

**CARBON DIOXIDE SEQUESTRATION BY
CYANOBACTERIUM *MICROCYSTIS AERUGINOSA* (KUTZING)**

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY**

R. LALREMDIKA

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DEPARTMENT OF BOTANY

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By

R. LALREMDIKA

DEPARTMENT OF BOTANY

SUPERVISOR: PROF. SURYA KANT MEHTA

SUBMITTED

**IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF
THE DEGREE OF DOCTOR OF PHILOSOPHY IN BOTANY OF
MIZORAM UNIVERSITY, AIZAWL**



MIZORAM UNIVERSITY
MIZORAM: AIZAWL - 796 004

Dr. S. K. MEHTA
Professor

Botany Department

Post Box No. 190
Gram: MZU
Phone: 0389-2330733
Fax: 0389-2330532
Email: mzut051@mzu.edu.in

CERTIFICATE

This is to certify that Mr. **R. Lalremdika** has submitted the thesis entitled “**Carbon dioxide sequestration by Cyanobacterium *Microcystis aeruginosa* (Kutzing)**” conducted under my supervision, to fulfill the requirements for award of the Degree of Doctor of Philosophy in the Department of Botany, Mizoram University, Aizawl. The work is authentic, content of the thesis is the original work of the Research Scholar, and the nature and the presentation of the work are the first of its kind.

It is further certified that no portion(s) or part(s) of the content of the thesis has been submitted for any degree in Mizoram University or any other University or Institute.

Date:

(PROF. SURYA KANT MEHTA)

Place: Mizoram University

Supervisor

Department of Botany

Mizoram University

DECLARATION

MIZORAM UNIVERSITY

May, 2024

I **R. Lalremdika**, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to do the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree at any other University/Institute.

This thesis is being submitted to the Mizoram University for the degree of Doctor of Philosophy in Botany.

Date:

(R. LALREMDIKA)

Place: Aizawl, Mizoram

Department of Botany

Mizoram University

Aizawl-796004

(PROF. F. LALNUNMAWIA)

(PROF. SURYA KANT MEHTA)

Head

Supervisor

Department of Botany

Department of Botany

Mizoram University

Mizoram University

Aizawl-796004

Aizawl-796004

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Date:

(R. LALREMDIKA)

PREFACE

The significant rise in atmospheric carbon dioxide levels driven by human activity, especially the burning of fossil fuels and industrial processes, has become a major worldwide concern and is a major factor in both climate change and environmental degradation. Unchecked, the rise in carbon dioxide emissions would have dire consequences for the environment, hence mitigation measures must be taken immediately and efficiently. Despite the advancement of these alternatives, fossil fuels remain the most practical option for meeting the current global energy demands due to their higher energy efficiency. Consequently, there is a pressing need for research into technologies that can mitigate the impact of fossil fuel usage. Among these technologies, carbon capture and sequestration has emerged as a pivotal tool for sustainably addressing atmospheric carbon dioxide emissions.

This thesis delves into the innovative and promising field of biological carbon capture and sequestration, particularly focusing on the utilization of cyanobacteria. Unlike physical methods of carbon capture, which often suffer from high operational costs and various technical challenges, the biological approach, especially using cyanobacteria, offers a more economical and sustainable alternative. Cyanobacteria are remarkably adaptable microorganisms with a high capacity for photosynthesis, enabling them to efficiently sequester carbon. The research focuses on promising cyanobacterial strain: *Microcystis aeruginosa*.

The subsequent chapters provide a comprehensive exploration of the potential of *Microcystis aeruginosa* in carbon dioxide sequestration, detailing their mechanisms of carbon fixation, growth, and cultivation techniques. Additionally, the research extends its focus by examining the downstream applications of cyanobacterial biomass, emphasizing its transformation into biochar—a process that not only facilitates long-term carbon storage but also offers valuable agricultural benefits.

The thesis is organized for ideal interpretation. Chapter I, "Introduction," which describes the issue of alarming rise of atmospheric carbon dioxide levels and the promise of carbon sequestration using cyanobacteria, its biomass transformation into

biochar and potential long term carbon storage and application in soil amendments. Chapter II, "Materials and Methods," acts as a framework, clarifying the experimental setup, materials utilized, and data collection methodologies. Chapter III, "Results," summarizes the study findings in a clear and succinct way, typically using figures and tables to aid visualization. Finally, Chapter IV, "Discussion," evaluates the results in light of existing research, emphasizing the most relevant innovations and their significance for carbon dioxide sequestration and application in agriculture. The major body of the thesis is preceded by an abstract, which provides a brief summary of the study objectives, methods, important findings, and conclusions. A "Summary" that follows Chapter IV restates the main research topics, highlights the important discoveries, and emphasizes the importance of this study.

By highlighting the advantages of using cyanobacteria over traditional methods and terrestrial plants for carbon dioxide sequestration, this research aims to contribute to the broader effort of developing sustainable and effective strategies for mitigating climate change. The findings presented herein underscore the significant potential of cyanobacteria as a viable solution to reduce atmospheric carbon dioxide levels, paving the way for further advancements in biotechnological applications for environmental sustainability.

Date:

(R. LALREMDIKA)

Mizoram University

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ABBREVIATION

AAS	Atomic absorption spectroscopy
Ca	Calcium
CaCl ₂	Calcium chloride
CaCO ₃	Calcium carbonate
CEC	Cation exchange capacity
CFU/g	Colony-forming unit per gram
Co	Cobalt
CO ₂	Carbon dioxide
Cu	Copper
CuSO ₄	Copper(II) sulfate
dS m ⁻¹	Decisiemens per metre
EC	Electrical conductivity
Fe	Iron
FTIR	Fourier Transform Infrared Spectroscopy
FWHM	Full width at half maximum
FYM	Farm yard Manure
H ₂ SO ₄	Sulfuric acid
HCl	Hydrochloric acid
HNO ₃	Nitric acid
ICSD	Inorganic Crystal Structure Database
K ₂ Cr ₂ O ₇	Potassium dichromate
K ₂ SO ₄	Potassium sulfate

KBr	Potassium Bromide
KMnO ₄	Potassium Permanganate
Mn	Manganese
Mo	Molybdenum
Na ₂ CO ₃	Sodium Carbonate
Na ₂ SO ₄	Sodium Sulfate
NaHCO ₃	Sodium bicarbonate
NaOH	Sodium hydroxide
NH ₃	Ammonia
NH ₄ OH	Ammonium hydroxide
RuBisCO	Ribulose biphosphate Carboxylase-Oxygenase
Se	Selenium
SEM	Scanning electron microscope
SnCl ₂	Tin(II) chloride,
TEM	Transmission electron microscopy
XRD	X-ray diffraction
Zn	Zinc

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CHAPTER 1

INTRODUCTION

The alarming rise in atmospheric carbon dioxide levels due to human activities, such as fossil fuel combustion and industrial processes, has become a pressing global concern, contributing significantly to climate change and environmental degradation. One of the main pollutants in the environment, carbon dioxide (CO₂), makes up 68% of all greenhouse gas emissions into the atmosphere. Carbon emissions have increased by almost 50% over the past three decades and have continued to rise due to continuous global industrialization and urbanization (Ajala et al., 2021). Allowing the continued release of this toxic gas into the atmosphere might have serious effects on the environment. Therefore, carbon emissions into the atmosphere need to be drastically decreased in order to reach tolerable levels. Nations worldwide, together with other interested entities, have investigated substitute energy sources that minimize carbon emissions into the atmosphere in order to reduce carbon emissions (Ighalo et al., 2021). Although alternative energy sources have poor energy efficiency, fossil fuels are now the most practical choice for successfully supplying the world's present energy needs. Since there is still a lot of reliance on fossil fuels, research must be done on carbon capture and sequestration technology to reduce carbon emissions.

