associated with higher photosynthetic activity. By contrast, a lower lightness value of 81.07 was observed under the 16:8 photoperiod at pH 9, suggesting darker pigmentation, potentially related to increased chlorophyll synthesis. Chromaticity (ΔE*Lab) analysis revealed the most significant color shift under the 16:8 photoperiod at pH 11, with a ΔE*Lab value of 4.99, approaching the threshold for perceivable color differences (>5). Other photoperiods exhibited more minor variations in color, indicating a stable pigment profile across different conditions, except for the 16:8 photoperiod, which induced notable color changes. Biomass production was maximized under the 16:8 photoperiod at pH 9, yielding a dry weight of 1.069 g/L. This photoperiod and pH combination also facilitated the highest carbon sequestration rate, measured at 1.675 g/L of CO2e. In contrast, acidic conditions such as pH 3 yielded no detectable growth, while pH 5 and pH 7 resulted in moderate biomass accumulation.

Phormidium demonstrated optimal growth and photosynthetic efficiency under the 16:8 photoperiod and pH 9 in BBM medium. This condition produced the highest chlorophyll content, biomass, and carbon sequestration rates, suggesting that both photoperiod and pH are critical factors influencing *Phormidium*'s growth dynamics, pigmentation, and overall photosynthetic performance.

Effect of pH, BG11 medium formulation and photoperiod on Phormidium Cell Culture Growth, biomass, Chromaticity, and Chlorophyll content

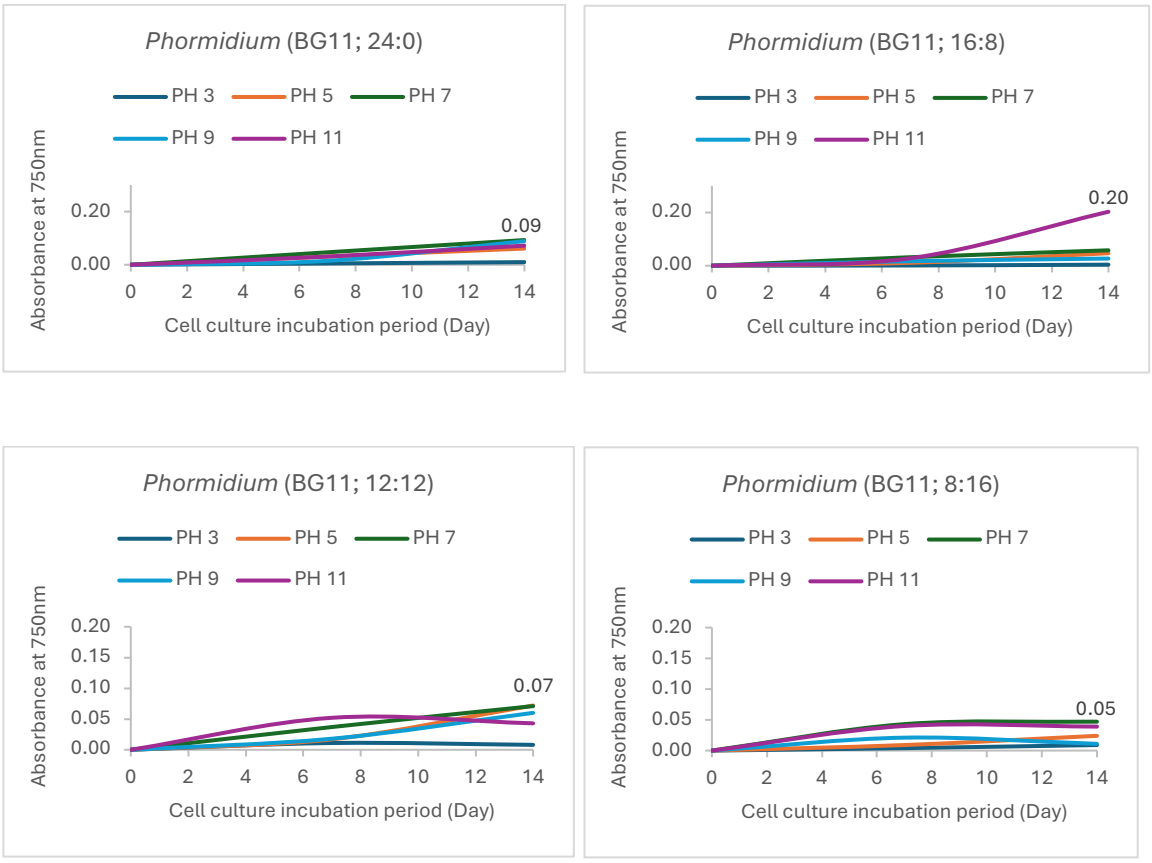


Figure 6.10 Effect of pH on cell culture growth of *Phormidium* in BG11 medium.

Table 6.9 The optimum cell density and day of growth of *Phormidium* in BG11 medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**Abs**
Phormidium	24:0	pH 7 (Day 14)	0.09
	16:8	pH 11 (Day 14)	0.20
	12:12	pH 7 (Day 14)	0.07
	8:16	pH 7 (Day 7)	0.05

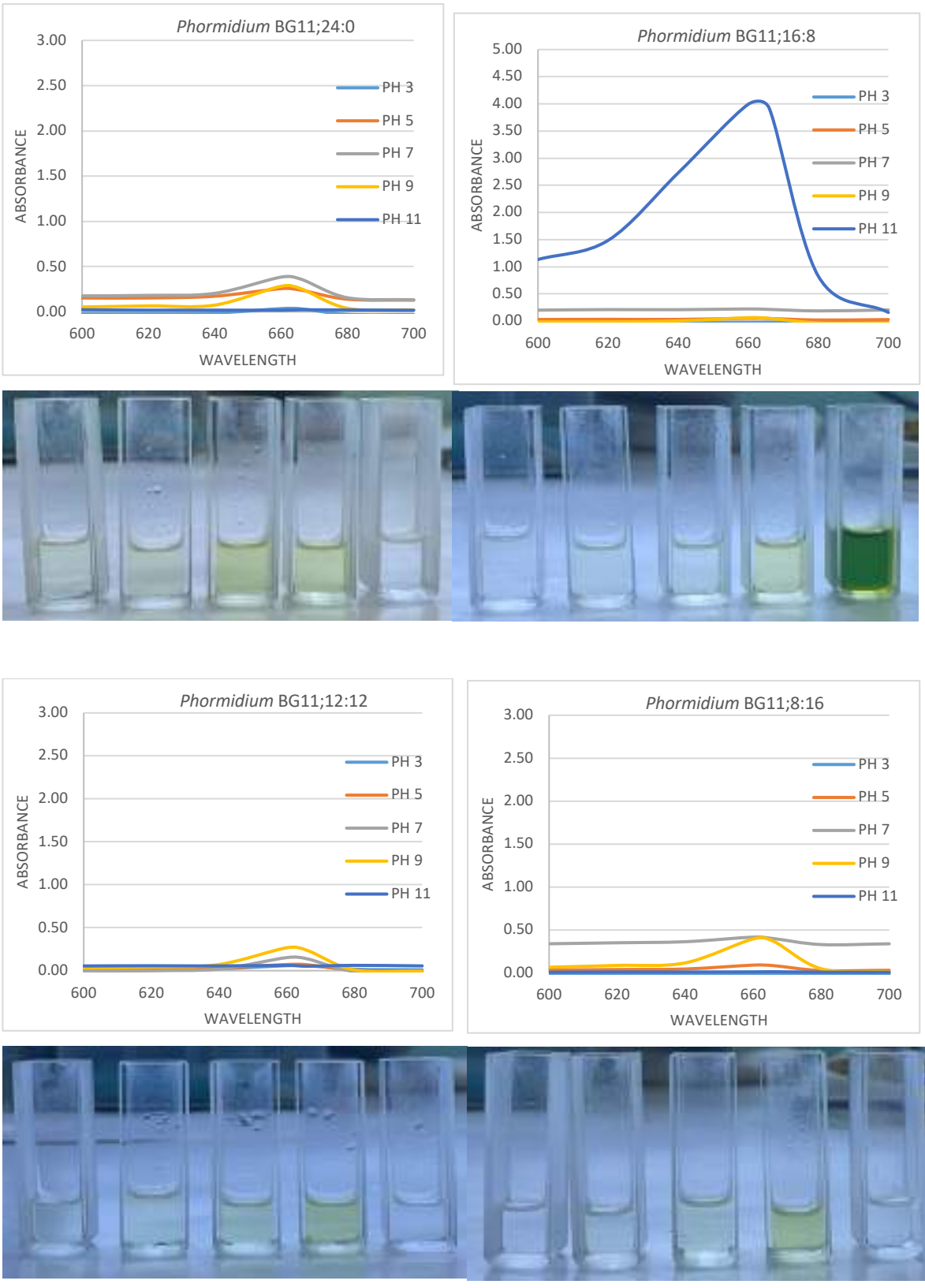


Figure 6.11 Effects of pH and photoperiod on chlorophyll content

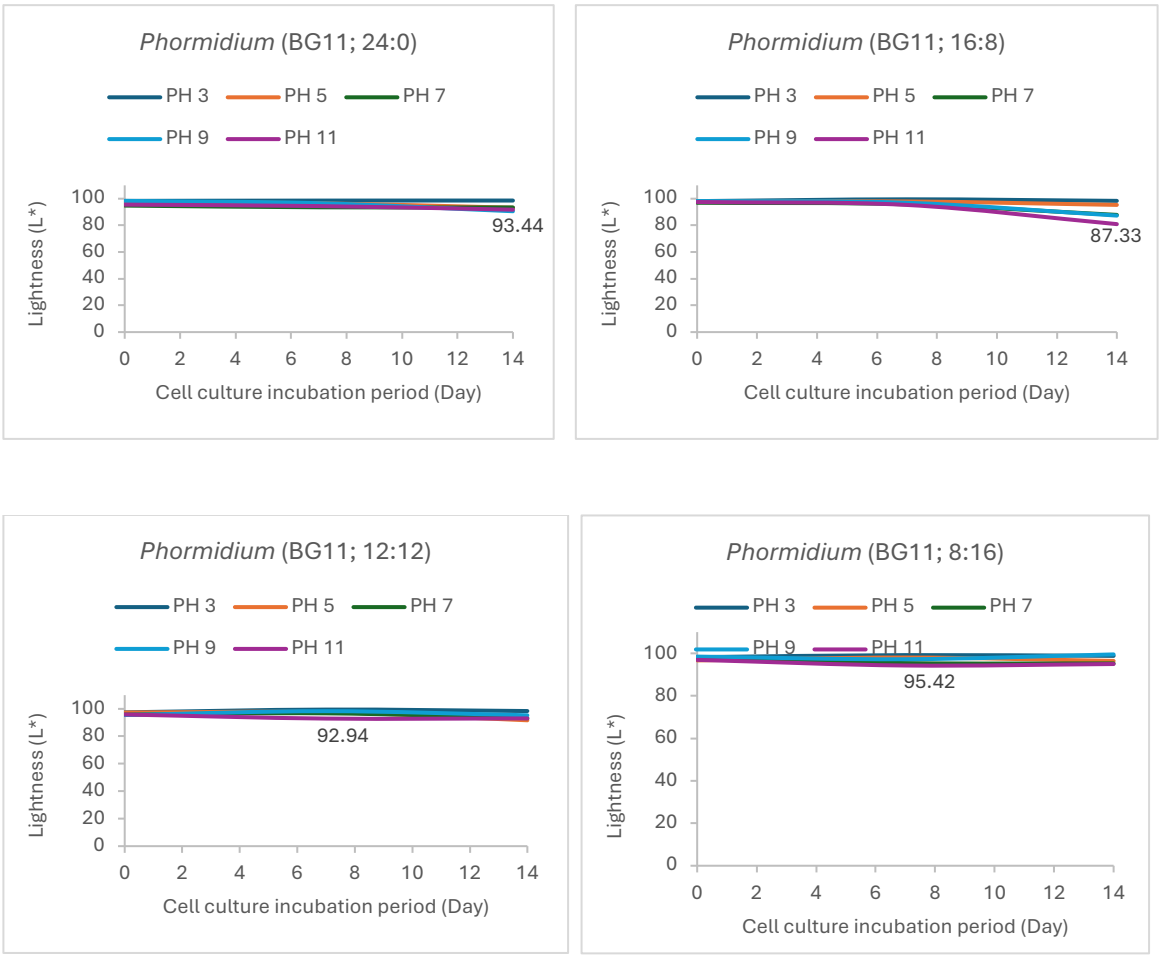


Figure 6.12 Effect of pH on lightness of *Phormidium* in BG11 medium

Table 6.10 The lightness (L*) of *Phormidium* cell culture growth in BG11 medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**L***
Syne.	24:0	pH 9 (Day 14)	93.44
	16:8	pH 11 (Day 14)	87.83
	12:12	pH 11 (Day 7)	92.24
	8:16	pH 11 (Day 7)	95.42

L, ranges from black (0) to white (100)*

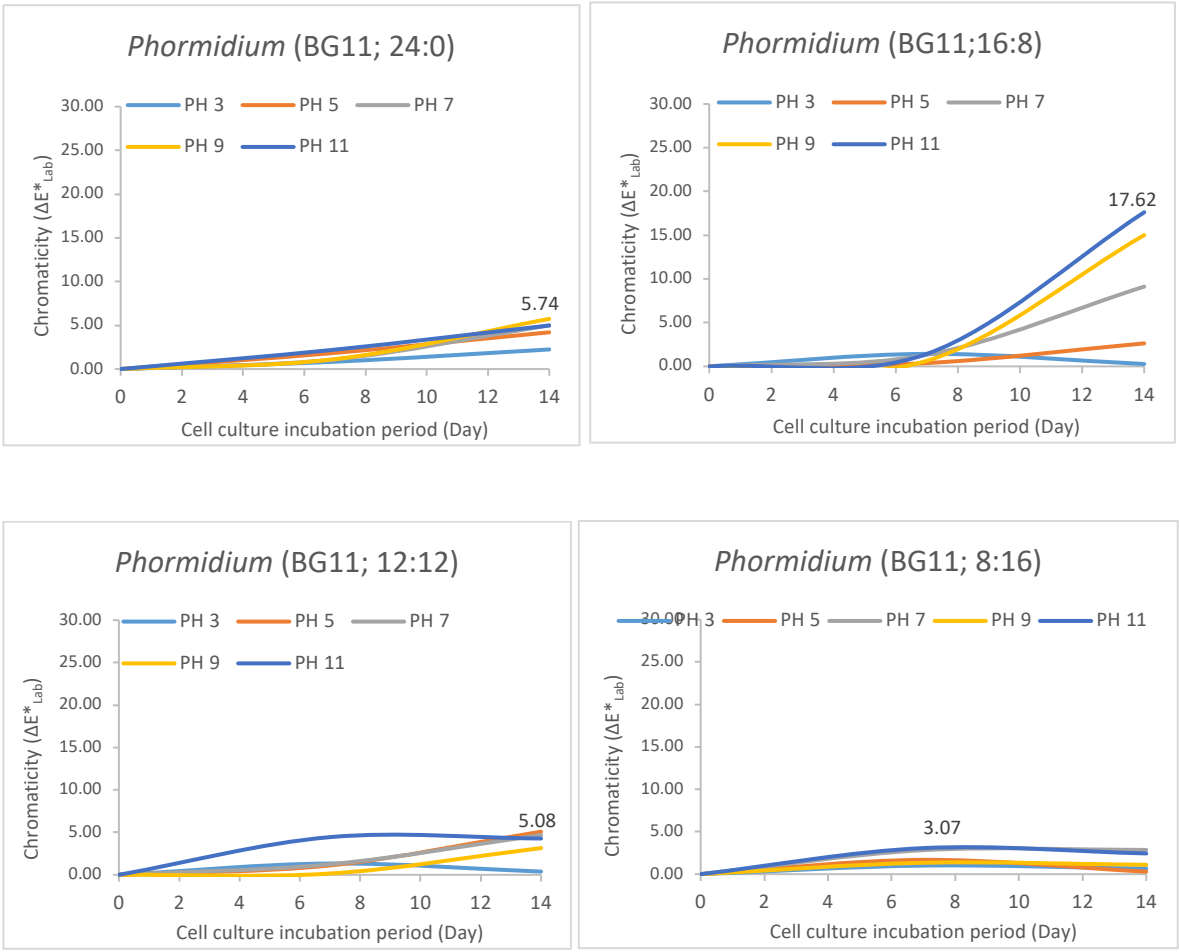
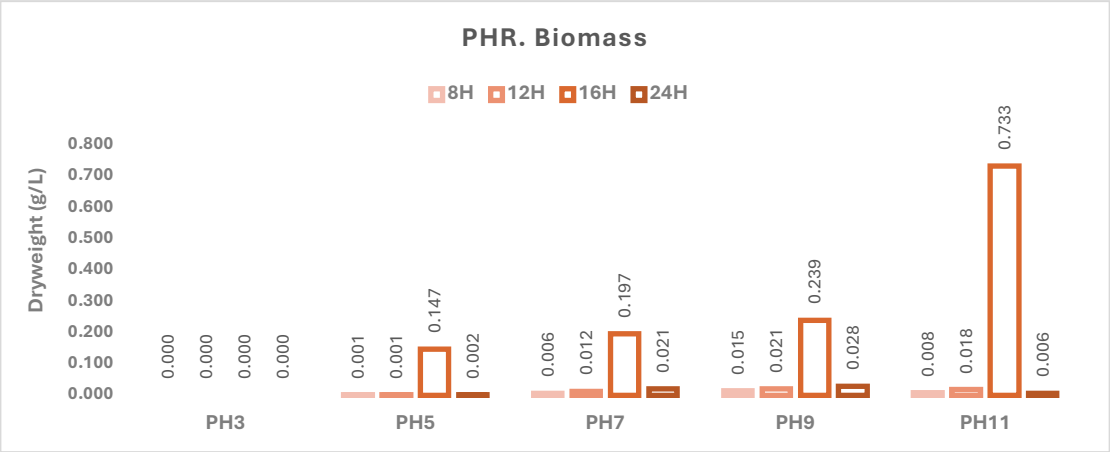


Figure 6.13 Effect of pH on Chromaticity of *Phormidium* in BG11 medium

Table 6.11 The CIE L*a*b* colour difference or Chromaticity (ΔE*Lab) of *Phormidium* cell culture growth in BG11 medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**ΔE^*_{Lab}**
Phormidium	24:0	pH 9 (Day 14)	5.74
	16:8	pH 11 (Day 14)	17.62
	12:12	pH 5 (Day 14)	5.08
	8:16	pH 11 (Day 14)	3.07

ΔE^*_{Lab} > 5.0 - indicate clearly two different colours



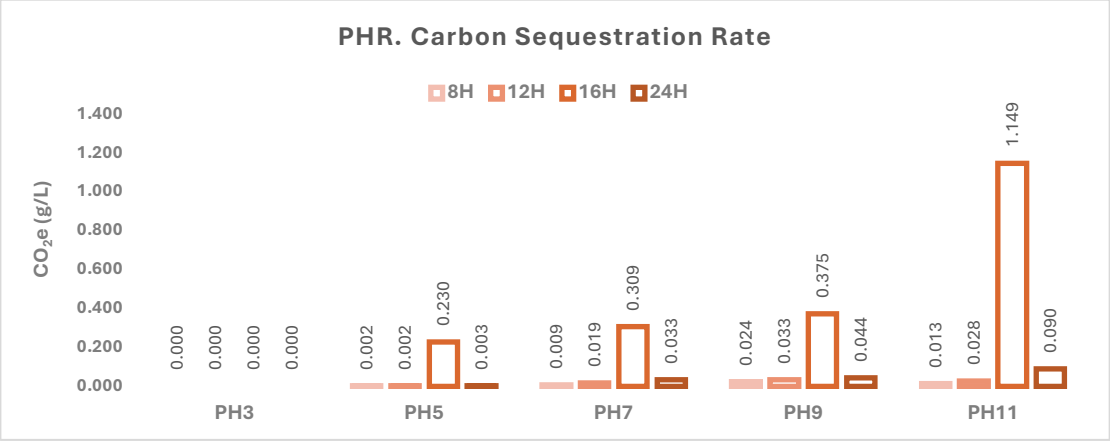


Figure 6.14 The biomass and carbon sequestration rate of *Phormidium*

Table 6.12 The biomass and carbon sequestration rate of *Phormidium* cell culture growth in BG11 medium at different pH and photoperiod.