The technology of carbon capture and sequestration has become a significant tool that can address the problem of atmospheric carbon emissions in a sustainable manner (Wilberforce et al., 2021). With the help of this technology, carbon may be captured or stored in many purposes. The two main carbon capture methods that have gained prominence recently are physical and biological carbon capture and sequestration technology (Zahed et al., 2021). The physical method has been found to have various drawbacks despite being a promising technology, including high operational costs due to its high energy consumption. Additionally carbon sequestering often includes the storage, transportation, mineralization, and injection of carbon into the ocean below the surface. Consequently, the physical technique of carbon capture is a laborious process that has several technological, environmental,

economic, and even safety concerns (Zaidi et al., 2022). Although there are several obstacles with the physical technique of carbon capture and sequestration, the biological approach has shown to be an economical and sustainable way to sequester and capture carbon. This process was originally carried out by terrestrial plants like trees, but more recently, it has been shown that cyanobacteria have a higher capacity for sequestering and capturing carbon than terrestrial plants. Thus, using cyanobacteria, to capture and sequester carbon has a number of benefits over alternative carbon capture and sequestration technologies (Agarwal et al., 2022). Advantages of employing cyanobacteria or microalgae over terrestrial plants for CO₂ biofixation are listed in Table 1.1.

Table 1.1 Benefits of CO₂ biofixation with microalgae rather than terrestrial plants.

Factors	Microalgae	Terrestrial plant	References
Adaptability to unfavorable weather conditions	High	Low	(Mata et al., 2010)
Traditional agricultural food production	No competition	Direct competition	(Radakovits et al., 2010)
Efficiency of photon conversion	High (8-10%)	Low (0.5%)	(Aresta et al., 2005)
Efficiency of CO ₂ fixation	High	Low	(Kumar et al., 2010)
Growth rate	Rapid	Slow	(Mata et al., 2010)
Production and harvesting	All year round	Seasonal	(Kumar et al., 2010)

Acid deposition	None	Fair	(Aresta et al., 2005)
Expand	Easy	Difficult	(Clarens et al., 2010)
Carbon rich biomass	High	Low	(Chisti, 2007)
Cultivation costs	Low	High	(Kumar et al., 2010)
Power plant-based direct sequestration of CO ₂	High	Low	(Ho et al., 2011)

1.1 Carbon dioxide sequestration by cyanobacteria

Cyanobacteria which are also known as blue green algae are colonizing microorganisms that are found throughout the world. They perform oxygenic photosynthetic and some of them are able to fix atmospheric nitrogen. These organisms are remarkably well adapted to a wide range of environmental conditions. Cyanobacteria are a source of human food, and chemical extracts from cyanobacteria are used in the manufacture of food and many other products. They are also “nuisance organisms” in municipal water supply systems. Cyanobacteria are microorganisms that utilise light energy for photosynthesis, which is the process of converting carbon dioxide into organic molecules. Being aquatic, they usually employ a variety of strategies that together make up a "CO₂ concentrating mechanism" that helps them take up inorganic carbon (CO₂ or bicarbonate). One of the more focused approaches is the broad distribution of the bacterial microcompartments called carboxysomes, which work in tandem with CO₂ and

bicarbonate active transporters to build up bicarbonate in the cytoplasm of the cell (Rae et al., 2013). These structures are considered to utilize metabolic channelling to attach the CO₂-fixing enzyme RuBisCO and the enzyme carbonic anhydrase to the inside of the shell. This increases the local CO₂ concentrations and, in turn, the enzyme's effectiveness (Yang et al., 2024). Cyanobacteria have a rapid rate of growth, which leads to high carbon sequestration. This is one of the green technologies designed for CO₂ assimilation (Miranda et al., 2021).

Cyanobacteria or microalgae can naturally sustain high CO₂ concentrations, making them appropriate for consuming CO₂ from power plant exhaust systems (Guillaume & Dos Santos, 2023). Numerous cyanobacterial or microalgal species are known to utilize carbonates like NaHCO₃ and Na₂CO₃ for cellular growth. Microalgae can therefore be used to store CO₂ and transform it into energy (Arenas & Vaz-Pinto, 2015). Numerous cellular lipids produced from microalgae demonstrate their ability to thrive in a wide range of carbon-rich conditions. The impacts of elevated CO₂ levels on a range of species were examined by researchers, with a particular emphasis on those that are able to withstand elevated levels and still generate large amounts of biomolecules, such as lipids and triglycerides (Saifuddin et al., 2015). Several species have been investigated for CO₂ sequestration, including *Scenedesmus obliquus*, *Dunaliella salina*, *Chlorococcum littorale*, *Nannochloropsis*, *Spirulina platensis*, *Isochrysis galbana*, *Dunaliella tertiolecta* and *Chlorella kessleri*. Carbon capture often involves the use of freshwater microalgae, specifically *Spirulina platensis*, *Scenedesmus*, and *Chlorella sp* (Alami et al., 2020). Cheah et al., (2015) found that cultivated microalgae possess an intense capacity for sequestering CO₂ and can fix 1.83 kg of CO₂ kg⁻¹. Algal biomass generation and CO₂ biofixation are highly variable processes that rely on the properties of microalgae species, culture system dynamics, and physicochemical processes. The effectiveness of CO₂ bioconversion for biomass generation depends on the cultivation of specific microalgae species. A significant biosequestration rate and a high susceptibility to CO₂, temperature, pH, nutrient levels, and pollutants are characteristics of an exceptional algal species (Thanigaivel et al., 2022). The CO₂ tolerance of significant microalgae species is considered throughout the cultivation process. *Scenedesmus sp.*

has CO₂ tolerance, enabling it to grow at CO₂ concentrations between 10% and 20% (v/v), despite the fact that 2% (v/v) is the ideal CO₂ concentration. The biomass production of the microalgal species is significantly influenced by the amount of CO₂ to which it was exposed (Zhang et al., 2015).

Several cyanobacteria and microalgae have been tested for their ability to sequester CO₂. The cyanobacteria and microalgae studied for CO₂ sequestration are listed in Table 1.2.

Table 1.2 The potential of cyanobacteria and microalgae in CO₂ sequestration.

Species	CO ₂ capture efficiency	Biomass yield	References
<i>Anabaena</i> sp. ATCC 33047	1.45 g/L/d	0.31 g/L/d	(López et al., 2009)
<i>Chlamydomonas reinhardtii</i>	113 mg/L/d	512 mg/L	(Banerjee et al., 2021)
<i>Chlorella pyrenoidosa</i>	92%	90.25 g/d	(Q. Yang et al., 2020)
<i>Chlorella sorokiniana</i> UTEX1602, <i>Chlorella</i> sp. L166, <i>Scenedesmus</i> sp. 336	52.21%	900.04 mg/L	(Han et al., 2021)
<i>Chlorella sorokiniana</i> , <i>Coelastrella</i> sp., <i>Chlorella pyrenoidosa</i>	567 mg/L/d	1.1 g/L	(Ding et al., 2020)
<i>Chlorella</i> sp. L166	93.7%	-	(Song et al., 2021)
<i>Chlorella</i> sp. L166, L38 and UTEX1602	85%	-	(Song et al., 2019)

<i>Chlorella vulgaris</i>	75%	1.28 g/L	(Yadav et al., 2020)
<i>Chlorella vulgaris</i>	40%	2.12 g/L	(Alhaboubi, 2021)
<i>Chlorella vulgaris</i> (UTEX 2714)	182 mg/L/d	0.219 g/L/d	(Hossain et al., 2022)
<i>Chlorogleopsis</i> sp.	20.45 mg/L/d	1.24 g/L	(Ono & Cuello, 2007)
<i>Chroococcidiopsis</i> sp. QUCCCM 26	147.4 mg/L/d	112.2 mg/L/d	(Schipper et al., 2019)
<i>Desmodesmus</i> sp.	210 mg/L/d	1.1 g/L	(Premaratne et al., 2021)
<i>Leptolyngbya</i> sp. QUCCCM 56	181.1 mg/L/d	91.4 mg/L/d	(Schipper et al., 2019)
<i>Parachlorella kessleri</i>	86.4%	65.8 mg/L/d	(Beigbeder et al., 2021)
<i>Phaeodactylum</i> <i>tricornutum</i>	2.47 g/L/d	-	(Sobczuk et al., 2000)
<i>Picochlorum</i> <i>maculatum</i> QUCCCM 127	184.8 mg/L/d	96.7 mg/L/d	(Schipper et al., 2019)
<i>Spirulina platensis</i>	178.46 mg/L/d	1.75 g/L	(Li et al., 2020)
<i>Synechocystis aquatic</i> SI-2	50 g/m ² /d	30 g/m ² /d	(K. Zhang et al., 2001)
<i>Tetraselmis</i> <i>subcordiformis</i> QUCCCM 51	270.8 mg/L/d	157.7 mg/L/d	(Schipper et al., 2019)