Blue-Green Algae	**pH**	**Biomass (g/L)**	**CSR (CO2e)**
Phormidium	pH 3	0.000	0.000
	pH 5 (16H)	0.147	0.230
	pH 7 (16H)	0.197	0.309
	pH 9 (16H)	0.239	0.375
	pH 11 (16H)	0.733	1.149

The highest absorbance value of 0.20, indicating peak growth, was observed under a 16:8 photoperiod at pH 11, measured on Day 14. This finding suggests that moderate light exposure combined with a higher pH significantly promotes the growth of *Phormidium* cells. Lower absorbance values were recorded for other photoperiods, such as 12:12 (absorbance: 0.07) and 8:16 (absorbance: 0.05), both at pH 7. Chlorophyll content was maximized under the 16:8 photoperiod at pH 11, as indicated by the higher absorbance. This corresponds to the enhanced photosynthetic efficiency observed under these conditions, which is also aligned with increased growth and lightness values.

The highest lightness (*L* value) of 95.42 was observed under the 8:16 photoperiod at pH 11. This suggests that even under reduced light exposure, the culture appeared relatively bright, which is indicative of a higher photosynthetic rate. The chromaticity (ΔE*Lab) value reached a maximum of 17.62 under the 16:8 photoperiod at pH 11, marking a significant shift in the culture's pigmentation. This change in color was more pronounced than those observed under other conditions. Biomass production reached its peak under the 16:8 photoperiod at pH 11, with a dry weight of 0.733 g/L. Concurrently, the carbon sequestration rate (CSR) reached a maximum of 1.149 g CO2e/L. These findings highlight the capacity of *Phormidium* In BG11 medium to sequester carbon effectively and grow efficiently under a combination of higher pH and moderate light exposure.

The analysis of *Phormidium* cultured in BG11 medium demonstrates that the 16:8 photoperiod at pH 11 provides optimal conditions for maximizing growth, chlorophyll content, color differentiation, biomass, and carbon sequestration. Reduced growth and chlorophyll content were observed under lower photoperiods, such as 8:16 and pH 7, suggesting that a careful balance between light exposure and pH is crucial for the successful cultivation of *Phormidium* in BG11 medium.

Effect of pH, Bristol medium formulation and photoperiod on Phormidium Cell Culture Growth, biomass, Chromaticity, and Chlorophyll content

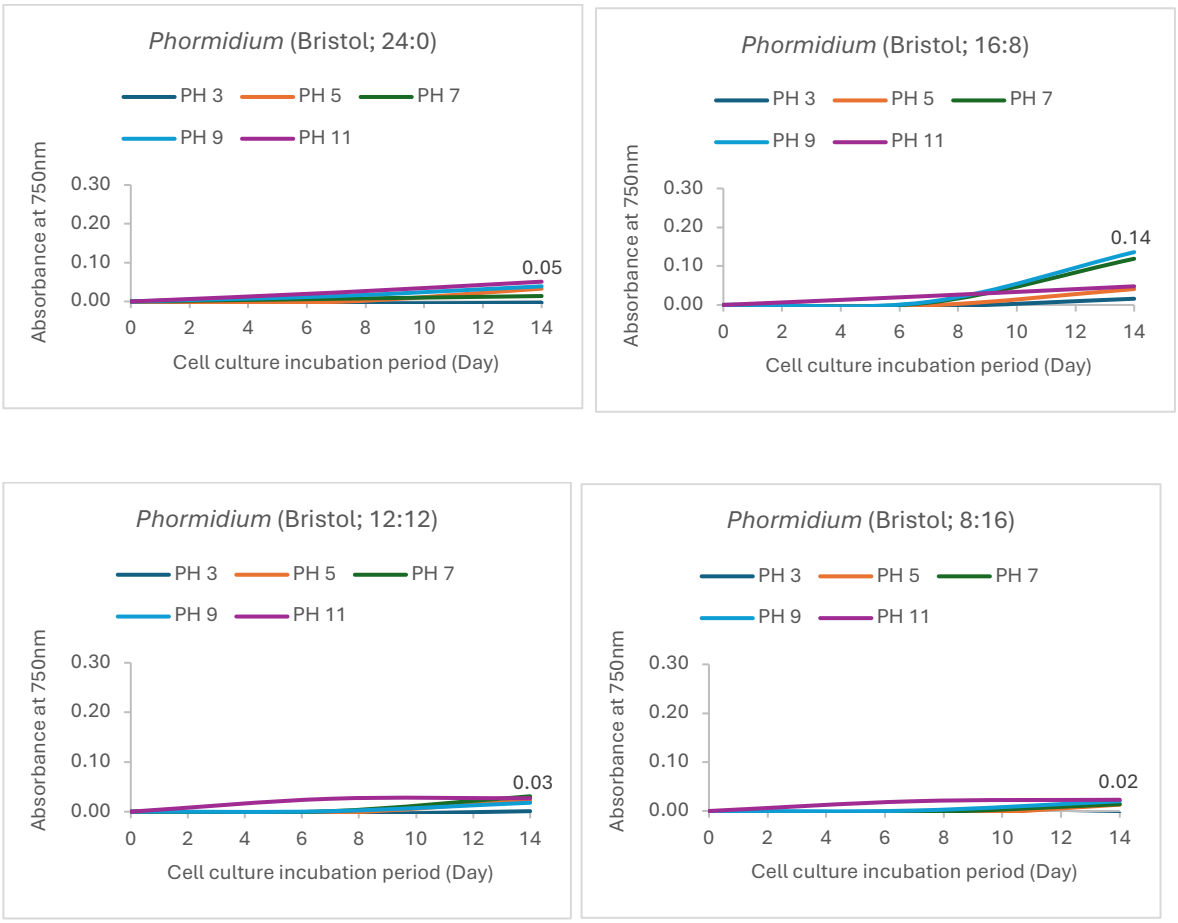


Figure 6.15 Effect of pH on cell culture growth of *Phormidium* in Bristol medium.

Table 6.13 The optimum cell density and day of growth of *Phormidium* in Bristol medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**Abs**
Phormidium	24:0	pH 11 (Day 14)	0.05
	16:8	pH 9 (Day 14)	0.14
	12:12	pH 11 (Day 14)	0.03
	8:16	pH 11 (Day 7)	0.02

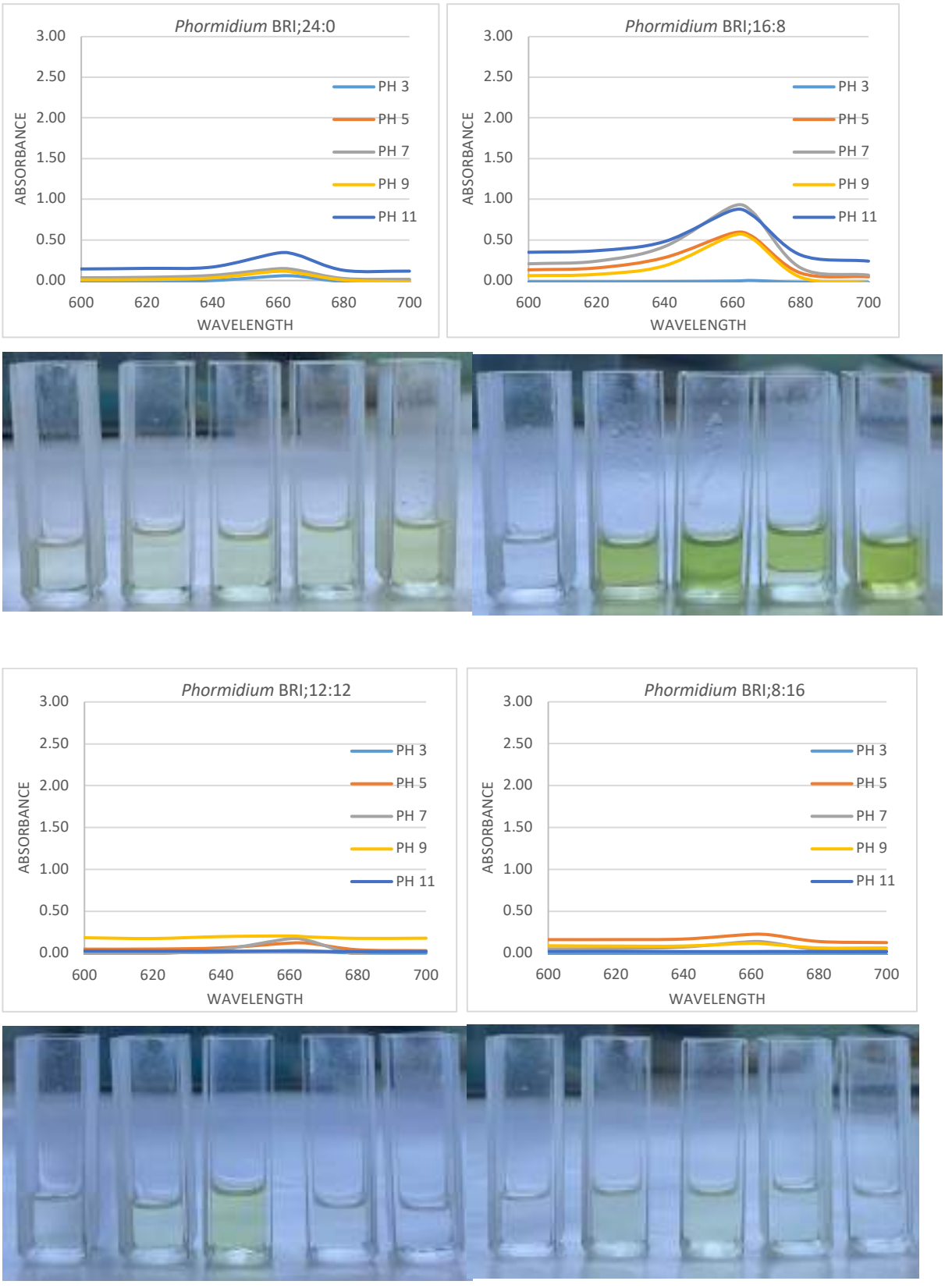


Figure 6.16 Effects of pH and photoperiod on chlorophyll content

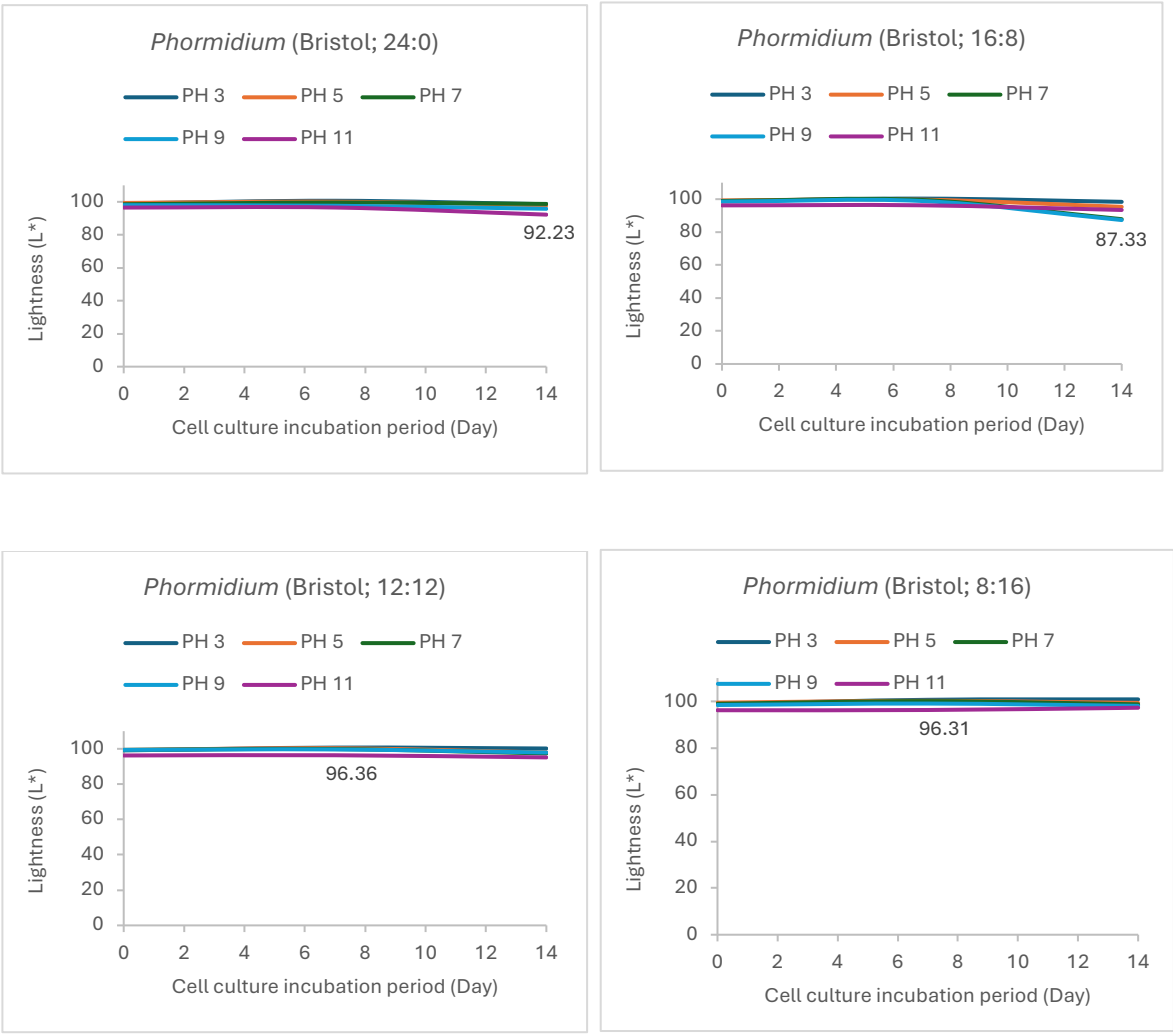


Figure 6.17 Effect of pH on lightness of *Phormidium* in Bristol medium

Table 6.14 The lightness (L*) of *Phormidium* cell culture growth in Bristol medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**L***
Syne.	24:0	pH 11 (Day 14)	92.23
	16:8	pH 9 (Day 14)	87.33
	12:12	pH 11 (Day 7)	96.36
	8:16	pH 11 (Day 7)	96.31