1.2 Culture technique of cyanobacteria

1.2.1 Batch culture

The simplest and most effective technique for cultivating cyanobacteria in a laboratory environment is batch culture. It is volume-restricted, low-cost closed system with limited resources. After inoculation into a sterilized culture medium, the cells are grown for a period of several days and finally harvested at the peak density of cyanobacterial population. Over time, other nutritional components of the culture reduce, but the density of the cell rises continuously until some limiting components diminished. The population shows distinctive sigmoid growth pattern. Batch culture methods are commonly utilized because of their simplicity and versatility. However, there are certain drawbacks, such as contamination and harvesting, cleaning, sterilizing, refilling, and inoculating the containers requires a lot of effort (Sánchez-Bayo et al., 2020).

1.2.2 Semi-continuous culture

The majority of growth-linked products such as biomass, are produced towards the end of the growth phase. The semi-continuous method of cyanobacteria cultivation employs bigger culture vessels in which the cultures are partially harvested and refilled with fresh culture media to the original volume. Nutrients are also supplied in this manner to attain the original amounts. The cultures are then allowed to expand to a specific density, following which partial harvesting is repeated occasionally. The semi-continuous culture method yields more than the batch culture since the culture is not harvested entirely, and the system can be set up both indoors and outdoors. Some drawbacks of semi-continuous culture compared to batch culture include that the system is technically more complicated to run, and longer culture period shows that contamination, mutation, wall development, and mechanical failure are more likely to occur (da Rosa et al., 2015).

1.2.3 Continuous culture

The continuous culture method involves pumping a constant supply of culture media into a growth chamber while also washing away any extra culture at the same time. The cultures are kept at a growth rate near to the maximal growth rate. Continuous cultures are efficient, produce a steady supply of high-quality cells, and are automated. However, they are quite costly and intricate (Sokolnikova et al., 2016).

1.3 Downstream cyanobacterial biomass applications

1.3.1 Biomass harvesting

Cyanobacterial cells are small (ranging from 2-70 μm) and have low densities in culture broth (ranging from 0.3-5 g L^{-1}). Harvesting cyanobacteria from the culture broth and dewatering them are energy demanding and hence a major barrier to industrial scale production and processing of cyanobacteria (Wu et al., 2012). The most common method employed for harvesting of biomass include flocculation, centrifugation, filtration and flotation (Tan et al., 2020).

1.3.1.1 Flocculation

Flocculation is a low-cost pretreatment method used for aggregating cyanobacterial cells with negative charges to create bigger clumps, which can enhance subsequent filtering, centrifugation, and sedimentation (Bracharz et al., 2018). Chemical compounds like aluminium sulfate, alum and ferric chloride induce cells to coagulate and precipitate at the bottom or float to the surface. The biomass is then retrieved by siphoning out the supernatant or skimming the cells off the surface.

1.3.1.2 Centrifugation

The most common technique for harvesting cyanobacterial biomass from photobioreactors is centrifugation. Centrifugation of large quantities of cyanobacterial culture is commonly conducted using a cream separator, with the flow rate regulated according to the algal species and the centrifugation rate of the

separator. Centrifugation does not require chemicals, although it consumes more electrical energy than flocculation (Singh & Patidar, 2018).

1.3.1.3 Filtration

The direct filtration method collects biomass by utilizing a microbial membrane that only enables cyanobacterial cells to pass through (Bejor et al., 2013). This technique is the simplest and most economical way to harvest cyanobacteria and is frequently used to collect cyanobacterial biomass from photobioreactor. However, this method is time-consuming and needs backwashing to keep the membrane filter effective.

1.3.1.4 Flotation

Cyanobacterial species with low specific gravities are capable of floating at the surface of water. Flotation can be facilitated by dissolved air flotation, which employs air bubbles to connect the flocs and then float quickly to the surface (Singh & Patidar, 2018). Cyanobacterial cells float as foam to the top of a treatment column when fine air bubbles are produced by air injection and bind to the cell. The water beneath the foam is drained or siphoned off and the foam with concentrated cyanobacteria is removed from the top.

1.3.2 Drying of biomass

Harvested cyanobacteria comprises 97-99% water, therefore removing the majority of the water is required for long-term storage of the cyanobacterial feedstock. It is usually suggested that the moisture content of biomass be kept less than 7% (Chen et al., 2010). Drying and dewatering are crucial factors in downstream extraction; thus, it is necessary to analyze the different approaches and their implications on the cost and energy, but most importantly, biomass quality, high value products and metabolites. Microalgae or cyanobacterial dewatering reduces the amount of water required for subsequent drying stages, enabling efficient processing downstream (Show et al., 2015), however, about 20–40% of the energy needed for the entire cyanobacterial harvesting process is required for dewatering. Furthermore,

contamination concerns must be minimized in the event of manufacture for human or animal use (Yazdanabad et al., 2021).

Dewatering the microalgae or cyanobacteria and drying are both essential processes. It is considered a vital step since the cyanobacterial slurry obtained during the upstream harvesting methods might be fragile. According to Patil et al. (Patil et al., 2008), over 80% of the total energy used in the production of algal-based products, such as biodiesel, is used in the drying process. As cyanobacteria are vulnerable to microbiological contamination, mechanical damage, and hostile storage conditions, which might reduce the quality of biomass, it is essential to dry cyanobacteria effectively for appropriate storage. Cyanobacteria may be dried using a variety of techniques, including traditional sun drying, hot air drying, freeze drying, oven drying, microwave drying, and spray drying (Brennan & Owende, 2010).

Traditional methods such as sun drying and oven drying are often used as these methods do not demand considerable energy and financial input. However, these procedures may not be preferable for reasons such as vulnerability to contamination from external sources in the case of sun drying. Moreover, this process is highly dependent on weather conditions and may not be effective in regions with heavy rain and limited sunlight. Another factor is the breakdown of pigments such as chlorophyll by direct sunlight (Wang et al., 2019).

In contrast, freeze drying and spray drying has become more widespread methods for drying cyanobacterial biomass. Spray-drying can be a cost-effective method that yields high-value products, while freeze-drying might be considered one of the most reliable methods to preserve significant byproducts that could otherwise be lost (Vilatte et al., 2023). These processes have several drawbacks such as expensive maintenance and operation. Also, the high-pressure mechanisms used in the spray-drying process have a risk to rupture cells and damage highly valuable components like pigments (Hosseinzand et al., 2018). Finally, selecting the method of drying relies on the financial and energy sources available, as well as the relevance of byproducts that must be effectively obtained from the cyanobacterial harvest (Chen et al., 2015).

1.4 Cyanobacterial biomass as a raw material for biochar

Cyanobacteria possess the capacity to absorb nutrients and grow rapidly, allowing them to dominate in nutrient-rich environments. Due to high nutritional content, cyanobacteria can be used as a feedstock for the production of biochar, which can then be used as a soil amendment and long-term carbon sequestration application. Using biomass for the production of carbon negative energy and environmental applications, algal biochar produced from wastewater treatment might be a significant asset in the future (Khan et al., 2022). Cyanobacteria are a viable source of renewable energy that can reduce greenhouse gas emissions without relying on fossil fuels.

The long-term benefits of biochar for sequestering carbon dioxide and its use as a soil amendment in agriculture have drawn increased attention. Biochar production is unique because it is carbon-negative, compared to other biomass energy production methods. Biomass is converted into biochar that contains more than 90% carbon as a long-term carbon storage method to reduce greenhouse gas accumulation (Yu et al., 2017). Microalgae-derived biochar is nutrient-rich, which makes it suitable for use as a fertilizer in agricultural land. Converting algal biomass into biochar is an economically viable option for algae production enterprises due to its numerous benefits (Park & Kim, 2024).