L, ranges from black (0) to white (100)*

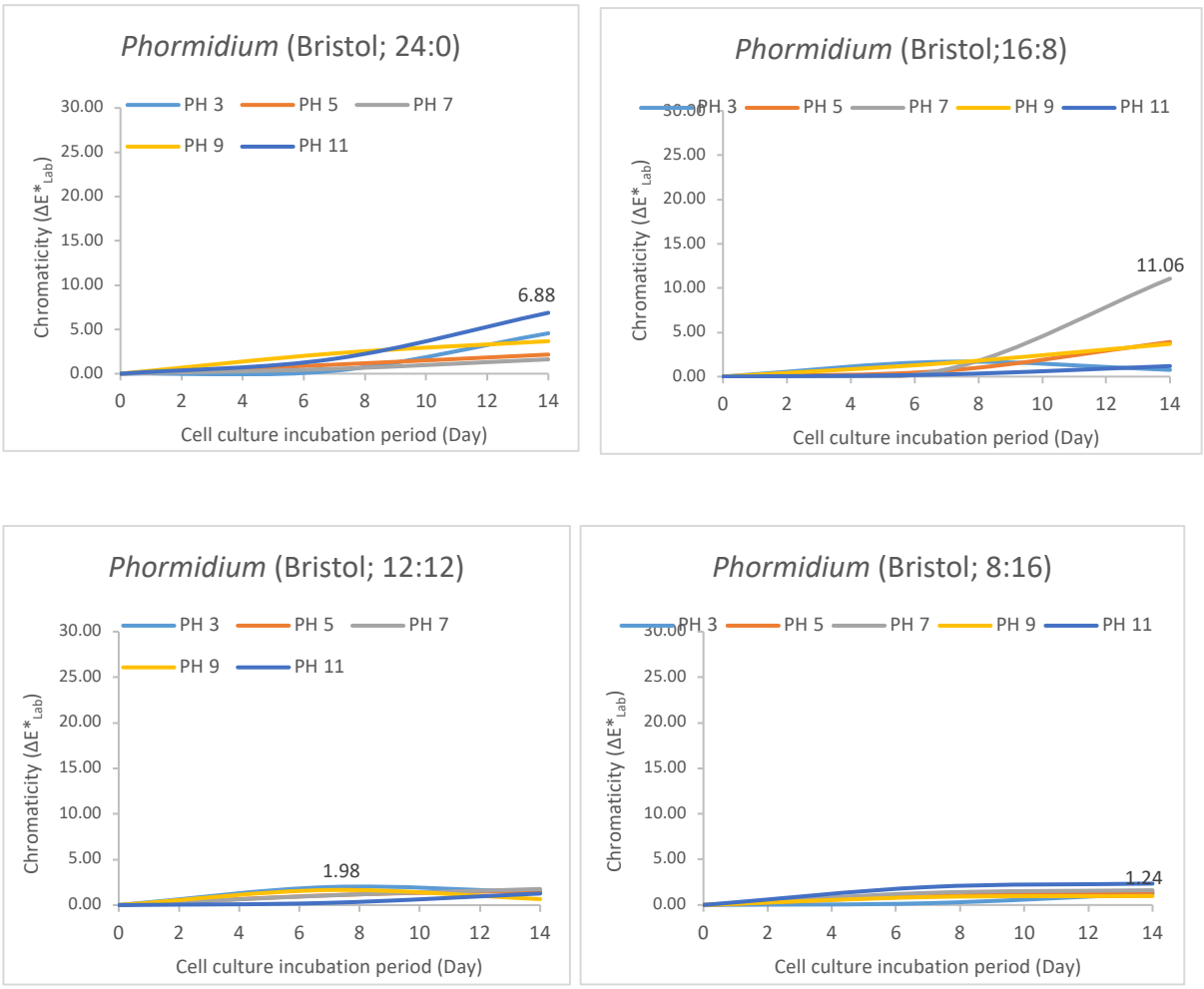


Figure 6.18 Effect of pH on Chromaticity of *Phormidium* in Bristol medium

Table 6.15 The CIE L*a*b* colour difference or Chromaticity (ΔE^*_{Lab}) of *Phormidium* cell culture growth in Bristol medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**ΔE^*_{Lab}**
Phormidium	24:0	pH 11 (Day 14)	6.88
	16:8	pH 7 (Day 14)	11.06
	12:12	pH 7 (Day 7)	1.98
	8:16	pH 11 (Day 14)	1.24

ΔE^*_{Lab} > 5.0 - indicate clearly two different colours

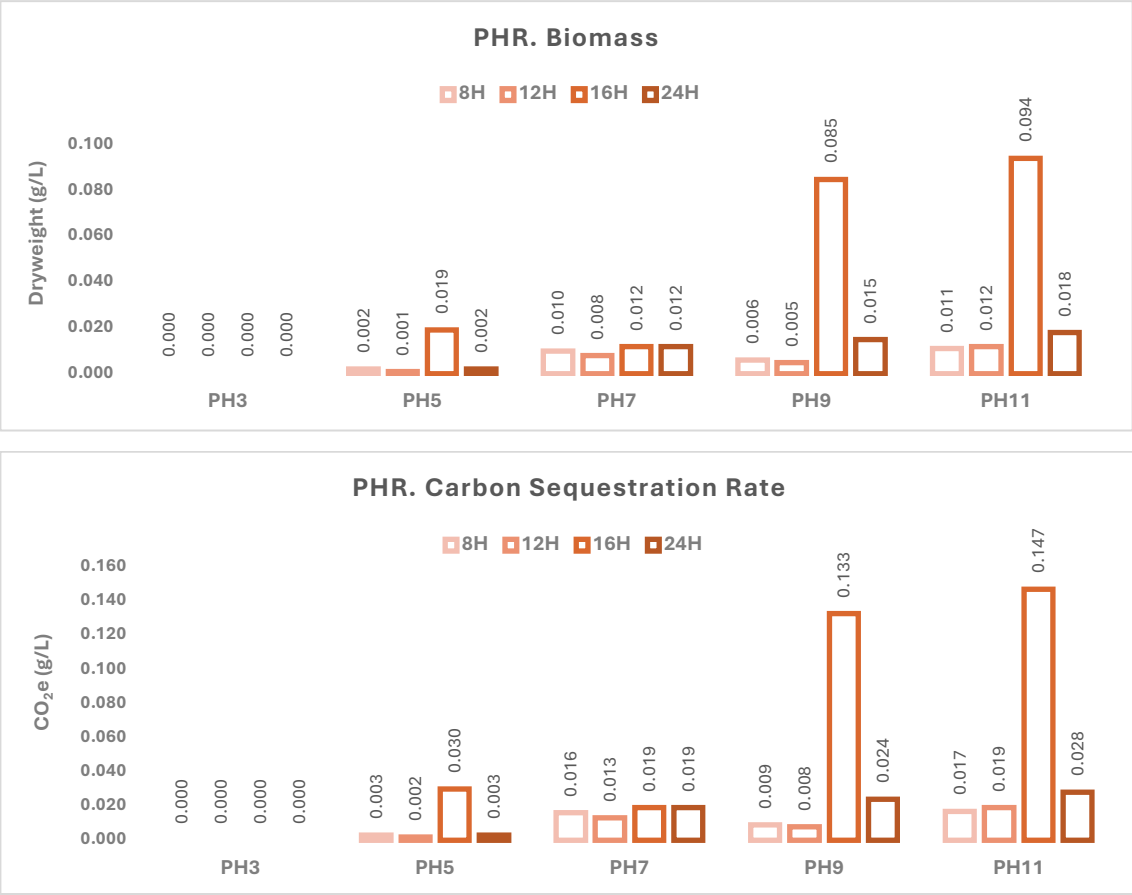


Figure 6.19 The biomass and carbon sequestration rate of *Phormidium*

Table 6.16 The biomass and carbon sequestration rate of *Phormidium* cell culture growth in Bristol medium at different pH and photoperiod.

Blue-Green Algae	**pH**	**Biomass (g/L)**	**CSR (CO2e)**
Phormidium	pH 3	0.000	0.000
	pH 5 (16H)	0.019	0.030
	pH 7 (16H and 24H)	0.012	0.019
	pH 9 (16H)	0.085	0.013
	pH 11 (16H)	0.094	0.147

The absorbance of *Phormidium*under various pH levels and photoperiods in *Bristol medium* indicates that the optimal conditions were at pH 9 and under the 16:8 photoperiod, where the absorbance peaked at 0.14. This suggests that a balance between light and dark cycles, combined with a pH closer to alkaline levels, promotes optimal growth in terms of cell density. UV-Vis absorbance results confirm that the 16:8 photoperiod at pH 9 yielded the highest absorbance value of 0.14, signifying the greatest total chlorophyll content in *Phormidium* cultures under these conditions in *Bristol medium*.

The highest lightness value (L*), measuring the brightness of the cultures, was observed under the 12:12 photoperiod with pH 11, recording a value of 96.36, closely followed by the 8:16 photoperiod at the same pH, which had an L* value of 96.31. This implies that longer dark cycles in *Bristol medium* tend to produce brighter cultures, particularly at higher pH values. The highest chromaticity value was recorded under the 16:8 photoperiod at pH 7, with a ΔE*Lab of 11.06, indicating a significant color difference under these conditions. Other photoperiods exhibited lower chromaticity values, with the lowest recorded under the 8:16 photoperiod with pH 11, suggesting less variation in color under those conditions.

In terms of biomass production, the highest recorded value in *Bristol medium* was 0.094 g/L, observed at pH 11 under the 16:8 photoperiod. Correspondingly, the highest carbon sequestration rate (CSR) was recorded under these conditions, with a value of 0.147 g/L CO2e. This suggests that higher pH levels combined with moderate light/dark cycles are most effective for maximizing biomass and carbon sequestration in *Phormidium* cultures grown in *Bristol medium*.

Phormidium cultured in *Bristol medium*, the optimal conditions for growth, as indicated by absorbance, lightness, chromaticity, biomass, and carbon sequestration, predominantly occurred at pH 9 under the 16:8 photoperiod. These results suggest that a slightly alkaline environment, coupled with balanced light and dark cycles, leads to the most effective growth and environmental performance for *Phormidium* when cultured in *Bristol medium*.

Effect of pH, Chu medium formulation and photoperiod on Phormidium Cell Culture Growth, biomass, Chromaticity, and Chlorophyll content

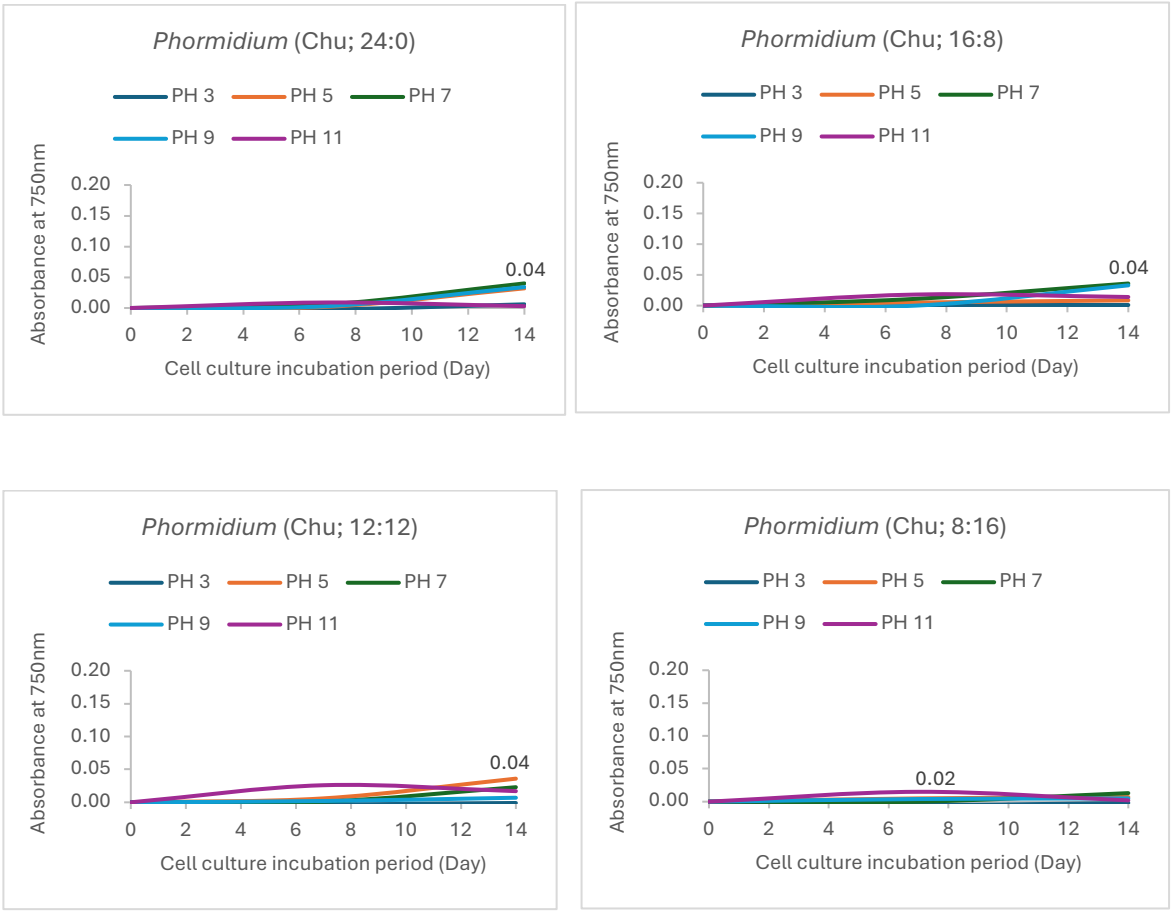


Figure 6.20 Effect of pH on cell culture growth of *Phormidium* in Chu medium.

Table 6.17 The optimum cell density and day of growth of *Phormidium* in Chu medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**Abs**
Phormidium	24:0	pH 7 (Day 14)	0.04
	16:8	pH 7 (Day 14)	0.04
	12:12	pH 5 (Day 14)	0.04
	8:16	pH 11 (Day 7)	0.02

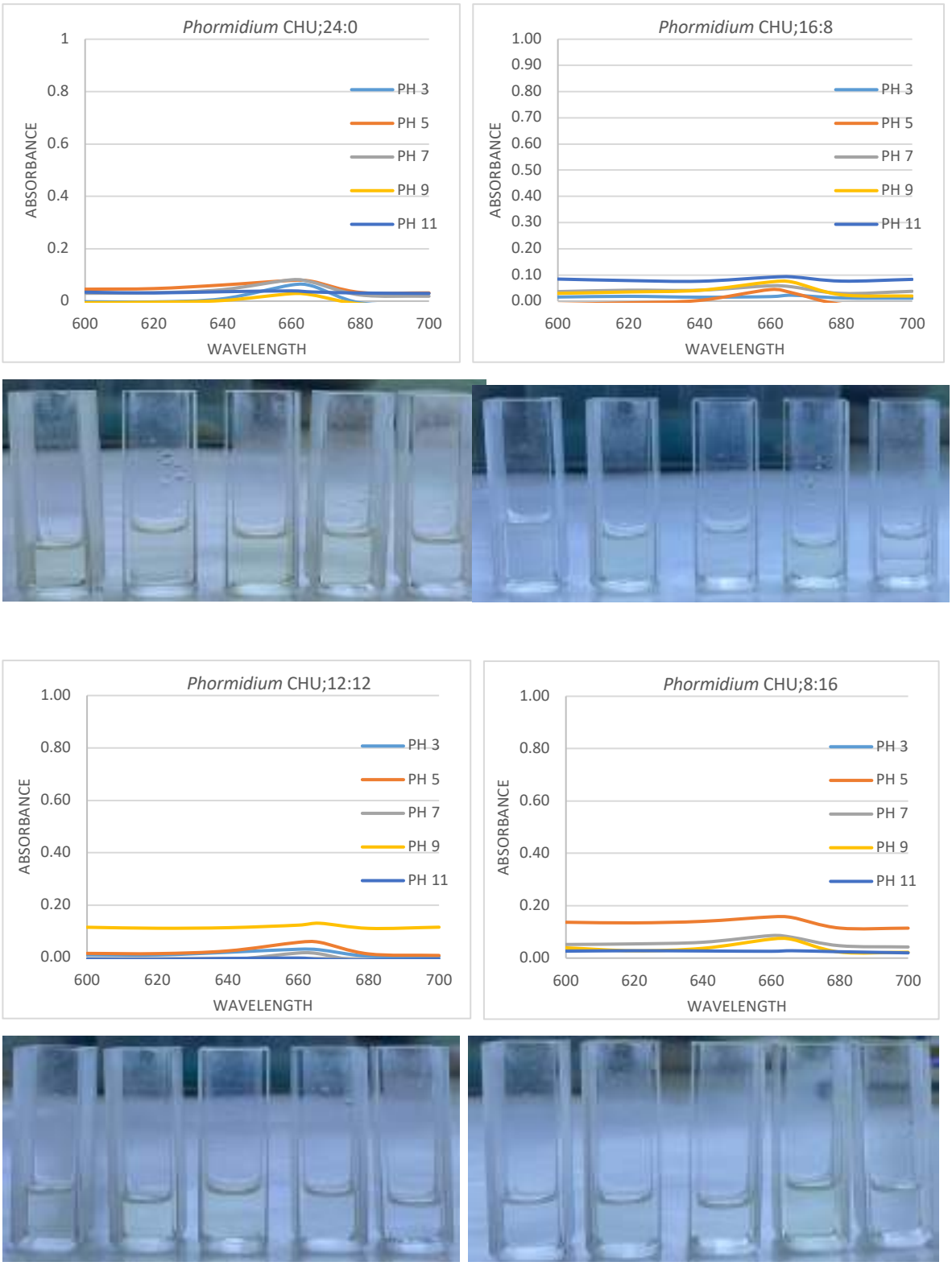


Figure 6.21 Effects of pH and photoperiod on chlorophyll content

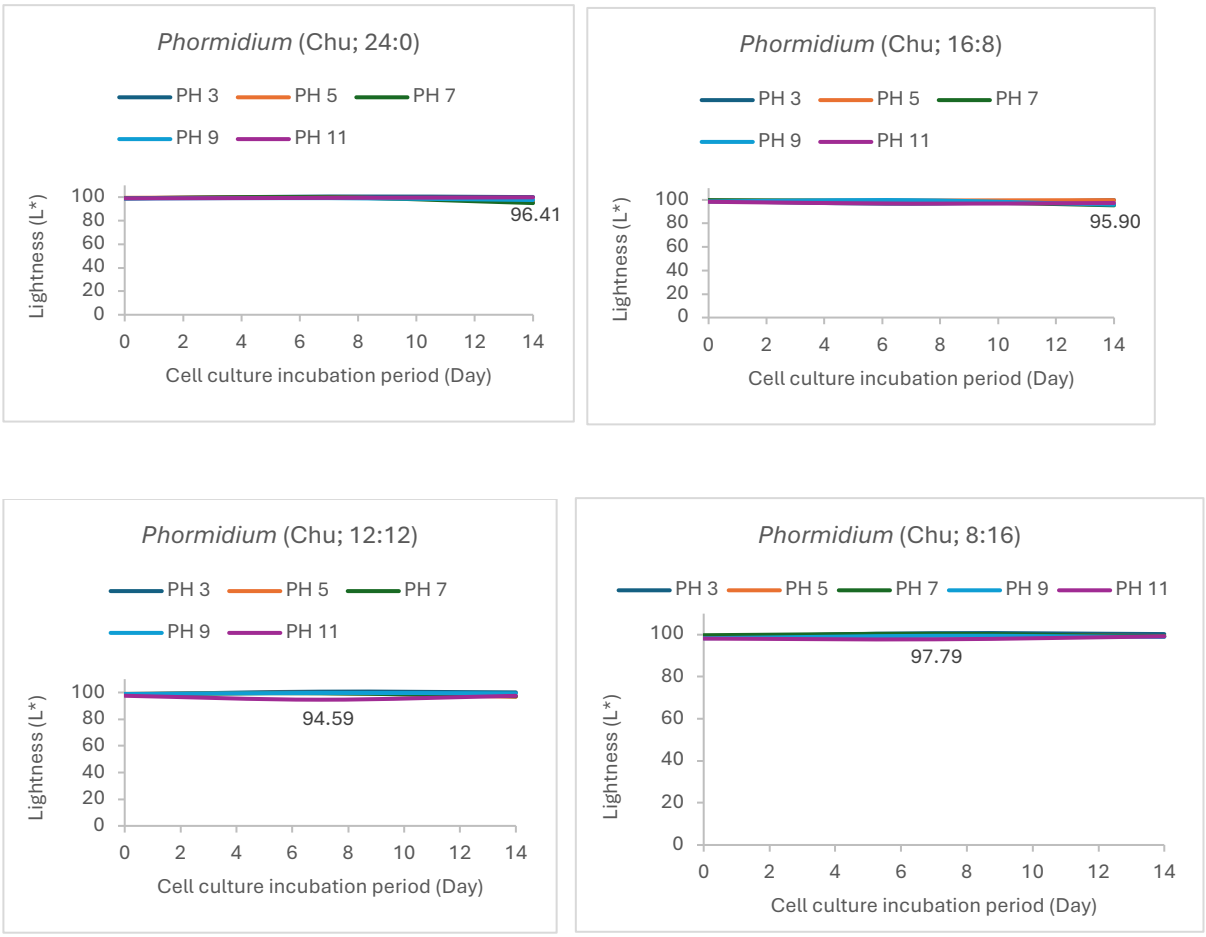


Figure 6.22 Effect of pH on lightness of *Phormidium* in Chu medium.