1.5 Cyanobacterial biochar production

In the future, cyanobacterial biochar produced by wastewater remediation from aquaculture, agriculture or saline wastewater sources could generate an important revenue stream through energy cogeneration, the sale of soil fertilizer and ameliorant and the carbon credits that result from long-term soil carbon sequestration. Cyanobacterial biochar is a viable target for slow pyrolysis-based bioenergy production, resulting in the synthesis of freshwater and saltwater cyanobacterial biochar and their effects on plant development. Pot experiments carried out on comparatively fertile agricultural soil revealed minor but significant advantages of the biochar supplement over the control (Tan et al., 2021).

The primary thermochemical processes that convert cyanobacteria into biochar include pyrolysis, hydrothermal carbonization, and torrefaction. The conventional method of producing biochar produces a high yield of char through slow pyrolysis. Hydrothermal carbonization produces a carbonaceous substance known as hydrochar. Torrefaction is a widely researched pre-treatment process for converting biomass into carbon-rich solid fuels like biochar (Kumar et al., 2017).

1.5.1 Conventional and microwave-assisted pyrolysis

One of the most promising methods for converting biomass into beneficial biofuels and biochar is pyrolysis. Based on the operation circumstances, pyrolysis may be classified into various types, including the recently generated microwave-assisted pyrolysis and conventional slow pyrolysis and fast pyrolysis. The composition of solid, liquid, and gaseous products produced by each form of pyrolysis depends on the parameters under which it operates, including temperature, heating rate, and residence duration. The maximum yield of biochar is produced by slow pyrolysis, whereas the highest yield of bio-oil containing biochar as a byproduct is produced by fast pyrolysis (Roy & Dias, 2017).

Generally slow pyrolysis is conducted at modest heating rates of 0.1–1 K/s with a residence duration of 450–550 s. The initial phase is pre-pyrolysis, which is followed by solid degradation in accordance with the high rate pyrolysis process to produce pyrolysis products. Biochar rich in carbon is produced when the char eventually decomposes at a very slow rate (Suganya et al., 2016). The majority of conventional slow pyrolysis processes employed fixed bed reactors, which generate heat through a heated surface. However, some research has investigated other heating techniques, including microwave heating (Chen et al., 2015).

The method of microwave-assisted pyrolysis is highly effective in producing biochar, bio-oil, and syngas from plant residues. It has also been successfully used to pyrolyze wood and sewage sludge (Lei et al., 2011). Microwave pyrolysis has several benefits, including reduced toxic chemical content in bio-oil, increased product output, and cost and energy savings.

The processes of microwave-assisted pyrolysis and traditional slow pyrolysis differ in a few ways that affect the production of biochar. Microwave pyrolysis requires pre-treatment and catalysts before heating, whereas conventional pyrolysis uses a standard heating method. Additionally microwave pyrolysis produces bio-oil, hot water, and non-condensable gases after condensation, whereas traditional pyrolysis produces pyrolysis gas as a byproduct. Many research have been conducted on the pyrolysis of lignocellulosic biomass, however there are few on the use of microwave heating to produce algal biochar (Mohit & Remya, 2023). The economical method of microwave-assisted pyrolysis makes it suitable for large-scale manufacture of biochar from cyanobacterial products in the future, which may be utilized as soil fertilizer.

1.5.2 Torrefaction

A thermochemical conversion process called torrefaction is carried out in an inert environment without oxygen at a temperature ranging from 200 and 300°C under atmospheric pressure (Chen et al., 2015). The process partially decomposes biomass to generate torrefied biomass or char, a solid product with high carbon content. Torrefaction is mostly used to produce biochar from microalgae, which is then utilized to manufacture biofuel. Biochar produced by torrefaction can possess a higher heating value or calorific value than fossil fuels, making it a viable feedstock substitute for the generation of clean energy. In order to produce a larger yield of solid product, torrefaction is often conducted at a low temperature and short residence time under low heating rate (Nhuchhen et al., 2014). Microalgae residue should be torrefied at 250°C or below to minimize weight loss and increase energy density (Chen et al., 2015).

1.5.3 Hydrothermal carbonization

Hydrothermal carbonization (HTC) is an innovative thermochemical process that is sustainable and cost-effective (Shyam et al., 2022). HTC is a unique process that converts biomass carbohydrate components into carbon-rich solids in water, producing biochar at lower temperatures (180-260°C) and higher steam or water

pressures. Using water as the solvent, the process is carried out in water at a self-generated pressure of less than 10 bar (Yu et al., 2017). This procedure is a viable substitute for the treatment of some waste streams as it may be used to concentrate carbon from wet biomass in situations where drying is not necessary before pyrolysis. Hydrochar is the term that produced from hydrothermal carbonization (Khan et al., 2022). The HTC technique has the benefit of lowering manufacturing costs and reducing the amount of time needed to produce biochar. This may be accomplished by using remaining algal biomass and processing it into more beneficial biofuels and other products.

1.6 Characteristics of biochar

Biochar is a form of charcoal produced from biomass, by a process known as pyrolysis. Pyrolysis means heating in the absence of oxygen, which prevents complete burning of the organic biomass (which happens in open fires). It is rich in a stable form of carbon which is not oxidized by soil micro-organisms. Biochar is best described as a “soil conditioner”. Biochar has unique properties that make it not only a valuable soil amendment to sustainably increase soil health and productivity, but also an appropriate tool for sequestering atmospheric carbon dioxide in soils for the long term in an attempt to mitigate global warming (Kurniawan et al., 2023).

1.7 Biochar potential for carbon sequestration and application in soil amendments

Renewable energy such as biomass has recently gained prominence as a sustainable energy source for the environment (Ruffing et al., 2022). Atmospheric CO₂ sequestration in soil as stable humus may provide a longer-term solution than temporarily storing CO₂ in standing biomass through reforestation and afforestation. Sustainable agricultural production through the development of novel biochar technologies; assess and analyze the effectiveness of applying biochar to vegetables and rice. The function of biochar is to improve soil fertility and compost efficiency for better crops and the environment (Liang et al., 2023). The long-term carbon sequestration capability of biochar and its ability to enhance crop production makes

it an attractive agricultural and environmental amendment. Regarding agronomic and environmental management, biochar has several advantages. It can help reduce the impact of climate change, improve crop production and plant productivity, and remediate the areas that are contaminated. Biochar commercialization has challenges in ensuring durability, variety, and economic sustainability (De Bhowmick et al., 2019). The applied application of biochar in soil is suggested as a unique way to assist terrestrial ecosystems generate a substantial, long-term sink for atmospheric carbon dioxide. In addition to providing a favorable impact on emissions reduction and greenhouse gas sequestration, the production of biochar and its application to soil will result in immediate advantages such as increased crop productivity and enhanced soil fertility (Losacco et al., 2022).

Soil has a vital function in terrestrial ecosystems. Deforestation, poor land management practices, and climate change have all contributed to soil degradation in recent decades, which poses serious risks to food security and the sustainability of agriculture (Chaudhary & Kumar, 2022). Recently, researchers becoming more interested in the application of biochar to soil improvement. Large surface area, reliable carbon content, and high nutrient content are some of the specific features that make biochar a potential product for soil remediation (Wang et al., 2022). Bird et al., (2011) reported earlier research explored the use of algal biochar for soil enhancements. Biochar was produced on a pilot scale using freshwater and saltwater macroalgae. The produced biochar was low in carbon, had a limited surface area, high in ash, and included a significant amount of nutrients. Pot experiments with soil deficient in nutrients and carbon were carried out, and it was established that the addition of biochar greatly increased plant development, indicating the great potential of algal biochar as a fertilizer and soil ameliorant. In certain instances, algal biochar contains significant levels of nutrients, including nitrogen and phosphorus, due to the high nutritional content of algal biomass.

In order to solve the issue of drought, the impact of adding algal biochar on crop development under deficit irrigation was investigated. Pot experiments were conducted to investigate the effects of plant growth-promoting rhizobacteria and

algal charcoal on maize growth (Ullah et al., 2020). After the addition of algal charcoal and plant growth-promoting rhizobacteria, it was found that maize growth and biomass output were greatly enhanced, and the physiological and nutritional absorption were also elevated. This study suggested utilizing algal biochar as a method to regulate deficit irrigation. Algal biochar applications in soil suggest the potential for improving soil quality, increasing plant development, and advancing sustainable agriculture.

1.8 Objectives

The main objectives of the present study were :

- I. To study the carbon sequestration ability of bloom forming cyanobacterium *M. aeruginosa*.
- II. To transform cyanobacterial biomass into biochar by thermal conversion, and study of composition and properties of cyanobacterial biochar.
- III. To study the soil applications of biochar by using seed germination technique and potted plant growth.

CHAPTER 2

MATERIALS AND METHODS

2.1 Test organism

Microcystis aeruginosa, the freshwater cyanobacteria was selected as the test organisms. The test organism was obtained from algal culture laboratory of the Department of Botany at Mizoram University, India. With the help of existing literature, the species were identified based on their morphological characteristics (Desikachary, T. V., 1959).

2.2 Cultivation and maintenance in laboratory

M. aeruginosa obtained from culture laboratory was isolate and purified by using standard microbial streaking technique in Chu-10 agar medium under aseptic conditions in a laminar air flow cabinet (Andersen, 2005). The pure culture collected from separate colonies in the agar plates was transferred to 250 ml Erlenmeyer's flasks containing 150 ml liquid Chu-10 medium (Chu, 1942) (Fig 2.1A) and the chemical composition of Chu-10 media is shown in Table 2.1. Cultures were maintained at 25°C and under 10 : 14 light : dark period using cool white fluorescent tubes (Fig 2.1B). The initial pH of the medium was adjusted to 7.5 using 0.1N HCl/NaOH. The cultures were manually agitated at least twice or thrice a day to ensure homogeneous growth.



Figure 2.1 (A) Photographs of culture grown in Chu-10 medium, (B) Culture of test organism maintained in culture room.

Table 2.1 Chemical composition of Chu-10 medium (pH 7.5).

Macronutrients	g L ⁻¹	Micronutrients	mg L ⁻¹
KNO ₃	0.04	MnCl ₂ .4H ₂ O	0.5
K ₂ HPO ₄	0.01	Na ₂ MoO ₄ .2H ₂ O	0.01
CaCl ₂	0.04	H ₃ BO ₃	0.5
MgSO ₄ .7H ₂ O	0.025	CuSO ₄ .5H ₂ O	0.02
Na ₂ SiO ₃	0.02	CoCl ₂	0.04
Ferric Citrate	0.003	ZnSO ₄ .7H ₂ O	0.05
Citric acid	0.003		

2.3 Extraction and estimation of photosynthetic pigments

2 ml of culture was centrifuged at 12000 rpm for 15 min in order to extract photosynthetic pigments. The pellet was then suspended in 2 ml of 90% acetone and left for 12 h at 4°C. The suspension was again centrifuged and absorbance of the supernatant was measured at 664, 647, 480, 663 and 645 nm. Pigment concentration was calculated by using the following equation:

$$\text{Chl a } (\mu\text{g ml}^{-1}) = 11.93 A_{664} - 1.93 A_{647} \text{ (Jeffrey \& Humphrey, 1975)}$$

$$\text{Carotenoid } (\mu\text{g ml}^{-1}) = A_{480} + (0.114 \times A_{663}) - (0.638 \times A_{645}) \text{ (Kirk \& Allen, 1965)}$$

2.4 Extraction and estimation of carbohydrate

Extraction and estimation of carbohydrate was performed according to Hedge & Hofreiter (1962). The samples (pellets) of *M. aeruginosa* was hydrolyzed in a boiling water bath for 3 h with 5 ml of 2.5N HCl and incubated at room temperature. Solid sodium carbonate was then added to the samples to neutralize it until the

effervescence ceased. The volume was made up to 100 ml and centrifuged. 4 ml of anthrone reagent (200 mg anthrone in 100 ml of ice-cold 95% H₂SO₄) was added and the optical density was observed at 630 nm. Carbohydrate content was determined using standard glucose curve.

2.5 Extraction and estimation of total protein

Total protein content was determined using the method given by Lowry et al. (1951). A protein standard curve was prepared using different concentrations of bovine serum albumin (BSA) solutions. It was soaked in hot water bath for 15 min to induce digestion, and then cooled. 0.2 ml of Folin's reagent was added, thoroughly mixed and allowed to stand for 30 min at room temperature and absorbance was measured at 660 nm.

2.6 Estimation of total lipid

The method of Bligh & Dyer (1959) was used to determine total lipid content. The samples were mixed with 30 ml of 2:1 chloroform-methanol and sonicated using an ultrasonicator (Labsonic, Sartorius) for 1 min at 200 V and kept overnight at room temperature. 15 ml of supernatant was then removed and 10 ml of chloroform was again added to the mixture, vortexed for 10 min and 10 ml of distilled water was added to it, and then centrifuged at 3000 rpm for 10 min. Using a Pasteur pipette, a clear lower phase of chloroform containing all of the lipid was carefully collected. After drying the solvent, the dry weight was determined, and the percentage dry weight of total lipids was estimated.

2.7 Growth measurement

Growth was measured on the basis of optical density (OD) by UV-VIS spectrophotometer (Systronics-117, India) at a wavelength of 663 nm. The specific growth rate was calculated using the following formula:

$$\mu \text{ (d}^{-1}\text{)} = \ln (n_2 - n_1)/\Delta t$$

where, μ denotes specific growth rate, n_1 and n_2 are culture suspension absorbance at the beginning and end of selected time intervals, Δt represents time interval of n_1 and n_2 in days (Stein & Guillard, 1973).

2.8 Estimation of biomass concentration and productivity

Homogeneous suspension of cells was extracted and centrifuged at 10000 rpm for 10 min at 4°C. The pellet was dried in pre-weighed aluminum foil after being cleansed with distilled water until a consistent weight was achieved. The biomass concentration and productivity were calculated by the following formula:

$$\text{Biomass concentration (g L}^{-1}\text{)} = (W_2 - W_1)/\text{volume}$$

$$\text{Biomass productivity (g L}^{-1}\text{ d}^{-1}\text{)} = (X_f - X_i)/t$$

Where, W_2 and W_1 are the final and empty weight of the aluminium foil. These weight were used to measure the biomass concentration (mg ml^{-1}) at the beginning and end of the cultivation period; X_i and X_f are the biomass concentration (g L^{-1}) at the beginning and end of growth period (t) respectively.

2.9 CO₂ fixation determination

The CO₂ fixation by a test organisms to convert the inorganic CO₂ into organic compound was calculated by the following equation (Dineshkumar et al., 2015).

$$\text{CO}_2 \text{ fixation rate (g L}^{-1}\text{ d}^{-1}\text{)} = \text{Carbon content (\%)} \times \text{biomass productivity} \times 44/12$$

2.10 Carbon dioxide sequestration experiments with *M. aeruginosa* in laboratory conditions

The test cyanobacterium was grown in the selected growth medium in 500 ml flasks. Sodium bicarbonate was used as a source of carbon. A stock solution of NaHCO₃ were prepared in medium by dissolving 1 g of NaHCO₃ viz., 0,0.5,1,2 and 5 g L⁻¹ were used. The culture was incubated for 5 days and growth rate were calculated for 20 days. The biomass productivity and CO₂ fixation rate were determined.

2.11 Semi-continuous outdoor cultivation of *M. aeruginosa* for biomass yield and harvesting

The semi-continuous outdoor cultivation was carried out in a 20 L plastic jar with 10 L culture medium with a time period of 20 days as shown in Fig 2.2. The jars were inoculated with 1g of fresh cyanobacterial biomass and put in the daylight with an air bubble pump for aeration. The biomass produced was estimated by biomass concentration and productivity which was calculated every 4 days interval with a time period of 20 days. After 20 days, the jars were left undisturbed overnight to settle the pellet in the bottom of the container. The next day the pellet was collected after centrifuging at 10,000 rpm for 15 min. The pellet was dried and the algal biomass was collected (Fig 2.3).