Table 6.18 The lightness (L*) of *Phormidium* cell culture growth in Chu medium at different photoperiod.

Blue-Green Algae	**Photoperiod**	**Peak pH (day)**	**L***
Syne.	24:0	pH 7 (Day 14)	94.94
	16:8	pH 9 (Day 14)	95.90
	12:12	pH 11 (Day 7)	94.59
	8:16	pH 11 (Day 7)	97.79

L, ranges from black (0) to white (100)*

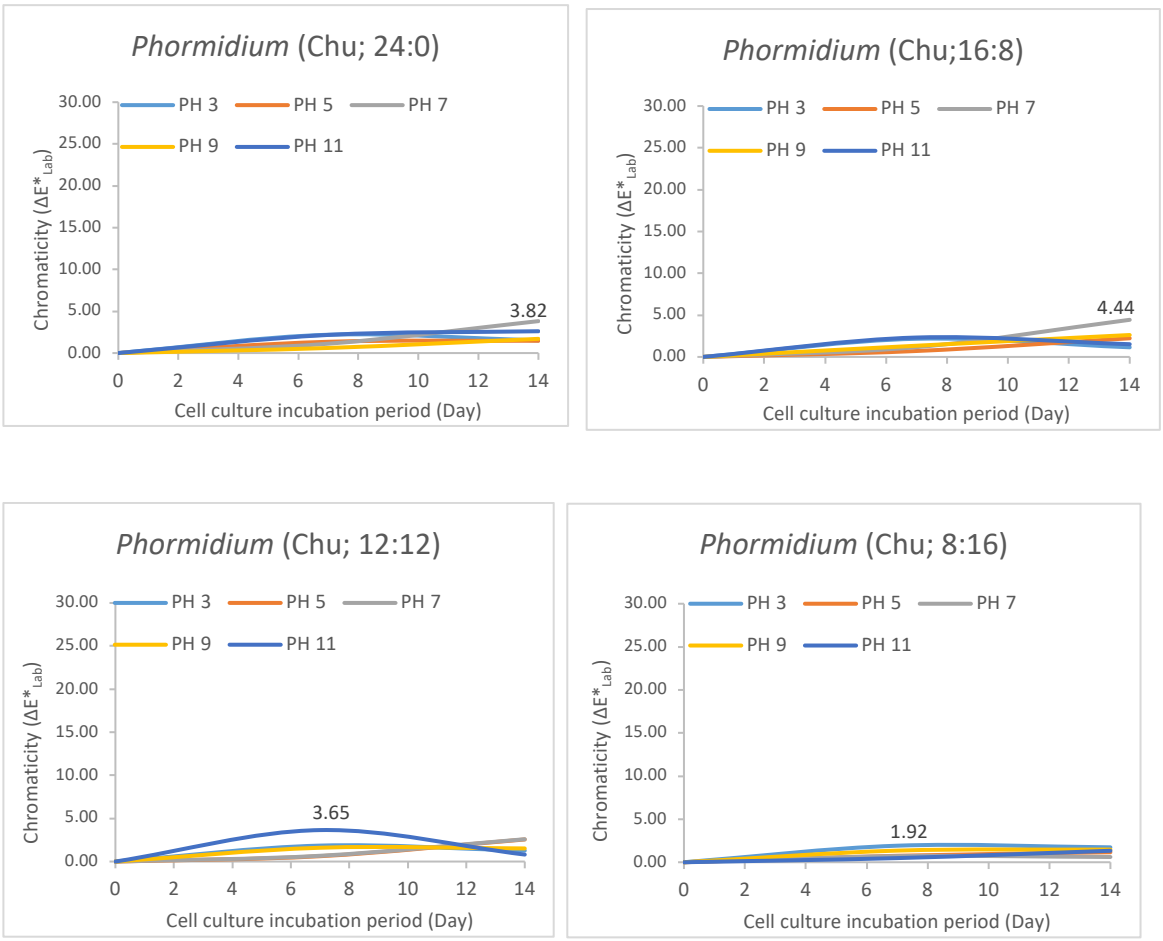


Figure 6.23 Effect of pH on Chromaticity of *Phormidium* in Chu medium.

Table 6.19 The CIE L*a*b* colour difference or Chromaticity (ΔE*Lab) of *Phormidium* cell culture growth in Chu medium at different photoperiod.

Green Algae	**Photoperiod**	**Peak pH (day)**	**ΔE^*_{Lab}**
Phormidium	24:0	pH 7 (Day 14)	3.82
	16:8	pH 7 (Day 14)	4.44
	12:12	pH 11 (Day 7)	3.65
	8:16	pH 3 (Day 7)	1.92

ΔE^*_{Lab} > 5.0 - indicate clearly two different colours

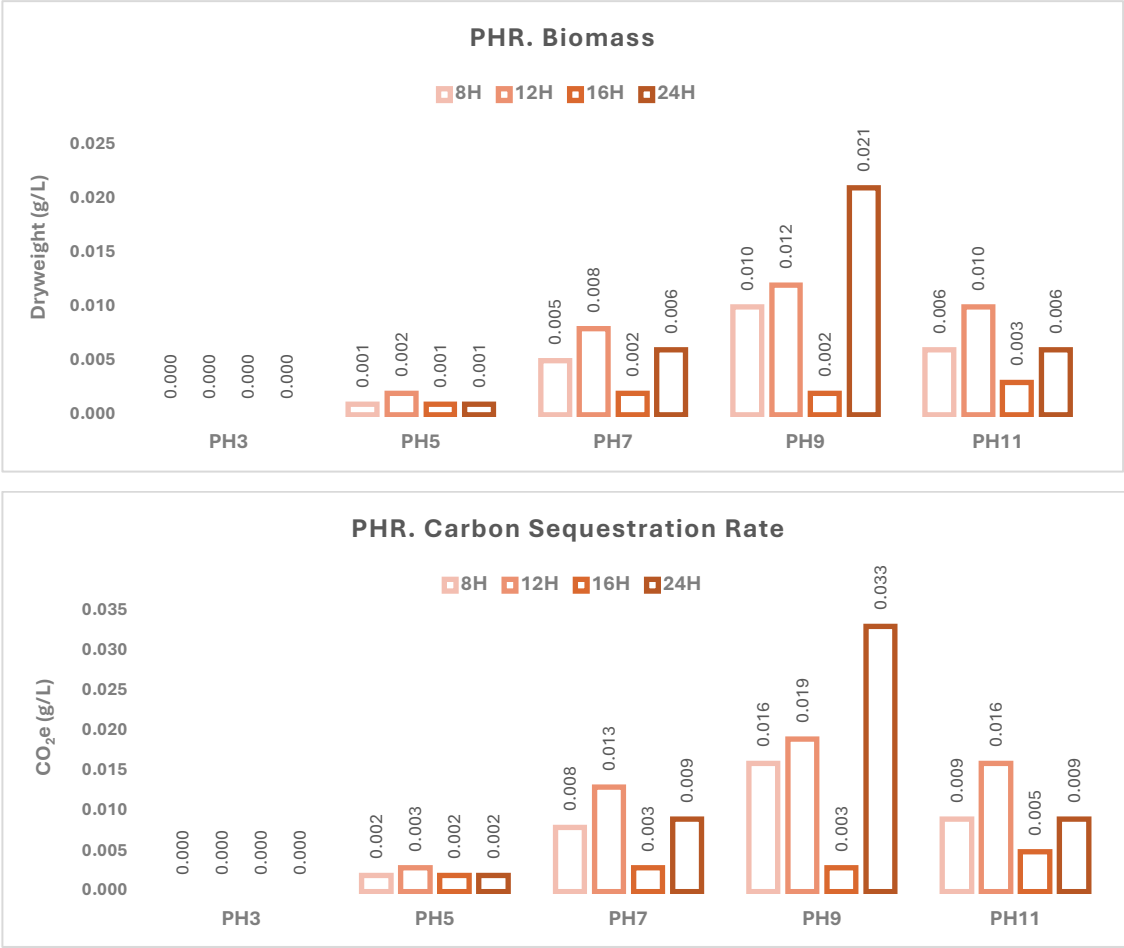


Figure 6.24 The biomass and carbon sequestration rate of *Phormidium*

Table 6.20 The biomass and carbon sequestration rate of *Phormidium* cell culture growth in Bristol medium at different pH and photoperiod.

Blue-Green Algae	**pH**	**Biomass (g/L)**	**CSR (CO2e)**
Phormidium	pH 3	0.000	0.000
	pH 5 (12H)	0.002	0.003
	pH 7 (12H)	0.008	0.013
	pH 9 (24H)	0.021	0.033
	pH 11 (12H)	0.010	0.016

Peak absorbance was consistently recorded at pH 7 on Day 14 for photoperiods 24:0, 16:8, and 12:12, with a value of 0.04. This indicates that pH 7 provides a more favorable condition for growth in *Chu* medium compared to other pH levels. However, under the 8:16 photoperiod at pH 11, absorbance dropped to 0.02, suggesting suboptimal growth under this condition. For total chlorophyll content, the overall absorbance values were relatively low, indicating limited biomass accumulation. While pH 7 may facilitate moderate growth in *Chu* medium, the results suggest that the medium may lack sufficient nutrients or conditions to support higher biomass yields.

The lightness (L value), which reflects the brightness or transparency of the culture, was highest at pH 11 with a photoperiod of 8:16, reaching 97.79. This indicates minimal pigment production and a lighter culture under these conditions. Conversely, the 16:8 photoperiod with pH 9 yielded a similarly high L value of 95.90, further supporting low pigment concentration. Across other photoperiods, lightness values remained high, such as 94.94 at pH 7 on Day 14 with a 24:0 photoperiod. In *Chu* medium, the highest ΔE*Lab value recorded was 4.44 at pH 7 (Day 14) under a 16:8 photoperiod, with other values remaining below 5, suggesting minimal color variation across the conditions.

Biomass production in *Chu* medium was low, with the highest yield observed at pH 9 under a 24:0 photoperiod, reaching 0.021 g/L. Other pH levels and photoperiod combinations exhibited much lower biomass production. This suggests that *Phormidium* could accumulate some biomass at pH 9, though overall yields remained modest. Similarly, carbon sequestration, as indicated by the carbon sequestration rate (CSR), was highest at 0.033 g CO2e/L under the same pH 9 (24:0) condition. However, this value remained relatively low, indicating that *Chu* medium did not support significant carbon capture by *Phormidium.*

Phormidium demonstrated limited growth and biomass production in *Chu* medium, with optimal results observed at pH 7 and pH 9 under specific photoperiods. While pH played a role in facilitating moderate growth, the low absorbance, biomass, and carbon sequestration values suggest that *Chu* medium may not be the most suitable environment for high-yield cultivation of *Phormidium.*

6.3 DISCUSSION

The growth performance of *Phormidium* in different culture media is closely linked to its potential as a carbon sequestration agent and ecological indicator. Optical density (OD_{750}) is a crucial parameter for assessing biomass accumulation, as it reflects cell density and overall growth performance (Hotos, 2021; Hotos et al., 2020). The variations in absorbance values across different media highlight the role of nutrient availability in facilitating *Phormidium's* growth. BG11 and BBM media, which contain balanced macronutrients and trace elements, often support higher biomass production than nutrient-limited formulations, suggesting their suitability for maximizing carbon sequestration (Hotos, 2022). Additionally, the duration of the culture period significantly impacts pigment accumulation, particularly chlorophyll and phycobiliproteins, which are directly linked to the efficiency of photosynthesis and carbon fixation (Hotos & Antoniadis, 2022). These pigments enhance the absorption of light energy, thereby optimizing CO_2 uptake and supporting *Phormidium's* role in mitigating atmospheric carbon levels.

The relationship between chlorophyll content and environmental conditions further underscores *Phormidium's* potential in climate mitigation. Higher chlorophyll concentrations, often observed in nutrient-rich media such as BG11 and Chu's medium, indicate enhanced photosynthetic efficiency, which directly contributes to carbon fixation capacity (Hotos, 2022). However, external factors such as pH fluctuations influence chlorophyll synthesis, with studies suggesting that neutral to slightly alkaline conditions (pH 7-9) promote optimal pigment production, whereas extreme pH levels can hinder growth (Hotos, 2022). Similarly, photoperiod plays a pivotal role in biomass accumulation, with an optimal 16:8 light-dark cycle supporting sustained growth and pigment synthesis, potentially due to a balance between photosynthetic activity and metabolic recovery (Hotos et al., 2020). Prolonged exposure to continuous light (24:0) may further enhance biomass yield, but it can also induce cellular stress, leading to diminished long-term productivity.

The implications of these findings extend beyond laboratory cultivation, as *Phormidium's* robust growth under optimized conditions highlights its feasibility for

carbon sequestration applications in aquatic ecosystems and engineered bioremediation systems. Its ability to adapt to variable environmental conditions while maintaining high biomass productivity positions it as a promising candidate for large-scale carbon capture initiatives. By optimizing media composition and environmental conditions, *Phormidium* could serve as an effective biological tool for climate mitigation, reinforcing its role as a key component in sustainable carbon sequestration strategies (Younesi et al., 2019).

6.4 MULTIVARIATE DATA ANALYSIS

Sampling Adequacy for Multivariate analysis

The results from 12 variables were analyzed using Multivariate Data Analysis to identify the key variables influencing the chromaticity stability of *Phormidium* cell culture growth. Prior to conducting the statistical analysis, data transformation was performed as part of the initial data preparation process. The entire dataset was normalized using variable transformation. Figure 6.24 presents a box and whisker plot to visually compare the distributions of the variables and to identify any outliers. The boxes represent the 25th percentile (lower quartile), the median, and the 75th percentile (upper quartile), with outliers located outside of these ranges. In this plot, the outliers were retained, as they are significant in illustrating the highest values for each variable. Among the 12 variables, the median value of H (Hue) is the highest at 98.377, while a has the lowest median at -0.037, highlighting the range of variation in the dataset.

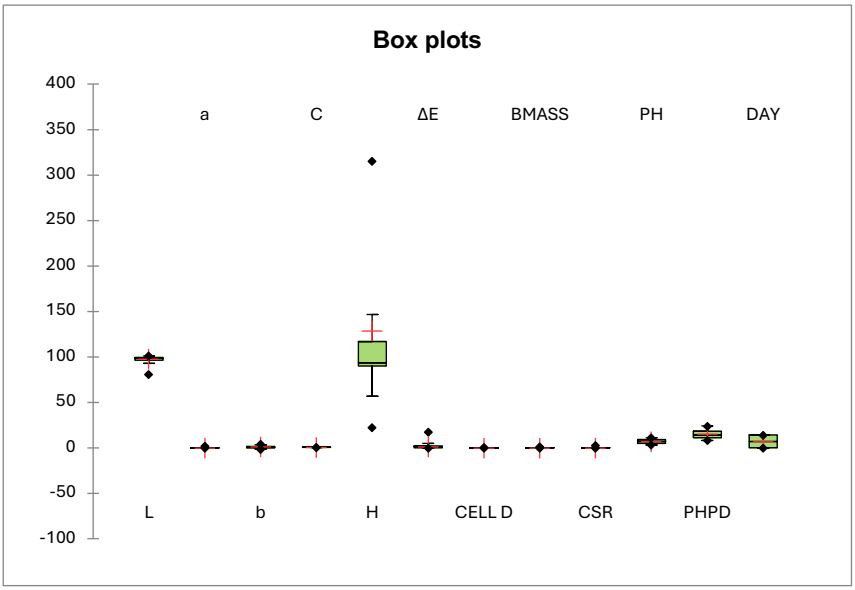


Figure 6.25: Box-and-whisker plots of *Phormidium* cell culture growth at different medium formulations, pH, photoperiod and cell culture growth time towards biomass, carbon sequestration rate and chromaticity

The KMO values for the 240 datasets produced a KMO score of 0.677 confirming the dataset is in good and adequate for PCA test. Barlett's Test of Sphericity is measured to ensure that the R-matrix does not equal the identity matrix. The *p*-value for *Phormidium3* cell culture growth chromaticity stability test exhibited highly significant differences at 0.0001.

Table 6.21 Kaiser-Meyer-Olkin and Bartlett's test for the *Phormidium* cell culture growth chromaticity stability test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy		**0.677**
Bartlett's Test of Sphericity	Chi-square (Observed value)	2047.017
	DF	66
	p-value (Two-tailed)	**< 0.0001**

A positive and negative correlation strength between two variables among *Phormidium* cell culture growth chromaticity stability test. A robust positive correlation was observed among C versus b (r^2 = 0.947). In contrast, other variables such as cell

density and biomass versus a, pH versus b, pH versus C, Cell Density and Day versus ΔE, Biomass, CSR, Day versus cell density and CSR versus biomass (r^2 = 0.517 to 0.683) have moderate positive correlations. The tables also showed the relationship of cell density versus L* produced a strong significant negative correlation (r^2 = -0.799). In general, *Phor* cell culture growth chromaticity stability test variables can be grouped into four clusters with two cluster with positive correlations and another two clusters with negative correlations as indicated in Figure 5.13.

Table 6.22 Correlation coefficient matrix for the *Phormidium* cell culture growth chromaticity stability test.

Variables	**L**	**a**	**b**	**C**	**H**	**ΔE**	**Cell Density**	**Biomass**	**CSR**	**pH**	**Photoperiod**	**Day**
L	**1**											
a	**-0.646**	**1**										
b	**-0.390**	-0.101	**1**									
C	**-0.490**	0.053	**0.947**	**1**								
H	**0.305**	**-0.025**	**-0.604**	**-0.550**	**1**							
ΔE	**-0.654**	**0.416**	-0.068	-0.020	0.115	**1**						
Cell Density	**-0.799**	**0.530**	**0.226**	**0.323**	-0.133	**0.564**	**1**					
Biomass	**-0.455**	**0.517**	0.123	**0.199**	-0.083	**0.254**	**0.580**	**1**				
CSR	**-0.410**	**0.375**	0.060	0.116	-0.063	**0.269**	**0.541**	**0.683**	**1**			
pH	**-0.386**	0.025	**0.569**	**0.571**	**-0.319**	0.066	**0.273**	0.137	0.128	**1**		
Photoperiod	-0.116	0.116	-0.054	-0.032	0.013	0.118	0.091	0.033	0.140	0.000	**1**	
Day												

Day	**-0.315**	**0.193**	-0.160	-0.110	**0.333**	**0.578**			**0.247**	0.000		
							0.526	**0.219**			0.000	**1**

Values in bold are different from 0 with a significance level alpha=0.01

The correlation coefficient (r^2) or the strength of the correlation between two variables *Phormidium* cell culture growth chromaticity stability test. The correlation coefficient is measured on a scale from -1 to +1 as described by Guilford (1973).

Table 6.23 The correlation coefficient (r2) strength between two variables among Phormidium cell culture growth chromaticity stability test

Variables	r^2	Size of Correlation	Interpretation
C x b	**0.947**	0.90 to 1.00	Very high positive correlation
-	-	0.70 to 0.90	High positive correlation
Cell D x a	**0.530**	0.50 to 0.70	Moderate positive correlation
Biomass x a	**0.517**		
pH x b	**0.569**		
pH x c	**0.571**		
Cell D x ΔE	**0.564**		
Day x ΔE	**0.578**		
Biomass x Cell D	**0.580**		
CSR x Cell D	**0.541**		
Day x Cell D	**0.526**		
CSR x Biomass	**0.683**		
H x L	**0.305**	0.30 to 0.50	Low positive correlation
ΔE x a	**0.416**		
CSR x a	**0.375**		
Cell D x C	**0.323**		
Day x H	**0.333**		
Cell D x L	**-0.799**	-0.70 to -0.90	High negative correlation
A x L	**-0.646**	-0.50 to -0.70	Moderate negative correlation
ΔE x L	**-0.654**		
H x b	**-0.604**		
H x C	**-0.550**		
b x L	**-0.390**	-0.30 to -0.50	Low negative correlation
C x L	**-0.490**		
Biomass x L	**-0.455**		
CSR x L	**-0.410**		
pH x L	**-0.386**		
Day x L	**-0.315**		
pH x C	**-0.319**		

Values in bold are different from 0 with a significance level alpha=0.01

The next step involved analyzing the data set using PCA to identify variables with high factor loadings that explain the principal components significantly. A factor loading greater than 0.75 was considered strong, between 0.50 and 0.75 was moderate, between 0.30 and 0.49 was weak, and less than 0.30 was negligible (Rani et al., 2017). Eigenvalues were used to determine the principal components with more cumulative dataset variations. An eigenvalue of 1 or greater was considered significant for the PCA (Le et al., 2017; Zubir et al., 2016; Yang et al., 2020). PCA has been applied to the 12 variables taken from *Phormidium* cell culture growth chromaticity stability test to study which variables significantly contributed to CV cell culture as detailed by Siudek & Frankowski (2018) and Vuong et al. (2020). The PCA exposed two principal components (PCs) entailing 12 variables that represent a cumulative variability of 58.407%. The eigenvalues (EV) were used to determine the principal components (PCs) with more cumulative dataset variations. The EV obtained for PC1 was 4.200, and for PC2 was 2.809 as shown in Table 5.8. In principle, variables far away from the axes F1 and F2 had a strong factor loading (FL). Referring to the Table 6.23, L*, b and C had strong FL (FL > 0.75) whereas pH had moderate FL (0.500 < FL < 0.749) and others had a weak FL (FL < 0.499).

Table 6.24 The principal components factor loading and eigenvalues of the correlation matrix for Phor cell culture growth chromaticity stability test

Variables	PC1	PC2
L	0.913	0.023
a	-0.616	-0.373
b	-0.470	0.814
C	-0.564	0.741
H	0.328	-0.680
ΔE	-0.586	-0.483
CELL D	-0.873	-0.223
BMASS	-0.677	-0.211
CSR	-0.621	-0.253
PH	-0.472	0.515
PHPD	-0.121	-0.134
DAY	-0.393	-0.565
Eigenvalue	4.200	2.809
Variability (%)	34.998	23.410
Cumulative (%)	34.998	**58.407**

The first principal PC1 accounted for 34.998% of the total variance, which comprised strong factor loadings of L*, b and C whereas for PC2 is b comprising of 23.410% of the total variance for the data. The PCA analysis allows for identifying factors and provides insight in *Phormidium* cell culture growth chromaticity stability test. Therefore, factor loadings can be utilised to determine the strength of the association between variables in *Phormidium* cell culture growth chromaticity stability test.

L	**Group 1**
H	**Group 2**
PHPD, Day, Cell D, a, ΔE, BMass, CSR	**Group 3**
B, pH, C	**Group 4**

All variables were positively correlated as long as they were grouped close together such as group 1, group 2 and group 3 variables. Group 1 and group 3 as well as group 2 and group 4 were negatively correlated as there were at the opposite positioned.

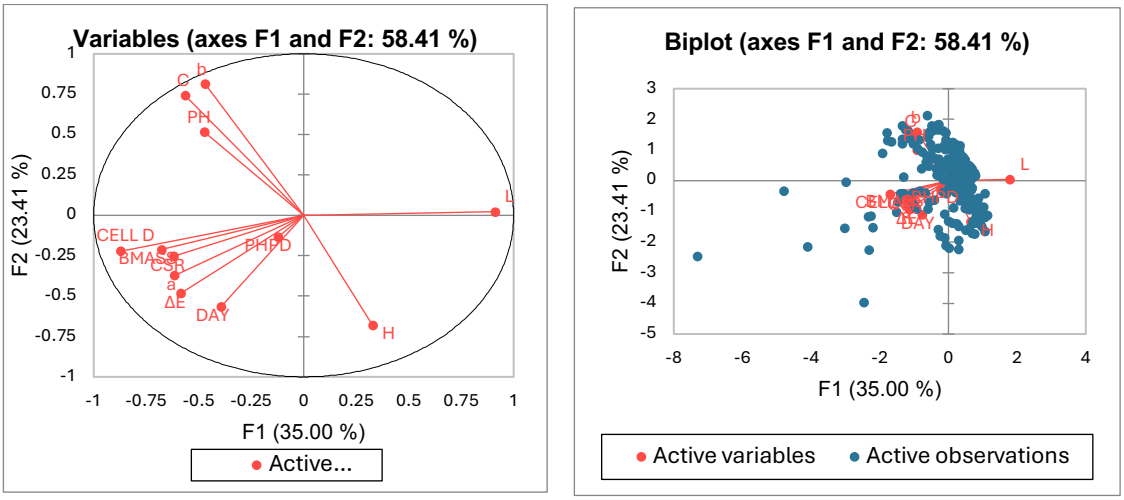


Figure 6.26 All variables were positively correlated as long as they were grouped close together as shown above

6.5 CHAPTER SUMMARY

Table below shows the comparison of *Phormidium* growth Parameters Across Different Media, Photoperiods, and pH Levels

Table 6.25 Comparison of *Phormidium* Growth Parameters Across Different Media, Photoperiods, and pH Levels"

Parameters	**BBM**	**BG11**	**Bristol**	**Chu**
Absorbance (750nm)	0.18 16:8 pH 9	0.20 16:8 pH 11	0.14 16:8 pH 9	0.004 24:0 pH 7
Total Chlorophyll Content (UV-Vis Peak)	pH 9 16:8	pH 11 16:8	pH 9 16:8	pH 7 24:0
Lightness (L*)	8:16 pH 11	8:16 pH 11	12:12 pH 11	8:16 pH 11
Chromaticity (ΔE*Lab)	16:8 pH 11	16:8 pH 11	16:8 pH 7	16:8 pH 7
Biomass (g/L)	1.069 16:8 pH 9	0.733 16:8 pH 11	0.094 16:8 pH 11	0.021 24:0 pH 9
Carbon sequestration rate (g/L C02e)	1.675 16:8 pH 9	1.149 16:8 pH 11	0.147 16:8 pH 11	0.033 24:0 pH 9

BBM showed the best overall performance, achieving the highest growth with an absorbance of 0.18 at 750nm, biomass of 1.069 g/L, and carbon sequestration rate (CSR) of 1.675 g/L CO2e under a 16:8 photoperiod at pH 9. In terms of lightness (L* = 96.86) and color variation (ΔE*Lab = 45.92), BG11 at pH 11 performed best, while also demonstrating a high CSR of 1.149 g/L CO2e under a 16-hour photoperiod.

Bristol exhibited moderate growth with an absorbance of 0.14, peaking in lightness (L* = 12:12) and biomass (0.094 g/L) at pH 9 under a 16:8 photoperiod. Chu displayed the lowest growth, with a maximum biomass of 0.021 g/L and CSR of 0.033 g/L CO2e at pH 9 under a 24:0 photoperiod.

The growth of *Phormidium* across BBM, BG11, Chu, and Bristol media under varying photoperiods demonstrates that continuous light maximizes growth parameters, especially in BBM and BG11. BBM under a 16:8 photoperiod and pH 9 provided the highest biomass and carbon sequestration rate. Shorter photoperiods or more extreme pH levels, particularly in Chu, led to reduced growth and chlorophyll content

CHAPTER SEVEN

HEAVY METAL ASSESSMENT

7.1 INTRODUCTION

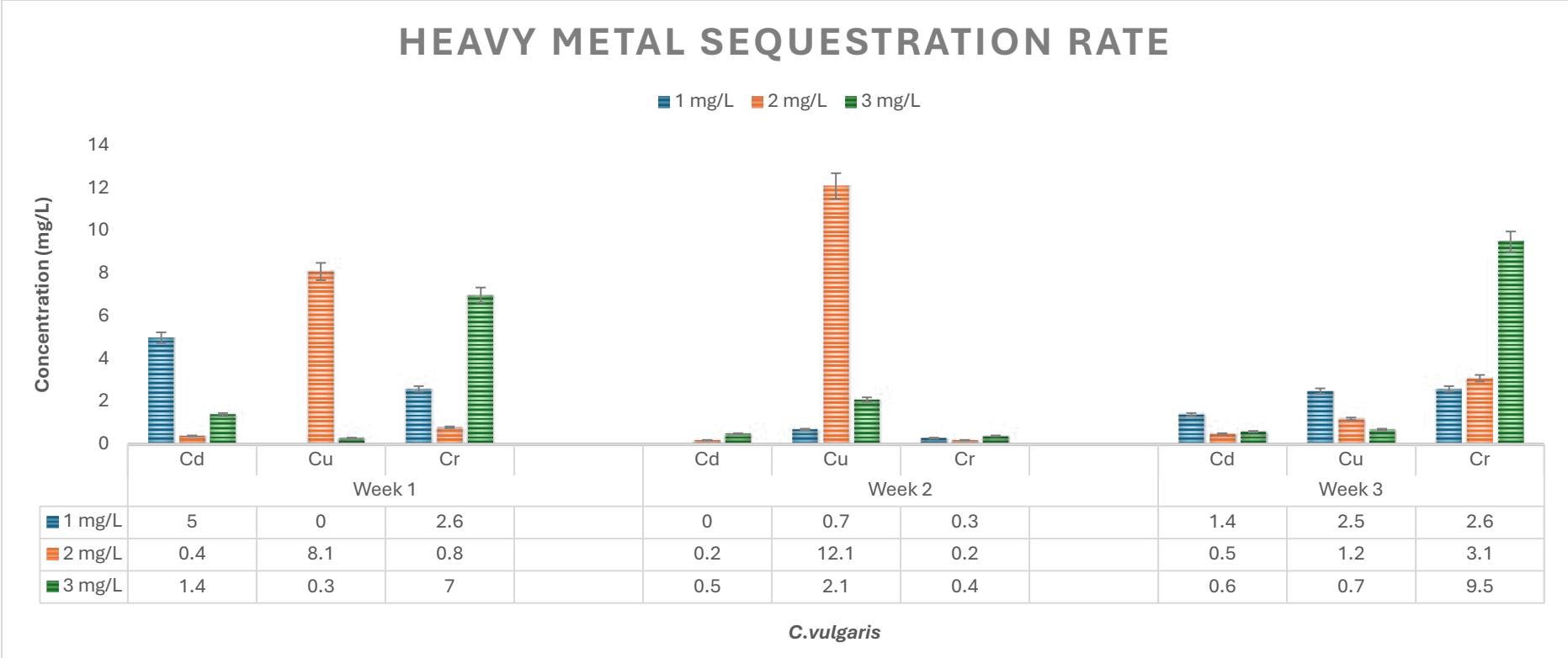
The assessment of heavy metal sequestration by microalgae, particularly *C.vulgaris* and *Synechococcus*, has garnered significant attention due to their potential in bioremediation processes. Microalgae are known for their ability to bioaccumulate various heavy metals, which can be harnessed for environmental cleanup efforts. This capability is primarily attributed to their unique physiological and biochemical properties, which facilitate the uptake and retention of heavy metals from contaminated water sources.

7.2 RESULT AND DISCUSSION

The Bio-Concentration Factor (BCF) measures the ability of *C. vulgaris and Synechococcus* to accumulate heavy metals from the surrounding environment. This section presents the BCF data for Cadmium (Cd), Copper (Cu), and Chromium (Cr) over three weeks at different initial concentrations.

7.2.1 Bio-concentration factors of *C. vulgaris*

The section presents data on the sequestration of three heavy metals. Cadmium (Cd), Copper (Cu), and Chromium (Cr) by *C. vulgaris* over three weeks. The graph below shows the concentrations of each metal in the algae after 1, 2, and 3 weeks of exposure at three different initial concentrations.



	Week 1				Week 2				Week 3		
	Cd	Cu	Cr		Cd	Cu	Cr		Cd	Cu	Cr
1 mg/L	5	0	2.6		0	0.7	0.3		1.4	2.5	2.6
2 mg/L	0.4	8.1	0.8		0.2	12.1	0.2		0.5	1.2	3.1
3 mg/L	1.4	0.3	7		0.5	2.1	0.4		0.6	0.7	9.5

Figure 7.1 BCF of *C. vulgaris* heavy metal sequestration rate at different period

C. vulgaris exhibits significant bioremediation potential with BCF values greater than 1, indicating efficient metal uptake and sequestration capabilities.

Table 7.1 Summarization of the *C.vulgaris* results BCF value greater than 1

HM	Week	Concentration (mg/L)	BCF Value
Cd	1	1	5
		3	1.4
Cu	1	1	8.1
	2	1	12.1
	3	1	2.5
Cr	1		2.6
	3	3	9.5

C. vulgaris shows significant uptake efficiency, particularly in the first and third weeks. In the case of cadmium, *C. Vulgaris* shows significant uptake efficiency, particularly in the first and third weeks. At a concentration of 1 mg/L, the BCF value reaches 5.0 in week 1 and 2.5 in week 3, while a concentration of 3 mg/L yields a BCF of 1.4 in week 1. These high BCF values highlight *C. vulgaris* ' ability to adapt to and efficiently sequester cadmium over different periods, making it highly effective for environments with moderate to high cadmium contamination. The sustained uptake over multiple weeks indicates its potential for long-term bioremediation projects.

Copper uptake by *C.vulgaris* is exceptionally efficient across various concentrations and weeks. The BCF value of 8.1 at a concentration of 2 mg/L in week 1, and the remarkably high BCF of 12.1 at 1 mg/L in week 2, demonstrate rapid and substantial copper sequestration. Even in week 3, with a BCF of 2.5 at 1 mg/L, the data suggests that *C. vulgaris* can continue to absorb copper efficiently over time. This high efficiency, particularly in the second week, positions *C.vulgaris* as an ideal candidate for remediating environments with significant copper contamination, allowing for both immediate and sustained cleanup efforts.

For chromium, *C. vulgaris* exhibits effective uptake capabilities, especially at higher concentrations. In week 1, a concentration of 1 mg/L results in a BCF of 2.6, and

a concentration of 3 mg/L yields a BCF of 7.0. By week 3, the BCF value at 3 mg/L increases to 9.5, indicating a robust ability to sequester chromium over an extended period. This significant sequestration capacity at higher concentrations makes *C. vulgaris* particularly suitable for environments with substantial chromium pollution, where it can reduce heavy metal levels effectively and maintain water quality.

Referring to BCF table (table 10), the order of BCF for heavy metal of *C. vulgaris* in descending order Cu>Cd>Cr. Only plant species with BCF greater than 1 have the potential to be used for metal hyperaccumulators. The hyperaccumulators of BCF greater than 1 sometimes reached 50 – 100 (Usman et al. 2019). The type, combination, and concentrations of heavy metal ions vary among wastewaters and it is a common situation rather than a relatively simple metal ions solution.