2.12 Biochar production

The biomass of *M. aeruginosa* was converted into biochar by traditional way through slow pyrolysis which gives a high char yield. The biomass was dried in direct sunlight, crushed and sieved to get the particle size ranging from 200 μm to 300 μm . Traditional pyrolysis was carried out in a ceramic pot (8cm x 8cm) with the bottom sealed with conical flask as shown in Fig. 2.4A. The conical flask was buried below soil level and a batch of 10 g of dried biomass was kept inside the ceramic pot and then sealed with lids completely so that oxygen flow was completely blocked. The pot was surrounded by tin sheet 30 cm away from the ceramic (Fig 2.4B). The space was filled with wood and plant debris and then burned and subjected to pyrolysis around 700 to 1000°C around 1 h (Fig 2.4C). This type of traditional pyrolysis was low cost and no electricity was employed. The ceramic provided biochar and the pyrolytic vapours produced during the pyrolysis were condensed and collected as pyrolysis oil in the conical flask and the conical flask act as a storage tank. At the end of pyrolysis, heating was halted and the ceramic was cooled. Soon after, the lids of the ceramic was then cautiously opened, and the solid, black residue (biochar) in the ceramic (Fig 2.4D) and pyrolytic oil (Fig 2.4E) in the conical flask was collected. The yield of biochar was calculate by the following formula:

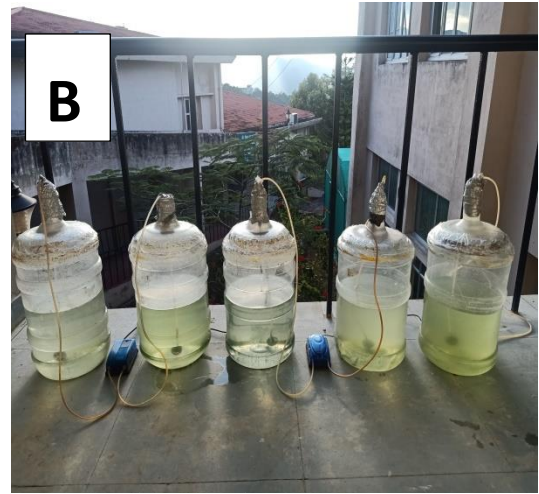
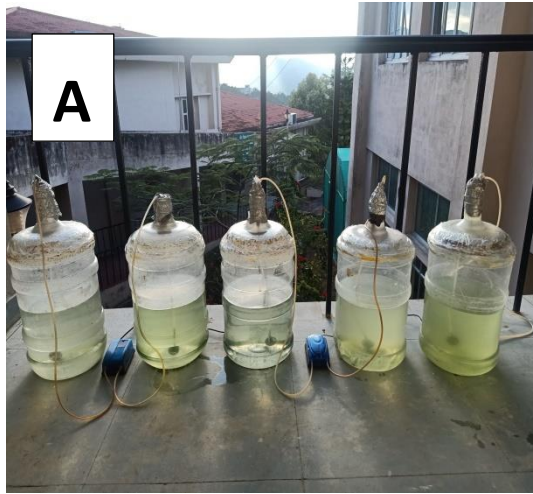


Figure 2.2 Semi-continuous culture of *M. aeruginosa* in outdoor conditions. (A) 0 day, (B) 4 days, (C) 8 days, (D) 12 days, (E) 16 days, (F) 20 days.

$$\text{Biochar yield (\%)} = \frac{W_{\text{Biochar}}}{W_{\text{Biomass}}} \times 100$$

Where, W_{Biochar} is weight of biochar (g) and W_{Biomass} is weight of biomass (g)

The pyrolytic oil produced from the biomass of *M. aeruginosa* was characterized by GC-MS. The yield of bio oil produced from pyrolysis of *M. aeruginosa* was calculated as follows:

$$\text{Bio oil yield (\%)} = \frac{W_{\text{Bio oil}}}{W_{\text{Biomass}}} \times 100$$

Where, $W_{\text{Bio oil}}$ is the weight of bio oil produced (g) and W_{Biomass} is the weight of biomass (g).

The biochar and bio oil yield (%) were determined by weighing the biochar and oil. 20 g carbonized samples were mixed with 100 ml 1.0M HCl in 250 ml beaker and then covered. The suspension were reciprocating shaker agitated at 180 rpm for 30 min at 25°C. The resultant is allowed to settle for 12 h and finally separated by vacuum filtration. The biochar was then oven dried for 24 h at 105°C, and then sieved through 0.4-0.25 mm mesh to get the particles in the size range of 70-200 µm. The biochar and oil were kept at -4°C for further used.

2.13 Bio oil characterization

The bio-oil fraction was analyzed by using Gas Chromatography–Mass Spectrometry (Thermo scientific ISQ single quadrupole). Compound identification was accomplished by comparing the recorded mass spectra of the compounds with those provided in the NIST2008 c2.0/Xcalibur data system library, which was made available by the instrument software. A silica capillary column was used to separate the bio-oil, and helium was used as the carrier gas (1.2 ml/min). A 20:1 split ratio and 260°C injector temperature were used with a 1 ml injection volume. The GC temperature sequence was start at 70°C, hold for 2 min, ramp up to 300°C at a rate of 10°C per min, and hold at 300°C for 5 min

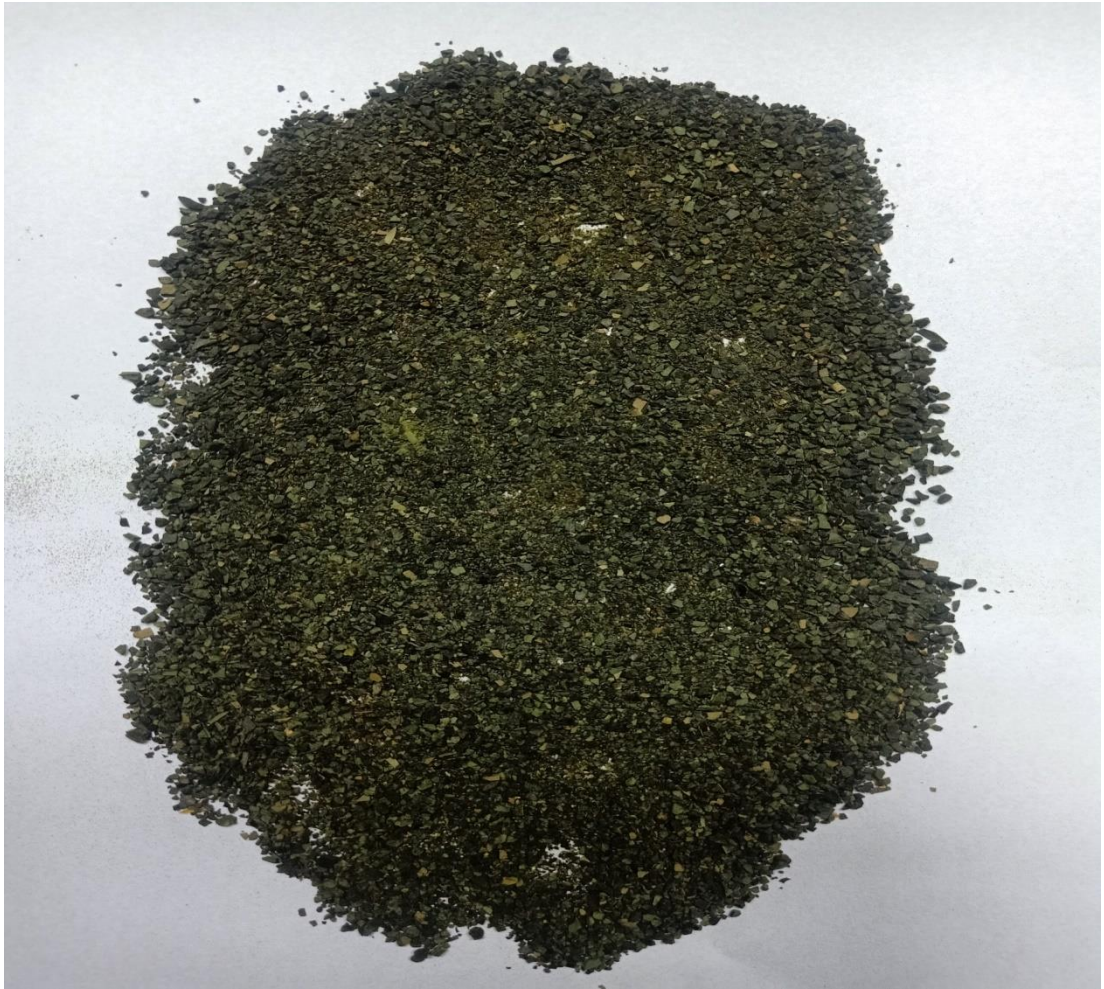


Figure 2.3 Photographs of dried biomass of *M. aeruginosa*.