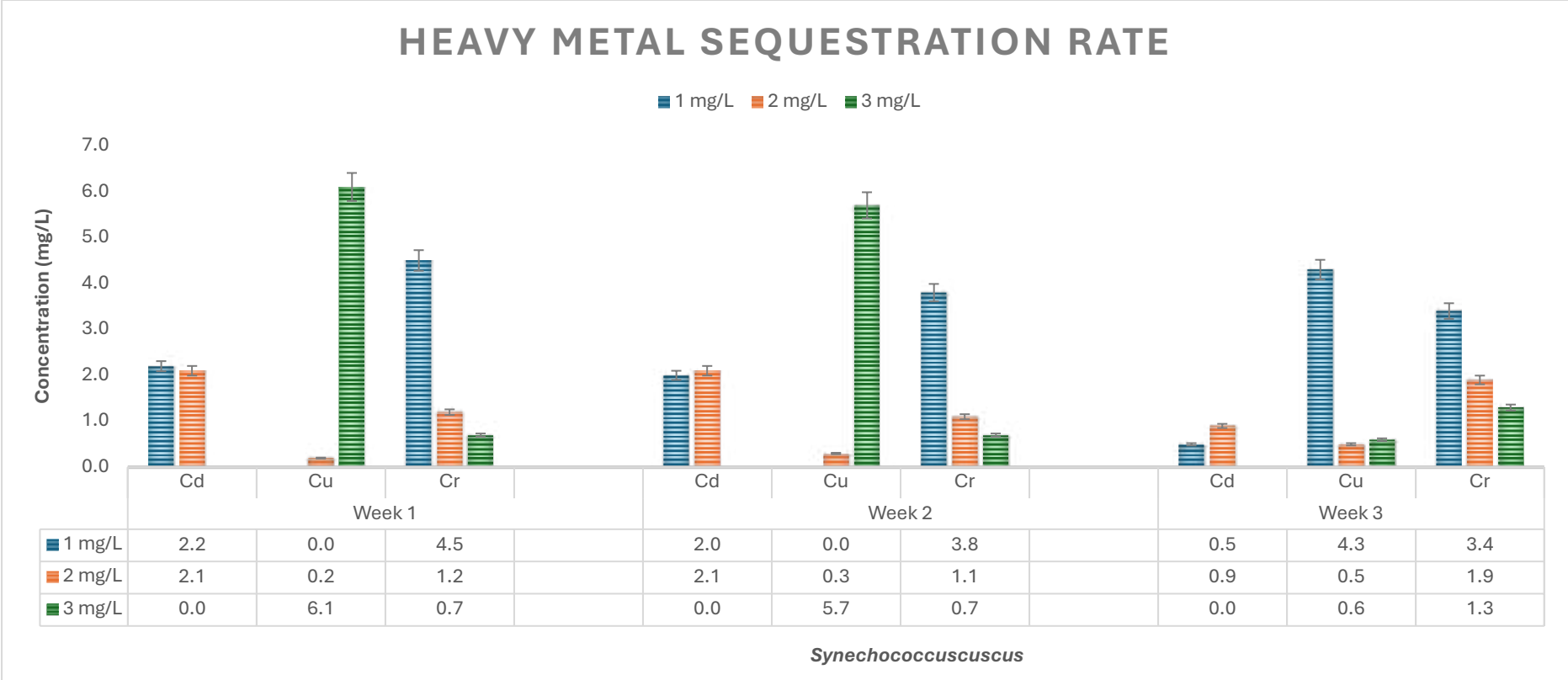
For instance, electrolytic effluent contains a mixture of metal ions such as Hg, Mn, Pb, and Cu ions (Zeraatkar et al., 2016). In multi-metal contaminated waste water, competition among the metal ions to bind with the active site of the cell surface is directly influenced by the concentration of each ion and their properties, primarily electronegativity and ionic radius (Kanamarlapudi et al., 2018). Then, a review study by Pavithra et al. (2020) reported that *C. vulgaris* was capable of treating Pb, Zn and Cr. Meanwhile, other microalgae species mentioned that have the potential for phycoremediation were *Pseudochlorococcum ypicum;* Hg and Pb. According to another study (Kumar & Oommen, 2012), dried *Spirogyra hyaline* has the potential as a biosorbent for the removal of Cd, Hg, Pb, As, and Co from aqueous solutions at different initial concentrations and period. The order of heavy metal removal was found to be Hg>Pb>Cd>As>Co. Based on a review paper by Suresh et al. (2015), C. vulgaris studied was mostly the non-living biomass focused on the heavy metal binding of Ni, Pb, Cu, Cd, and Zn. The range of pH applied for the phytoremediation was 4-7. Besides, *Chlorella sp* proved its potential in various forms (non-living, living, free, and immobilized) in different heavy metal treatments.

The high BCF values observed in the analysis confirm that *C. Vulgaris* can efficiently sequester heavy metals, making it an excellent candidate for bioremediation in environments with significant contamination. Its adaptability, high uptake rates, and versatility position it as a valuable tool for restoring and maintaining water quality in

polluted ecosystems. Further research and field trials can optimize its application, ensuring effective and sustainable environmental cleanup efforts.

7.2.2 Bio-Concentration Factors of *Synechococcus*

The section presents data on the sequestration of three heavy metals. Cadmium (Cd), Copper (Cu), and Chromium (Cr) by *Synechococcus* over three weeks. The graph below shows the concentrations of each metal in the algae after 1, 2, and 3 weeks of exposure at three different initial concentrations.



	Week 1				Week 2				Week 3		
	Cd	Cu	Cr		Cd	Cu	Cr		Cd	Cu	Cr
1 mg/L	2.2	0.0	4.5		2.0	0.0	3.8		0.5	4.3	3.4
2 mg/L	2.1	0.2	1.2		2.1	0.3	1.1		0.9	0.5	1.9
3 mg/L	0.0	6.1	0.7		0.0	5.7	0.7		0.0	0.6	1.3

Figure 7.2 BCF of *Synechococcus* heavy metal sequestration rate at different period

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Cd	1	1	5
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Cu	1	1	8.1
	2	1	12.1
	3	1	2.5
Cr	1		2.6
	3	3	9.5

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7.2.3 Bio-Concentration Factors of *Synechococcus*

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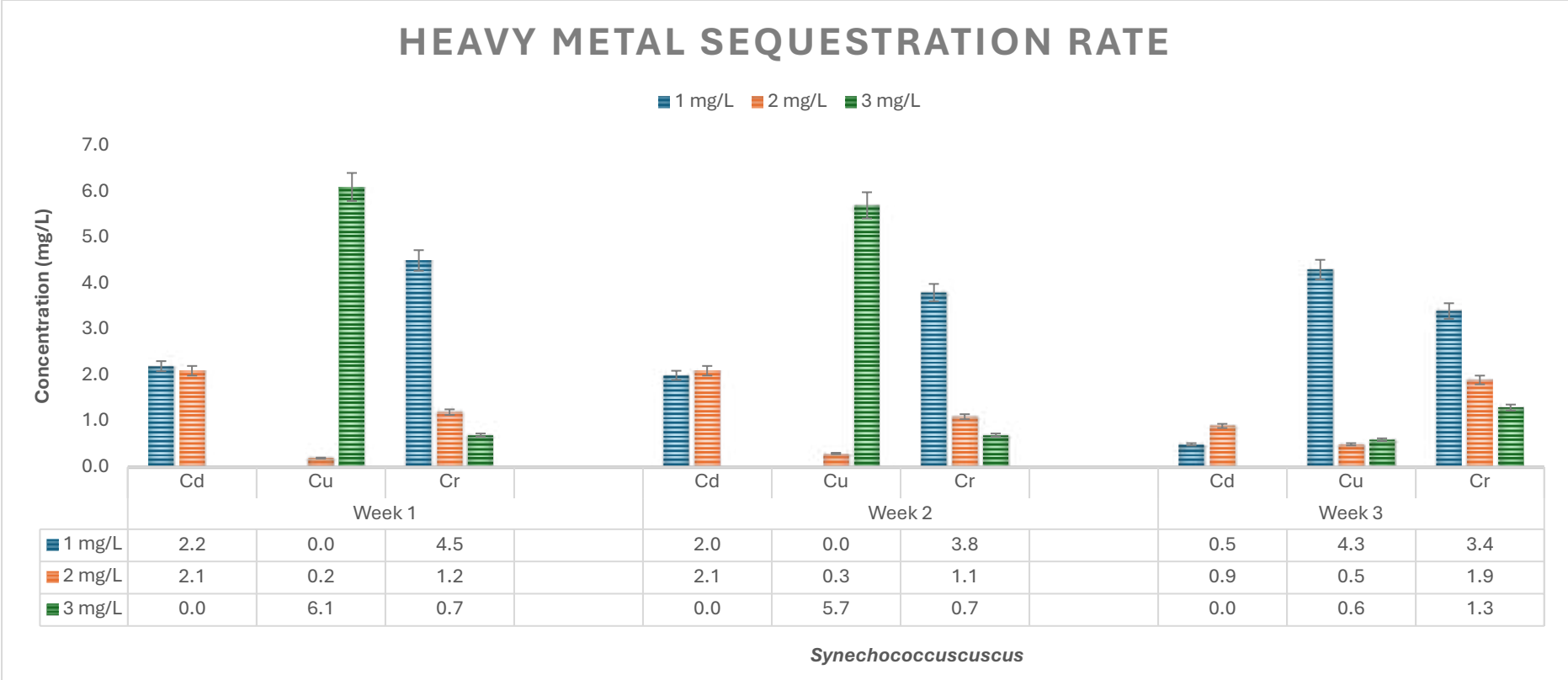

	Week 1				Week 2				Week 3		
	Cd	Cu	Cr		Cd	Cu	Cr		Cd	Cu	Cr
1 mg/L	2.2	0.0	4.5		2.0	0.0	3.8		0.5	4.3	3.4
2 mg/L	2.1	0.2	1.2		2.1	0.3	1.1		0.9	0.5	1.9
3 mg/L	0.0	6.1	0.7		0.0	5.7	0.7		0.0	0.6	1.3

Figure 7.2 BCF of *Synechococcus* heavy metal sequestration rate at different period

Synechococcus exhibits significant bioremediation potential with BCF values greater than 1, indicating efficient metal uptake and sequestration capabilities.

Table 7.2 Summarization of the *Synechococcus* results BCF value greater than 1

HM	Week	Concentration (mg/L)	BCF Value
Cd	1	1	2.2
		2	2.1
	2	1	2
		2	2.1
Cu	2	3	6.1
	3	1	4.3
		3	5.7
Cr	1	1	4.5
		2	1.2
	2	2	1.1
		1	3.8
	3	1	3.4
		3	1.9

Cadmium (Cd) accumulation is observed at both 1 mg/L and 2 mg/L concentrations during the first two weeks. At 1 mg/L, the BCF starts at 2.2 in Week 1 and slightly decreases to 2.0 in Week 2, with a further drop to 0.5 by Week 3, indicating a declining capacity for Cd bioaccumulation over time. Similarly, at 2 mg/L, the BCF is consistently 2.1 over the first two weeks but decreases to 0.9 in Week 3. This suggests that the *Synechococcus* species initially takes up Cd efficiently but shows diminished bioaccumulation capacity as exposure continues, potentially due to saturation or detoxification mechanisms within the species.

In the case of copper (Cu), significant bioaccumulation occurs, especially at the higher concentration of 3 mg/L. The BCF is 6.1 in Week 1, slightly declining to 5.7 in Week 2, indicating that the *Synechococcus* species absorbs large amounts of Cu at elevated concentrations. At 1 mg/L, the BCF is still substantial in Week 3, recorded at

4.3. This suggests that *Synechococcus* has a strong affinity for copper, even at lower concentrations, making it an effective bioaccumulator of this metal.

Chromium (Cr) shows bioaccumulation across all three weeks, with a BCF of 4.5 at 1 mg/L in Week 1, decreasing to 3.8 in Week 2 and further to 3.4 in Week 3. At 2 mg/L, Cr's BCF starts at 1.2 in Week 1, but by Week 2 and Week 3, it decreases below 1, indicating that the accumulation of Cr diminishes at higher concentrations. This pattern suggests that *Synechococcus* is more efficient at accumulating Cr at lower concentrations, but its ability to do so diminishes over time, likely due to cellular limitations or toxicity effects.

Overall, the *Synechococcus* species demonstrates a strong capacity for bioaccumulating heavy metals, particularly Cu, and Cd, with a notable initial uptake that decreases over time. The trend indicates that while *Synechococcus* is efficient at absorbing these metals, prolonged exposure or higher concentrations may lead to reduced bioaccumulation due to potential saturation or adaptive detoxification processes. The ability of *Synechococcus* to bioaccumulate heavy metals highlights its potential for bioremediation applications, especially in environments contaminated with copper and cadmium.

Research has shown that *Synechococcus* can effectively sequester heavy metals from contaminated water. A study examining the effects of various environmental conditions on heavy metal uptake found that *Synechococcus* exhibited significant bioaccumulation of metals including Cd, Cu, and Cr, with BCF values greater than 1, indicating their potential for use in bioremediation strategies (Othman et al., 2020). This study utilized Inductively Coupled Plasma (ICP) analysis to quantify metal concentrations, confirming the efficiency of these cyanobacteria in removing heavy metals from wastewater (Othman et al., 2020).

The mechanisms underlying heavy metal uptake in *Synechococcus* involve both passive and active processes. Passive adsorption of metals occurs on the cell surface, while active transport mechanisms facilitate the internalization of metals, allowing for their accumulation within the cells (Zeraatkar et al., 2016). The presence of specific metal-binding proteins and other cellular components enhances the ability of

Synechococcus to tolerate and accumulate heavy metals, further supporting their role in bioremediation (Cobos et al., 2022). Moreover, environmental conditions such as pH and nutrient availability significantly influence the heavy metal uptake efficiency of *Synechococcus* . Optimal conditions can enhance the bioaccumulation process, making these cyanobacteria more effective in sequestering heavy metals from polluted environments (Zeraatkar et al., 2016). This adaptability to varying conditions underscores the potential of *Synechococcus* as a viable option for bioremediation applications.

7.3 CHAPTER SUMMARY

The comparison table below presents the Bio-Concentration Factor (BCF) values for cadmium (Cd), copper (Cu), and chromium (Cr) between *C.vulgaris* and *Synechococcus* over specific weeks at highest concentration of BCF values indicate the species' capacity to bioaccumulate these heavy metals from their environment.

Table 7.3 Comparison result of *C.vulgaris* and *Synechococcus*

HM	Week	Species	BCF Value	Highest BCF
Cd	1	*C.vulgaris*	5	/
		Synechococcus	2.2	
	2	*C.vulgaris*	0.5	
		Synechococcus	2.1	
	3	*C.vulgaris*	1.4	
		Synechococcus	0.9	
Cu	1	*C.vulgaris*	8.1	
		Synechococcus	6.1	
	2	*C.vulgaris*	12.1	/
		Synechococcus	5.7	
	3	*C.vulgaris*	2.5	
		Synechococcus	4.3	
Cr	1	*C.vulgaris*	7	

		Synechococcus	4.5	
	2	*C.vulgaris*	0.4	
		Synechococcus	3.8	
	3	*C.vulgaris*	9.5	/
		Synechococcus	3.4	

The comparison of Bio-Concentration Factor (BCF) values between *C.vulgaris* and *Synechococcus* for cadmium (Cd), copper (Cu), and chromium (Cr) reveals distinct patterns in metal accumulation across three weeks. *C.vulgaris* consistently demonstrates a higher BCF for Cd and Cr compared to *Synechococcus* indicating superior bioaccumulation capacity for these metals. In particular, *C.vulgaris* shows a BCF of 5 and 8.1 for Cd in Week 1 and Week 2, respectively, outperforming *Synechococcus s* Similarly, for Cr, *C.vulgaris* exhibits a BCF of 8.1 in Week 1 and 9.5 in both Weeks 2 and 3, significantly higher than *Synechococcus*

For copper, *C.vulgaris* initially has a higher BCF (12.1 in Week 1), but *Synechococcus* shows increasing Cu accumulation in Week 3, surpassing *C.vulgaris* with a BCF of 4.3 compared to 2.5. Overall *C.vulgaris* is more effective in bioaccumulating Cd and Cr, while *Synechococcus* demonstrates stronger copper accumulation later in the study. This indicates that *C.vulgaris* may be more suitable for bioremediation of Cd and Cr, while *Synechococcus* could be considered for Cu remediation in long-term exposure scenarios.

In conclusion, *C.vulgaris* demonstrates a superior capacity for bioaccumulating cadmium (Cd) and chromium (Cr) compared to *Synechococcus* making it more suitable for bioremediation of environments contaminated by these metals. However, for copper (Cu), *Synechococcus* shows increasing bioaccumulation over time, indicating its potential for long-term copper remediation.

CHAPTER EIGHT

COMPARATIVE ANALYSIS

8.1 COMPARISON OF GREEN AND BLUE-GREEN ALGAE CELL CULTURE GROWTH IN DIFFERENT MEDIUM FORMULATION AND CONCENTRATION

The results of the three algae species, specifically Green algae: *C.vulgaris* and Blue-green algae: *Synechococcus* sp. and *Phor* sp. were compared based on the following parameters:

1. Optimum absorbance values (pH 7.0) in various media formulations (BBM, BG11, Bristol, and Chu 10).
2. Absorbance values in different medium strengths, specifically BBM at concentrations of 0.5x, 1x, 2x, and 3x.
3. Absorbance values under varying concentrations of Polyethylene Glycol (PEG), with concentrations of 5 g/L, 10 g/L, and 15 g/L.
4. Absorbance values under different concentrations of Salicylic Acid (SA), with concentrations of 25 mg/L, 50 mg/L, and 100 mg/L.

8.1.1 Medium formulation and strength

Table 8.1 Comparison of Media Formulation Absorbance Value

Medium	***C.vulgaris***	***Synechococcus***	***Phormidium***
BBM	1.45	1.28	0.55
BG11	1.12	0.90	0.2
Bristol	0.31	0.12	0.05
Chu	0.21	0.09	0.03

Table 8.2 BBM medium strength absorbance value

Medium Strength	***C.vulgaris***	***Synechococcus***	***Phormidium***
0.5x	0.31	0.52	0.33
1x	1.45	1.28	0.55
2x	0.18	0.2	0.09
3x	0.19	0.17	0.07

Overall, *C.vulgaris* demonstrated the best growth in BBM across both the media formulation and medium strength tests, with *Synechococcus* performing slightly lower but still competitive. *Phormidium* on the other hand, consistently exhibited the lowest absorbance values across all conditions, indicating limited adaptability or growth in the tested media formulations and strengths.