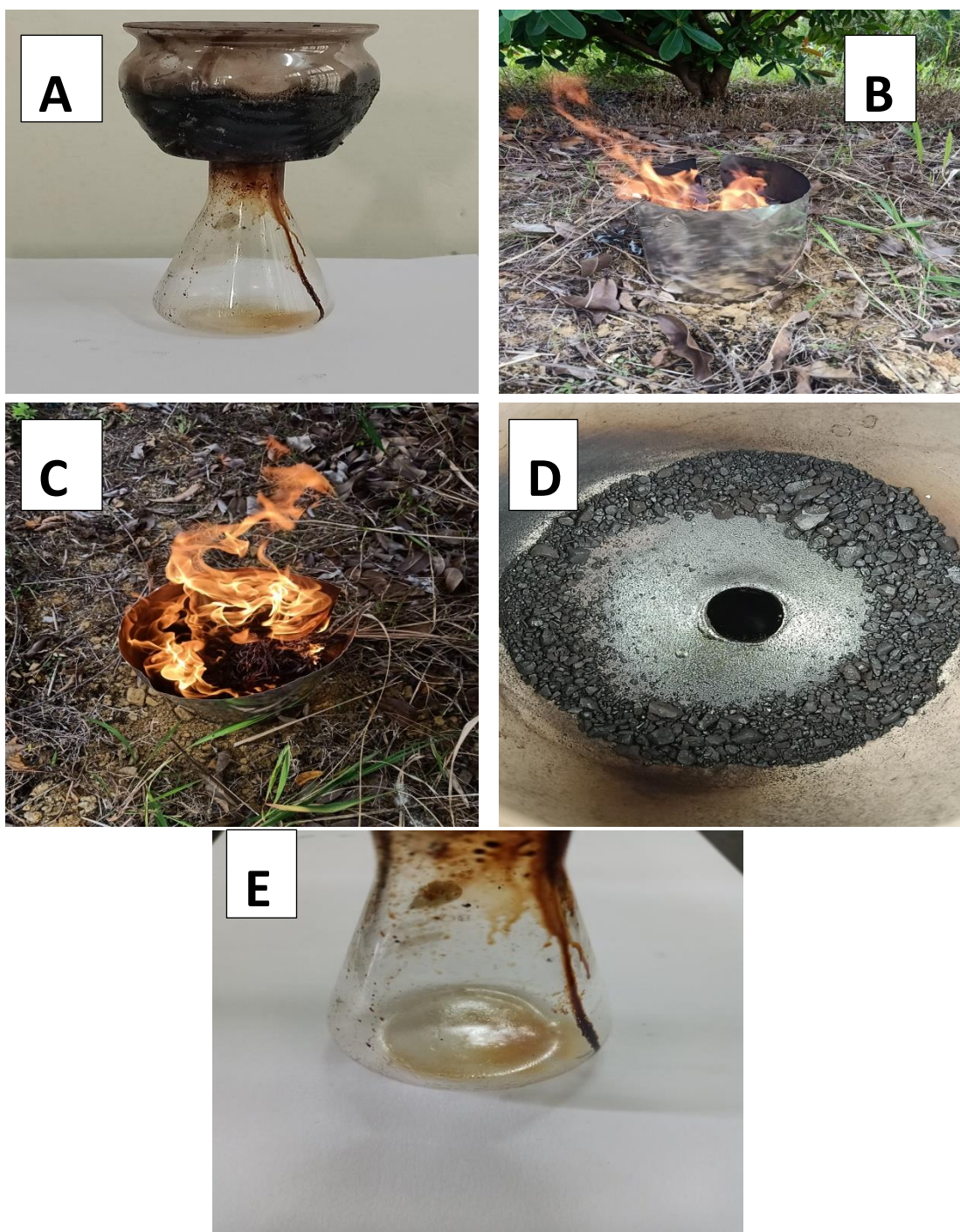


Figure 2.4 Production of Biochar from *M. aeruginosa* biomass. (A) Ceramic pot with the bottom sealed with conical flask, (B) Ceramic surrounded by tin sheet 30 cm away, (C) Space burned with plant debris, (D) Biochar produced in ceramic, (E) Pyrolytic oil produced in conical flask.

2.14 Biochar characterization

Biochar of a test organism *M. aeruginosa* was analyzed and characterized by different methods including proximate and ultimate analysis (pH, EC, CEC, moisture, ash, volatile, water holding, carbon, nitrogen, phosphorus and potassium), FTIR, XRD, SEM, TEM and AAS.

2.14.1 pH

Biochar pH was measured in 1 : 20 biochar : water (deionized water) ratio after 1 h shaking on a reciprocating shaker at 25°C. The samples were then allowed to stand for 30 min and then pH was measured using a digital pH meter (Mettler Toledo, Five Easy Plus, India). The pH 7 and 10 buffers were used to calibrate the pH meter (Singh et al., 2017).

2.14.2 Electrical conductivity

Electrical conductivity (EC) of biochar were measured in 1 : 10 biochar : water ratio after 24 h shaking on a reciprocating shaker at 25°C. After 30 min, the EC was measured using a pre-calibrated EC meter (Singh et al., 2017).

2.14.3 Cation exchange capacity

The cation exchange capacity was determined by ammonium acetate method. The biochar was mixed with 100 ml of 1M ammonium acetate solution (pH-7) and agitated thoroughly. The mixture was allowed to stand for overnight at room temperature and then the mixture was filtered through Buchner funnel fitted with moistened filter paper. The biochar residue collected was washed four times with 25 ml aliquots of ammonium acetate followed by four times washing with 25 ml aliquots of ammonium chloride. The residue was again washed with 95% ethanol and formation of cracked was prevented. Ammonium absorbed biochar residue was extracted by leaching the residue with 200 ml of 10% acidified sodium chloride solution. The filtrate was collected and transferred to Kjeldahl flask and to that 25 ml of 1N sodium hydroxide was added. The flask was refluxed at 70-75°C and the

distillate was collected in 50 ml of 2% boric acid. The boric acid solution was used for titration, where the solution was loaded with 10 drops (200 µl) of the mixed indicator (Bromocresol green-Methyl red). Then the solution was titrated against 0.1N sulfuric acid solution, till the initial color of bluish green turns to pink (Schollenberger & Simon, 1945).

2.14.4 Moisture, volatile and ash

The biochar sample was weighed and placed in a crucible close with a tightly fitted cover to prevent carbon deposits from bituminous, subbituminous, and lignite from burning away from the underneath and directly inserted into the furnace chamber which was maintained at a temperature of $950 \pm 20^{\circ}\text{C}$. After heated for 7 min, the crucible was removed from the furnace and allowed to cooled, weighed as soon as cold. The percentage of ratio of weight lost by sample weight gives the moisture content and the percentage loss of weight minus the percentage moisture equals the volatile matter (ASTM Standard: D3173, 2011). For determination of ash of biochar, the sample was material pulverized to pass 250 mm sieve and then transferred to crucible and placed in cold furnace and gradually heated at $500 \pm 10^{\circ}\text{C}$ about 1 h. The crucible was removed from muffle furnace, cooled under conditions to minimize moisture pickup and weighed.