8.1.2 PEG and SA Concentration

Table 8.3 PEG concentration absorbance value

PEG Conc. (g/L)	***C.vulgaris***	***Synechococcus***	***Phormidium***
5	0.71	1.16	0.17
10	0.62	0.68	0.29
15	0.56	1.07	0.46

Table 8.4 Salicylic acid concentration absorbance value

PEG Conc. (mg/L)	***C.vulgaris***	***Synechococcus***	***Phormidium***
25	0.19	0.24	0.13
50	0.16	0.52	0.30
100	0.14	0.51	0.10

Overall, *Synechococcus* displayed the highest absorbance across both PEG and SA concentrations, indicating its superior growth performance under these stress conditions. *C.vulgaris* showed a moderate response, with higher absorbance in PEG compared to SA, suggesting that PEG may be a more favorable medium for its growth. *Phormidium* however, exhibited the lowest absorbance values in all tested conditions, indicating its limited growth potential when subjected to both PEG and SA stressors. This suggests that *Phormidium* may require more optimal conditions for growth or that it is more sensitive to the chemical stress induced by PEG and SA treatments.

8.2 COMPARISON OF THE EFFECTS OF PH, MEDIUM FORMULATION, AND PHOTOPERIOD ON CELL CULTURE GROWTH, BIOMASS, CHROMATICITY, AND CHLOROPHYLL CONTENT

This section compares the behavior of green algae *C.vulgaris* and blue-green algae (*Synechococcus* sp. and *Phormidiumr* sp.) cell culture growth in four different media: BBM, BG11, Chu-10, and Bristol. The cultures were tested under varying pH levels (3.0, 5.0, 7.0, 9.0, and 11.0) and photoperiods (24:0, 16:8, 12:12, and 8:16). The parameters assessed include:

1. Absorbance (750 nm)
2. Effects of pH and photoperiod on UV-Vis spectra absorbance values for chlorophyll content
3. Lightness (L*) of *C. vulgaris* cell culture growth and The CIE L*a*b* color difference or Chromaticity (ΔE*Lab) of cell culture growth
4. Assessment of biomass as a carbon sequestration agent

Table 8.5 Comparison of the highest and best result for each parameter

Parameters	**Algae sp.**	**Media**	**pH**	**Photoperiod**
Absorbance (750nm)	*C.vulgaris*	BBM	9	16:8
	Synechococcus	BBM	11	16:8
	Phormidium	BG11	11	16:8
Total Chlorophyll Content (UV-Vis Peak)	*C.vulgaris*	BBM	9	16:8
	Synechococcus	BBM	9	16:8
	Phormidium	BG11	11	16:8
Lightness (L*)	*C.vulgaris*	Bristol	11	12:12
	Synechococcus	Chu	11	8:16
	Phormidium	BG11	11	8:16
Chromaticity (ΔE*Lab)	*C.vulgaris*	BBM	9	16:8
	Synechococcus	BBM	11	16:8
	Phormidium	BG11	11	16:8
Biomass (g/L)	*C.vulgaris*	BBM	9	16:8
	Synechococcus	BBM	9	16:8
	Phormidium	BBM	9	16:8
Carbon sequestration rate (g/L C02e)	*C.vulgaris*	BBM	9	16:8
	Synechococcus	BBM	9	16:8
	Phormidium	BBM	9	16:8

BBM medium consistently demonstrated superior performance for *C.vulgaris*, *Synechococcus* sp., and *Phormidium* sp., particularly under a 16:8 photoperiod at pH levels of 9 or 11. While BG11 medium supported high growth rates, it was slightly less efficient in biomass production and carbon sequestration than BBM. Bristol medium facilitated moderate growth, especially in terms of lightness and chromaticity, but was less effective in biomass accumulation and carbon sequestration. Chu medium yielded the lowest values across most parameters, highlighting its limitations in promoting growth, biomass production, and carbon sequestration.

8.3 COMPARISON OF HEAVY METAL ASSESSMENT

Table 8.6 below shows the summary of the best findings for heavy metal bioaccumulation, highlighting the species with the highest bioaccumulation factor (BCF) for each heavy metal and each week:

Table 8.6 Comparison of the highest bioaccumulation factor (BCF) for each heavy metal and each week:

Heavy Metal	Week	Sp.	BCF Value
Cd	1	*C.vulgaris*	5.0
	2	*Synechococcus*	2.1
	3	*C.vulgaris*	1.4
Cu	1	*C.vulgaris*	8.1
	2	*C.vulgaris.*	12.1
	3	*Synechococcus*	4.3
Cr	1	*C.vulgaris*	7.0
	2	*Synechococcus*	3.8
	3	*C.vulgaris*	9.5

C.vulgaris consistently exhibited the highest BCF values for cadmium (Cd) and copper (Cu) during the first and second weeks. *Synechococcus* displayed the highest BCF value for cadmium (Cd) in week 2 and for chromium (Cr) in week 2, indicating a higher accumulation rate in the mid-term. *C. vulgaris* outperformed *Synechococcus* for chromium (Cr) in weeks 1 and 3, while *Synechococcus* showed stronger performance during week 2.

Both species demonstrate potential for use in assessing heavy metal contamination in aquatic environments, but their performance varies depending on the metal and exposure duration. *C. vulgaris* appears to be a more reliable indicator for short- and long-term contamination, whereas *Synechococcus* offers value in monitoring environments with continuous or mid-term exposure to pollutants.

8.4 CHAPTER SUMMARY

The study highlights the distinct capabilities of *C. vulgaris, Synechococcus sp., and Phormidium sp.* as potential landscape ecological indicators by analyzing their growth, chlorophyll content, biomass production, carbon sequestration, and heavy metal accumulation under varying environmental conditions. The results demonstrate that different algae species respond uniquely to culture conditions, reinforcing the need for species-specific applications in environmental sustainability efforts.

The growth performance of each species varied depending on the culture medium, pH, and photoperiod. *C. vulgaris* exhibited the highest absorbance at 750 nm in BBM medium under pH 9 and a 16:8 photoperiod, indicating optimal conditions for its biomass production and photosynthetic activity. Similarly, chlorophyll content peaked under these conditions, suggesting high photosynthetic efficiency. In contrast, *Synechococcus sp*. and *Phormidium sp.* displayed moderate growth under BG11 and Chu-10 media, with their absorbance and chlorophyll content fluctuating under different pH levels. These variations highlight the adaptability of each species to specific environmental conditions, which is crucial when considering their application in ecological monitoring.

Lightness (L*) and chromaticity (ΔE*Lab) were also assessed to determine pigment composition and stability under different conditions. *C. vulgaris* exhibited higher lightness values in Bristol medium under pH 11, which may be associated with reduced pigmentation and higher photosynthetic activity. The highest chromaticity variations were observed in *C. vulgaris* under BBM medium, indicating notable pigment shifts in response to environmental stressors. Meanwhile, *Synechococcus* sp. and *Phormidium* sp. exhibited relatively stable chromaticity, suggesting potential resilience in fluctuating environmental conditions.

In terms of carbon sequestration, *C. vulgaris* recorded the highest sequestration rate of 1.675 g/L CO_2e under BBM medium at pH 9 and a 16:8 photoperiod, making it an effective agent for carbon capture. *Synechococcus* sp. achieved a slightly higher sequestration rate of 2.006 g/L CO_2e under similar conditions, indicating its strong carbon fixation potential in specific environments. *Phormidium* sp., while showing

lower carbon sequestration overall, demonstrated stability in alkaline conditions, which could be advantageous in carbon mitigation strategies under extreme pH environments.

The study also evaluated heavy metal bioaccumulation, with *C. vulgaris* demonstrating the highest bioconcentration factor (BCF) for copper (12.1 in week 2) and cadmium (5.0 in week 1). *Synechococcus* sp. showed strong chromium uptake, with a peak BCF of 7.0 in week 3, while *Phormidium* sp. exhibited moderate metal accumulation across all tested metals. These findings suggest that different algae species can be strategically applied in phycoremediation efforts, depending on the specific contaminants present in an environment.

Rather than identifying a single superior species, this study underscores the importance of leveraging the unique strengths of each algae species for targeted environmental applications. *C. vulgaris* is well-suited for carbon sequestration and broad-spectrum heavy metal accumulation, making it a strong candidate for pollution mitigation. *Synechococcus* sp. demonstrates promising carbon fixation and selective heavy metal uptake, which could be beneficial in localized remediation efforts. *Phormidium* sp., with its resilience across different conditions, may serve as a stable bioindicator in fluctuating environments. By understanding these specific capabilities, these algae species can be effectively assigned to address distinct environmental challenges, optimizing their role in landscape ecological monitoring, pollution mitigation, and climate resilience.

CHAPTER NINE

RECOMMEDATION

9.1 INTRODUCTION

This chapter consolidates the key findings of the study titled "Assessment of Selected Blue-Green and Green Algae Species as Potential Landscape Ecological Indicator Agents for a Sustainable Environment." The research examined the potential of *C. vulgaris, Synechococcus,* and *Phormidium* to serve as ecological indicators by assessing their growth performance, biomass production, carbon sequestration potential, and heavy metal bioaccumulation capacity. These species were evaluated under varying environmental conditions, with a particular focus on their role in promoting environmental sustainability and enhancing urban landscape management.

The recommendations outlined in this chapter seek to improve the scalability and practical implementation of algae-based systems for sustainable landscape and urban ecosystem management. These insights are intended to inform both future research and policy development in the fields of environmental sustainability and green infrastructure. Following these recommendations, the conclusion will summarise the broader implications of this study in advancing ecological sustainability, particularly in alignment with the Sustainable Development Goals (SDGs).

9.2 ALGAE AS LANDSCAPE ECOLOGICAL INDICATORS: AN INDEX FOR ENVIRONMENTAL ASSESSMENT

These findings highlight the role of algae in sustainable landscapes, pollution control, and climate adaptation strategies, positioning them as valuable bioindicators for ecological assessments. *C.vulgaris* demonstrates strong capabilities in heavy metal sequestration, with a high bio-concentration factor (BCF = 12.10 mg/L), indicating its suitability for pollution mitigation. Additionally, its chromaticity values suggest stable

environmental conditions. *Synechococcus* exhibits the most robust performance across multiple indicators, excelling in carbon sequestration (51.00%), biomass production (1.28 g/L), and photosynthetic efficiency (chlorophyll intensity = 3.90 Abs). Its strong green intensity (a* = 0.78) and lower lightness (L* = 75.07) further reinforce its potential as a bioindicator and *Phormidium* demonstrates moderate potential as an ecological indicator but may be more susceptible to environmental stress, as indicated by its chromaticity values (ΔE = 17.60).

Table 9.1 Algae as potential Ecological Indicator

Unit	Abs	L*	a*	b*	ΔE	mg/L	%	g/L	g/LC02e	Chl
C.vulgaris	**0.20**	79.96	2.57	1.46	8.20	**12.10**	20.00	0.50	0.78	2.85
Synechococcus	0.16	**75.07**	**0.78**	**4.57**	12.10	9.50	**51.00**	**1.28**	**2.01**	**3.90**
Phormidium	0.20	80.94	0.01	3.83	**17.60**		30.00	0.73	1.15	3.20

Therefore, from this finding an index demonstrating the potential of algae as natural bioindicators for environmental conditions, evaluated through key parameters including cell growth, CIELAB colour properties, heavy metal sequestration, and carbon efficiency had been established based on this results.

Table 9.2 Index for Environmental Assessment

Parameter	Sub-parameter	Unit	Quality Index	Landscape Ecological Indicator
Cell growth	Absorbance	Abs	> 0.1 < 0.5	Good growth and productivity
CIELAB Component	Lightness	L*	< 80.0	Healthy Environment
	Green-intensity	a*	< 1.0	Strong photosynthesis and ecosystem health
	Yellow-intensity	b*	< 3.0	Less environmental stress
	Chromaticity	ΔE	< 5.0	Stable environmental conditions
Heavy Metal Sequestration	BCF	mg/L	> 1.0	Good heavy metal absorption
Carbon Efficiency	Dry Weight	%	> 30	Efficient carbon storage
	Biomass	g/L	> 1.0	Strong carbon fixation
	CSR	g/LC02e	> 1.0	Good for climate change mitigation
	Chl intensity	Abs	> 3.0	High photosynthetic activity

Cell Growth is an absorbance measurements indicate optimal growth and productivity, signifying the suitability of specific algal species for ecological monitoring. CIELAB Colour Components shows variations in *lightness (L), green intensity (a*), yellow intensity (b*), and chromaticity (ΔE)** serve as indicators of photosynthetic efficiency, ecosystem health, and environmental stability. Heavy Metal Sequestration indicates a bio-concentration factor (BCF) exceeding 1.0 mg/L confirms the capacity of algae for heavy metal absorption, highlighting their potential for pollution mitigation. Carbon Efficiency shows key parameters such as dry weight,

biomass accumulation, carbon storage rate (CSR), and chlorophyll intensity reflect the effectiveness of algae in carbon fixation and climate change mitigation strategies.

9.2 CHAPTER SUMMARY

The systematic evaluation of *C. vulgaris, Synechococcus, and Phor*midium as potential landscape ecological indicator agents highlights distinct strengths and limitations among the three algal species. *Synechococcus* sp. demonstrates the highest carbon sequestration efficiency, excelling in biomass production and carbon sequestration rate, while also exhibiting high adaptability under varying photoperiods. This suggests its strong potential for climate mitigation applications. C. vulgaris, on the other hand, emerges as a robust candidate for heavy metal remediation, displaying high bioaccumulation capacity and strong resilience under varying pH conditions, making it well-suited for pollution control efforts. Meanwhile, Phormidium sp. shows moderate ecological indicator potential but exhibits weaker performance in growth stability, particularly under changing environmental conditions.

Overall, the findings reinforce the role of algae in sustainable landscape management, environmental monitoring, and climate adaptation strategies. The distinct ecological functions of each species suggest that their application should be tailored to specific environmental objectives, whether in carbon sequestration, heavy metal remediation, or environmental stress tolerance. Future research should explore their long-term stability and effectiveness in varying ecological settings to enhance their application as bioindicators for sustainable environmental management.

Table 9.3 Systematic Evaluation Review

Parameter	Sub-Parameter	*C. vulgaris*	*Synechococcus*	*Phormidium*
Ecological Indicator Potential	Pigmentation stability	High	Moderate	High
	Sensitivity to environmental stress	Moderate	Considerable	High
Carbon Sequestration Efficiency	Biomass production	Moderate	High	Low
	Carbon sequestration rate	Moderate	High	Low
Heavy Metal Remediation	Bioaccumulation capacity	High	Considerable	N/A
	Suitability for monitoring	High	Moderate	N/A
Environmental Stress Tolerance	Growth under varying pH	High	Moderate	Moderate
	Growth under varying photoperiod	Moderate	High	Low

CHAPTER TEN

CONCLUSION

This study provides a novel contribution to the field of landscape ecology and environmental sustainability by establishing a systematic evaluation of blue-green and green algae species as potential ecological indicator agents. The research introduces an integrated assessment framework that examines the growth performance, biomass production, carbon sequestration potential, heavy metal bioaccumulation, and environmental stress tolerance of *C. vulgaris, Synechococcus*, and *Phormidium* under varying environmental conditions. The findings offer new insights into the application of algae as bioindicators for landscape health monitoring and sustainable urban ecosystem management, bridging the gap between microalgae research and ecological landscape planning.

A key novelty of this study lies in the development of an ecological indicator scorecard, which provides a quantitative and comparative assessment of the selected algae species across multiple environmental parameters. This scorecard serves as a practical tool for policymakers, urban planners, and environmental scientists to assess and implement algae-based ecological monitoring systems. The results indicate that *Synechococcu*s exhibits the highest potential for carbon sequestration and biomass production, while *C. vulgaris* demonstrates superior heavy metal bioaccumulation capacity. *Phormidium,* although showing moderate potential, is more susceptible to environmental stressors, highlighting species-specific strengths and limitations in different ecological contexts. Furthermore, t the existing body of knowledge by evaluating the photoperiod and pH tolerance of these algae species, which has not been comprehensively explored in previous research within the context of landscape ecological indicators. The integration of chromaticity analysis and chlorophyll intensity measurements further strengthens the study's novelty by providing an additional layer of precision in assessing the physiological responses of algae under diverse environmental conditions.

The implications of these findings extend beyond laboratory settings, offering practical applications for sustainable landscape design, climate adaptation strategies, and urban greening initiatives. By demonstrating how algae species can be harnessed as natural indicators of environmental stability, this study paves the way for future research in biomonitoring, carbon offset strategies, and eco-sensitive urban development. In conclusion, this research establishes a new paradigm in landscape ecological monitoring by positioning microalgae as viable tools for sustainable environmental management. The holistic evaluation framework and ecological indicator scorecard developed in this study provide a scientific foundation for integrating algae-based systems into green infrastructure planning and ecological risk assessment models. These contributions align with global sustainability goals, particularly the Sustainable Development Goals (SDGs), reinforcing the role of algae in climate resilience, pollution mitigation, and sustainable urban landscapes.

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APPENDIX: PUBLICATION

Algae as an Ecological Indicator in Creating a Halal Sustainable and Ecological Environment

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ABSTRACT

Environmental problems, including global warming and climate change widely acknowledged as one of the top global threats with far-reaching consequences. Despite the importance of decreasing CO_2 emissions or developing carbon-free energy sources, carbon sequestration should be a key issue since the amount of carbon dioxide that already exists in the atmosphere is great enough to cause global warming. Algae can fix carbon dioxide 10-50 times more than other terrestrial plants. It also has a rapid growth and multiplying rate much greater than higher plants. The study is aimed to explore algae characteristics and behaviour as potential ecological indicators and sequester agents for an unhealthy environment in an urban context. It was conducted by cultivating algae in controlled environments, which include different formulations, photoperiod and pH. Then, the selected species of algae will be evaluated and compared for their efficiency as potential carbon sequestration agents and lastly, to identify the colour changes of the algae that indicate the surrounding environment. These algae will be optimized and applied in landscape ecological design as phycoindicator and as a phycosequestration. Besides being

a marker in creating a halal sustainable and ecological environment, it could help reduce the planet's natural impact by employing biological and sustainable initiatives aligned with maqasid shariah's objectives. The principle of *Al Darar Yuzal* is very close to prevent all kind of harm in order to protect human live, wealth as well as future environment for next generation as what is being concluded in objectives of *shariah*.

Keywords: *Ecological Indicator, Environment, Maqasid shariah, Al Darra Yuzal, Carbon Sequestration*

INTRODUCTION

Climate change is widely acknowledged as one of the top global threats with far-reaching consequences. These include; rising sea levels, elevation in the concentration of atmospheric greenhouse gases, elongated heat waves with more frequency, loss of mass in Greenland and Antarctic ice sheets and biodiversity loss (Helen et al., 2021). In some cases, cultures have been affected, while some coastal cities have been flooded due to rising sea levels affecting more than 150 million people (Xu et al., 2019; Ekwebelem et al., 2020). Hence, a biological technique involving the application of microorganisms to capture and convert carbon dioxide to food, chemicals or fuel will be environmentally welcoming, greener, and cost-effective. Carbon Dioxide is captured using absorbent materials and released into the environment. Biological capture and sequestration of carbon using algae have been recognised as one of the world's most important and effective carbon sequestration methods (Moreira & Pires, 2016; Alami et al., 2021). Helen et al. (2021) also summarise that bio-capturing carbon using algae has been deemed environmentally friendly, economically feasible, and sustainable in the long run.

Algae can fix carbon dioxide 10-50 times more than other terrestrial plants. It also has a rapid growth and multiplying rate (a few hours), much greater than higher plants. Additionally, traditional manufacturing processes are unsustainable, utilising non-renewable feedstock and releasing large quantities of greenhouse gases, toxic side streams, and waste products into the environment (Arun et al., 2020). Implementing an algae-based biological as a carbon-capture approach facilitates carbon footprint mitigation and bioenergy production. (Choi et al., 2019). Therefore, all these are evidence that algae have strong environmental flexibility as they can tolerate and adapt to various extreme environmental conditions and enhance their applicability. Halal provides various guidelines which also include creating halal environment.

LITERATURE REVIEW

Algae has been used for centuries in environmental assessments, especially in water bodies, as an indicator of a good ecosystem. Algae are also a major source of problems threatening many ecosystem goods and services. Besides capturing carbon dioxide through photosynthesis, they can respond to future challenges regarding its availability; algae can produce more oxygen than plants can produce. It is also directly responsible for almost 50% of the photosynthesis on earth (Cenci et al., 2017).

Architectural applications use algae to reduce the impact on the planet by employing biology to solve some of the world's biggest problems. The use of algae has many benefits over more expensive traditional treatment technologies. In comparison, traditional treatment requires enormous resources and discharges huge toxic byproducts, while algae are used to minimise environmental pollutants in an eco-friendly way (Priyadharshini et al., 2021). The efficiency of carbon captured by algae varies depending on their algal

physiology, pond chemistry, and temperature. A study by Keffer and Kleinheinez (2002) revealed that carbon capture efficiencies are as high as 80% to 99% under normal conditions, with gas residence time obtained within two seconds. Algae efficiently utilize carbon dioxide, water and nutrients more than terrestrial plants because of their simple cellular structure (Pacheco et al., 2020). Hence, algae are considered to have high carbon mitigating and photosynthetic efficiencies, reducing atmospheric carbon emissions more rapidly than terrestrial.

Ecological civilization is a way of approaching social and ecological reform and represents a new standard of human existence that may be sustainable well into the future. Various landscape ecology measures have been used extensively throughout the landscape ecology community (Frohn, 2018). Algae is one the new measures that have the ability as a future landscape indicator due to many reasons proven by several studies, which are:

Table 1: Ability of Algae as a Future Landscape Indicator

Nos	Advantages	Author	Year
1.	Algae have the potential to address several issues concurrently, which is not feasible in traditional treatments in an environmentally sustainable way.	Daneshvar et al.	2019
2.	The ability of algae to sequester carbon is a promising alternative to the risk of global warming. The oxygen emitted will then be used to help purify the atmosphere.	Lutzu et al.	2021
3.	Remediation using algae is a cost-effective treatment that does not require large number of toxic substances or electricity to operate.	Sirakov et al.	2013

The table above demonstrates that algae can be used as future landscape indicators in creating more sustainable environments.

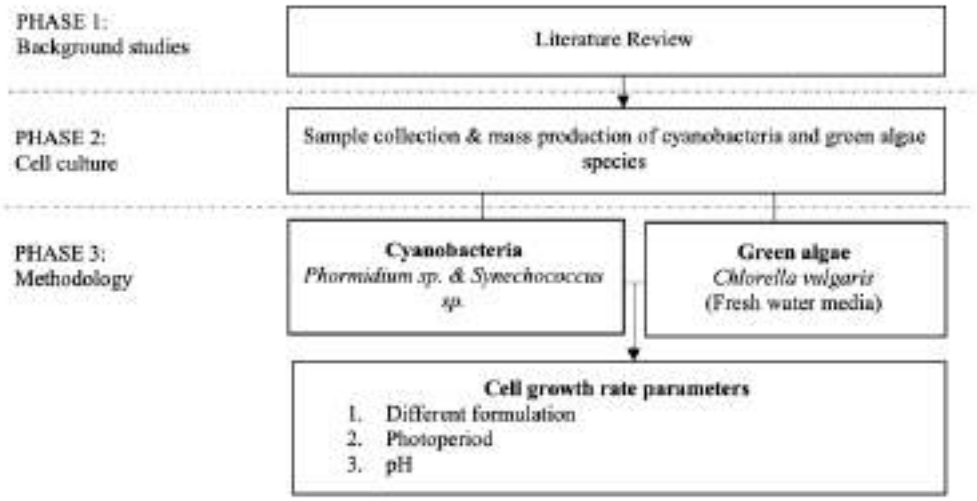
Algae are key primary producers in aquatic environments and represent several emerging genetic model systems (Hopes et al., 2016) Apart from playing an important role in human nutrition (FAO, 2014), photosynthesis of algal could provide about one-half of the oxygen (Price et al., 2012; Cenci et al.,2017). According to Mishra et al. (2022), Algae often occur in extreme environments, which reflects their ability to endure various natural and anthropogenic stresses. They need to survive with low concentrations of few essential nutrients in natural water and low availability of light and temperature. Apart from the limiting factors, they must survive and grow in adverse habitats enriched with salts, toxic metals and pesticides, elevated temperature, pH, UV-B radiation, and light intensity. Algae are the primary producers of water bodies (from rain ponds to oceans). Therefore, the tolerance range of algae to diverse stress factors has huge significance from an ecological standpoint. Also, the basic metabolic mechanism of algae is similar to higher plants.

The concept of Halal-Tayyiban (clean and pure) takes into account protection of health, food safety, animal rights, tincluding the environment, social justice and welfare in the food production, fair business practices, and ethics. It is seen as a more comprehensive system that aims to accomplish international standards compliance, making it universally acceptable. Tayyiban. There is also an environmentally friendly approach at the core of Halal Tayyiban that respects Mother Nature and also protects and cares for the environment. Rooted in the Islamic teaching, it is clearly noted that corruption of all kinds, including environmental corruption, which includes industrial pollution, environmental damage, and reckless exploitation and mismanagement of natural resources are disliked by Allah (SubhanahuWaTa'ala) (Idris, 2021).

To conclude, in this study, these algae will be optimized and applied in creating halal sustainable and ecological environment as phycoindicator (algae that indicate the health of the ecosystem), phytoremediation (algae is used to clean up contaminated environment), and as a phycosequestration (to sequester carbon involving algae).

METHODOLOGY

Table 2: Methodology



The culture was kept under Philips fluorescent light at 1800 lux of light intensity 25 µmol photons.$m^{-2}s^{-1}$ at intended photoperiod (light:dark) cycle, at 24:0, 12:12, 18:6, 6:8, and 0:24. The cultures were put under the temperature of 24 ± 1 °C for two weeks. Below is the list of all parameters conducted:

i. Algae strain: *Chlorella vulgaris* (Freshwater and marine), *Phormidium*, & *Synechococcus*
ii. Medium: Freshwater (BBM, Bristol, BG11, and Chu10)
iii. Photoperiod: All species in the different medium were put under five photoperiods (light: dark) cycle, at 24:0, 12:12,18:6, 6:8, and 0:24. The best photoperiod were chosen according to the fast growth and normal pigmentation of algae for next experiment of pH.
iv. pH: Different pH (3.0,5.0,7.0,9.0 & 11.0) was tested to determine the suitable pH for different species of algae.
v. Observed responses as in table 3

Table 3: Observed Responses

Parameters	Unit
Cell growth rate per day	ABS
Density of cell	$\times 10^4$ ML^{-1}
Cell chromaticity component:	CIELAB UNIT
Bluish green (-)/red purple (+)	A*
Yellow (+)/blue (-)	B*
Hue	H°
Biomass weight	%

EMPIRICAL RESULTS

Algae production grew more rapidly under the evaluated environment. This finding shows that they do not have to occupy arable land to grow, unlike terrestrial plants where they need large acres of land to plant and later, it takes years for them to grow. Figure 1 below shows the growth curves of the alga in a controlled environment. In 14 days, algae could have reached their stationary phase and later be used as a remediation agent in creating a sustainable environment.

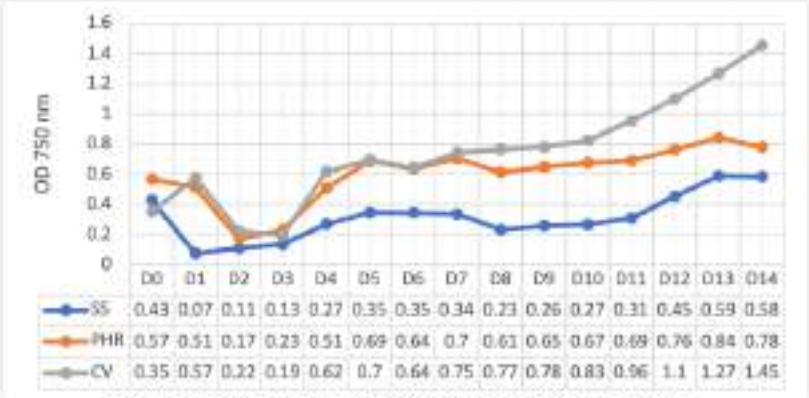


Figure 1: Growth Curves of the Microalgae Species in 14 days

Different pH range will significantly affect microalgae growth. The effect of pH on algal growth shows that the growth was influenced by pH at different ranges. Figure 2 below shows the intensity of the green color of microalgae (from the top: *synchococcos, phormidium and chlorella vulgaris).* The intensity become darker at pH 7 and above and decreased at pH5 and below.

Figure 2.: Growth Observation of (from the top) *synchococcos, phormidium and chlorella vulgaris*

All species were observed with the highest growth at a higher pH (pH 7 and above). They were grown at the lowest with pH 3. Figures 3 and 4 below display the different pigmentation of green depend on the culture growth. The highest growth is observed in pH 9 and pH 11. The pigmentation decreases accordingly as the pH level decreases. As the pH increases, the intensity of the algae is also influenced by the alkaline condition. It shows that algae can be a good indicator of the aquatic system's environmental conditions and bio-indicator pollution levels based on their changing color according to their pH.

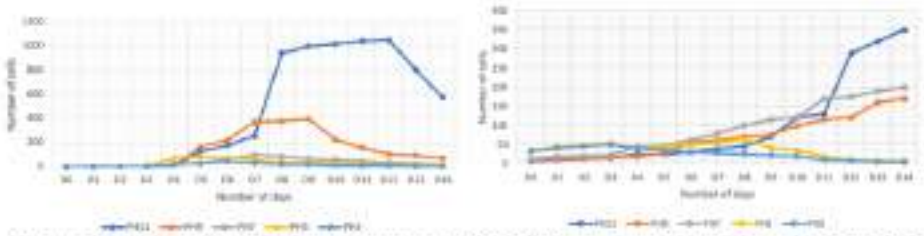


Figure 3: Cell Growth of (left) *synchococcos* and (right) *phormidium* from pH3 to pH 11 in BBM

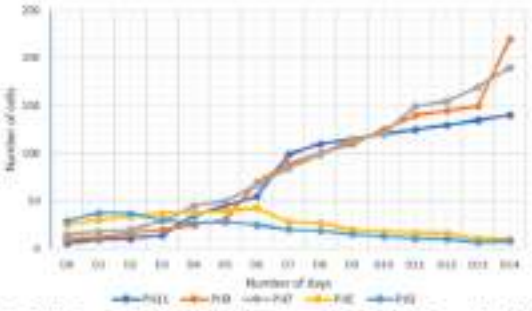


Figure 4: Cell Growth of *chlorella vulgaris* from pH 3 to pH 11 in BBM

Some other key features of microalgae are the high photosynthetic efficiency. Microalgae have the ability to fix carbon dioxide 10-50 times more than other terrestrial plants (Moreira and Pires, 2016). Different environmental factors could influence their growth rates.
Next, Algae production was found to be significantly affected by medium formulation. The effect of time on cell growth is shown in Table below. Different media show a significant effect on algae growth. Table 4 and 5 illustrates the results of the effect of BBM on three different species: *chlorella vulgaris, phormidium*, and *synechococcus* and it is evident that the time growth for each species is different even it is in the same medium formulation (BBM).

Table 4: Comparison Between Algae Species in the Same Formulation (BBM) (D0-D7)

Species	D0	D1	D2	D3	D4	D5	D6	D7
SS	0.4281	0.1744	0.1097	0.1345	0.2688	0.3475	0.4455	0.5365
PHR	0.5672	0.5143	0.1705	0.231	0.5075	0.6879	0.6355	0.7045
CV	0.3515	0.5771	0.2214	0.1936	0.6164	0.6954	0.6441	0.7452

Table 5: Comparison Between Algae Species in the Same Formulation (BBM) (D8-D14)

Species	D8	D9	D10	D11	D12	D13	D14
SS	0.5315	0.6578	0.7677	0.8079	1.0548	1.1896	1.2844
PHR	0.712	0.7492	0.8746	0.8904	1.0613	1.1242	1.2779
CV	0.7655	0.7809	0.8251	0.9553	1.1004	1.2669	**1.4548**

These three species started with low absorbent values and gradually increase until it reaches 1.0 starting on day 12 (indicated in red font). Therefore, it is concluded that *chlorella vulgaris* shows the highest value, especially on day 14 (1.4548) compares to other species.
Next, table below shows the correlation between carbon weight and its chromaticity. Pollution can lead to changes in water color. Therefore, the pollution degree can be monitored by measuring the chromacity of water. In order to create halal sustainable environment, algae can be used as indicator to determine the environment conditions. Such as algae bloom which can become a serius public's health and environmental problem.

Table 6: Correlation Between Carbon Weight and Chromacity for *chlorella vulgaris* in BBM

	carbon weight		Chromacity (D14)		
CV (BBM)	Weight	Cell Density	Δa*	Δb*	s
pH 3	0.39	0.296	0.69	-1.4	0.2
pH 5	1.53	0.556	1.45	-2.68	0.15
pH 7	4.3	1.458	1.25	-2.71	0.16
pH 9	4.63	1.466	1.6	-1.89	0.18
pH 11	**8.43**	**1.468**	**0.41**	**-1.51**	**0.31**

* Positive a* indicates a hue of red purple and negative a*, of bluish green. Whereas positive b* indicates yellow and negative b* blue. The colour of achromatic or grey is when a* and b* = 0.

Table 6 data highlight the major effect the pH can have on the presence and accumulation of green pigment intensity and cell weight. There was a positive relationship between pH conditions, saturation and green colour intensity of algae cell culture as shown in figure 5 below.

Figure 5: Positive Relationship between pH Conditions, Saturation and Green Colour Intensity

	ph 3	ph5	ph7	ph9	ph11
Δa*	0.69	1.45	1.25	1.6	0.41
Δb*	-1.4	-2.68	-2.71	-1.89	-1.51
s	0.2	0.15	0.16	0.18	0.31

In general, it can be concluded from the above results that environmental factors can influence the expression of green-intensity pigments, cell density and total weight og algae cells.

Algae cultivation aims to create a halal sustainable environment. Since algae is a fast-growing species, the effort we would need to spend to cultivate them is very little. It is proved that the ability to grow fast is not a hassle since some algae reproduce quickly, and fast-growing algae is also a quality that can be used to absorb carbon dioxide on a large scale. Land-based plants contribute 52% of the total carbon dioxide absorbed by the earth's biosphere. In comparison, ocean-based algae contributed 45% to 50% of that, which means that despite their small size, algae can absorb carbon dioxide efficiently because of their comparatively short life cycles (Cai, 2018). Thus, algae can reduce the amount of carbon dioxide in the atmosphere if supported by an appropriate environment.

The assessment of algae as indicators for aquatic environments is widely used, but their potentials are not yet fully recognized. Algae provide an integrated measure of water quality as experienced by the aquatic biota and have many biological attributes that make them ideal for biological monitoring (Barinove et al. 2010). They also respond quickly to changes in water chemistry. For example, increases in water acidity due to acid-forming chemicals that influence lake pH levels and heavy metals discharged from industrial areas affect the colour changes of the algae itself. Hence, this could impact drinking water supplies, aquatic life, and recreational water quality by supporting excessive algae growth. Nutrients reach waterbodies through agricultural and urban runoff, sewage discharges and detergents containing phosphorus.

CONCLUSION

Algae is outstanding among all the types of biological solutions to natural problems according to its ability, as mentioned before. Besides being a marker in creating a halal

sustainable and ecological environment, it could help reduce the planet's natural impact by employing biological and halal sustainable initiatives. The halal sustainable also provides a conducive ecosystem that can govern a healthy and sustainable lifestyle within the framework of permissible and lawful as defined by the concept of halal.

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