2.14.5 Water holding capacity

Water holding capacity of biochar was measured using the keen box method as described by Piper (1945). Whatman No. 1 filter paper was placed over the bottom of a keen-box that had a perforated hole (about 0.75 mm in diameter), and the weight was observed. The biochar sample was taken and placed on the bottom of the keen box and weighed. The keen box along with biochar were partially submerged in water and kept overnight. The keen box was then taken out and allowed to stand to drain off the excess water. The water holding capacity of biochar was determined by the following formula:

$$\text{Water holding capacity (\%)} = \frac{B-(C+D)}{(C-A)} \times 100$$

Where, A is weight of empty keen's box, B is weight of saturated biochar and keen's box, C is weight of oven dried biochar and keen's box and D is weight of wet filter paper.

2.14.6 Carbon content

The titration method described by Walkley & Black (1934) was used to determine organic carbon in biochar (Walkley & Black, 1934). 1 g of biochar sample was mixed with 10 ml of 1N $K_2Cr_2O_7$ and 20 ml of concentrated H_2SO_4 , and the mixture was allowed to stand for 30 min. It was diluted with 200 ml of distilled water and then 10 ml of orthophosphoric acid and 1 ml of diphenylamine indicator were added. Blue-violet color appeared in the solution. The solution was titrated with 0.5N ferrous ammonium sulfate till the color changes to brilliant green. A blank titration was also run simultaneously. The amount of organic carbon in percentage was calculated by using the following formula:

$$\text{Organic Carbon (\%)} = \frac{10 (B-T)}{B} \times 0.003 \times \frac{100}{S}$$

Where, B is volume of ferrous ammonium sulphate required for blank titration in ml, T is volume of ferrous ammonium sulphate needed for the sample in ml, and S is weight of biochar in gram.

2.14.7 Nitrogen content

The nitrogen content of biochar sample was estimated by the Kjeldahl method (Bremner, 1960), where the sample was kept for acidic digestion followed by neutralization and finally the titration. 5 g of samples was introduced in digestion flask and to that 10 ml of concentrated H_2SO_4 and 5 g of digestion mixture of K_2SO_4 : $CuSO_4$: Na_2SO_4 (equal ratio) was added. The flask was swirled in order to mix the contents thoroughly placed on heater to start digestion till the mixture become clear (blue green in color). It took 3 h to complete the process. The digest was cooled and transferred to 100 ml volumetric flask. The volume was topped up to mark using distilled water. Then from those 10 ml of the digest was introduced in the

distillation tube where 10 ml of 0.5 N NaOH was gradually added. Distillation continued for 10 minutes and NH₃ (Ammonia gas) produced was collected as NH₄OH (Ammonium hydroxide) in a conical flask containing 20 ml of 4% boric acid solution with a drop of modified methyl red indicator. During distillation yellowish colour appeared due to NH₄OH. The distillate was then titrated against standard HCl solution of 0.25 mol/l concentration till a pink color observed. At this step the initial and final reading were noted to calculate the amount of titrant used and noted as Vs. A blank was also run as previous to the sample and for the blank nitrogen content of acetanilide or tryptophan after addition of 1 g of saccharose was determined at the titration step and the volume of titrant used was noted as Vb. The percentage of Nitrogen in samples was calculated via the given formula (Bremner, 1960):

$$\text{Nitrogen (\%)} = \frac{(Vs - Vb \times F \times C \times M(N))}{(m \times 1000) \times 100}$$

Where, Vs is volume of titrant used for sample, Vb is volume of titrant used for blank, F is molar reaction factor of titrant (HCl-1 and H₂SO₄-2), C is concentration of titrant (mol/l), M(N) is molecular weight of nitrogen, m is weight of sample, and 1000 is conversion factor (ml into l).

2.14.8 Phosphorus content

Phosphorus content was determined by ammonium molybdate method given by Olsen (1954). 2.5 g of the sample was mixed with 50ml of Olsen's reagent (0.5M NaHCO₃; pH 8.5), the mixture was shaken for 30 min and then filtered through Whatman No.1 filter paper. 5 ml of the clear filtrate was taken in to a volumetric flask. To this solution, 5 ml of ammonium molybdate solution (containing 400 ml of 10N HCl per litre) was slowly added. To liberate CO₂ from the solution, the components were gently shook. To make the volume around 22 ml, distilled water was added and poured down the sides. 1 ml of freshly diluted SnCl₂ solution was added and the contents were agitated briefly and the volume was made up to 25 ml with distilled water. Blue color intensity was measured at 660 nm against a blank. The phosphorus content was calculated by the following formula:

$$\text{Phosphorus content (kg ha}^{-1}\text{)} = R \times \frac{V}{v} \times \frac{1}{S} \times \frac{(2.44 \times 10^6)}{10^6}$$

Where, V is total volume of extractant (ml), v is volume of aliquot taken for analysis (ml), S is weight of sample (g), and R is weight of phosphorus in the aliquot in μg (from standard graph).

2.14.9 Potassium content

Potassium content of the sample was determined by the flame photometer method as described by Ghosh et al. (1983). 5 g of sample was mixed with 25 ml of 1N ammonium acetate solution, agitated for 20-30 min and filtered through Whatman filter paper. The potassium concentration in the extract was determined by using flame photometer.

$$\text{Potassium content (kg ha}^{-1}\text{)} = R \times \frac{V}{W} \times 224 \times \frac{10^6}{10^6}$$

2.14.10 Fourier-Transform Infrared (FTIR) Spectroscopy

Fourier-transform infrared spectroscopy (FTIR) of biochar samples were analyzed using a PerkinElmer Spectrum IR Version 10.6.1 over 400-4000 cm^{-1} wavenumbers for identification of various functional groups present in biochar. 0.1% biochar samples were blended and pulverized with solid KBr to produce KBr-pellet thin films for analysis. The functional groups and vibrations present in different peaks were identified based on academic literature that was readily accessible (Coates, 2000; Koçer et al., 2020; Nandiyanto et al., 2019; Yu et al., 2017). The results of the analysis were obtained as a plot of transmittance percentage (%) versus wavenumber (cm^{-1}). The spectra showed peaks that appeared in correlation with the absorption of infrared radiation. These peaks indicates a specific link with a molecular environment within the biochar (Zhao et al., 2019). Variation in the spectrum involving the appearance or disappearance of the peak implies the structure of functional group has changed inside the molecule.

2.14.11 X-ray Diffraction (XRD)

The powdered biochar was characterized by X-ray diffraction (Panalytical X'Pert Pro) with CuK α radiation at a wavelength of 1.54060 Å. Scanning was run from 10° to 80° (2 Θ) at a step size of 0.02° with the scanning rate at 2° min⁻¹ at 40 kV and 40 mA. The generated XRD pattern was compared and indexed with ICSD standard.

2.14.12 Scanning Electron Microscopy (SEM)

The topographies of biochar was examined by scanning electron microscopy (JEOL-JSM-6360) at very high magnifications of up to more than 300,000 X. SEM analysis involved gold-sputtered and scanned samples at various magnifications.

2.14.13 Transmission Electron Microscopy (TEM)

TEM (JEOL-JEM-2100) was used to visualize the internal morphology of a material in the range of micrometer to nanometer. The operating voltage for sample analysis was 200 kV. Biochar samples were sonicated in ethanol and then drop casted onto a carbon-coated copper grid.

2.14.14 Atomic Absorption Spectroscopy

Biochar analysis was carried out using atomic absorption spectroscopy (AAS) technique to determine the metal and other elements present in the biochar (Enders & Lehmann, 2012). Biochar samples were dried at 105°C and 1.0 g was converted to ash. Biochar ash was treated with concentrated HNO₃ and allowed to stand overnight. The sample treated with acid was heated under controlled conditions until the production of brown fumes ceased to occur. The residue was diluted with distilled water to reach the appropriate volume. Drops of dilute HNO₃ were added to obtain the final volume. These liquid samples were filtered through Whatman No 1 filter paper and the filtered solution was used for AAS analysis.

2.15 Application of biochar in agronomy

The application of cyanobacterium *M. aeruginosa* biochar in agriculture was studied in three ways such as soil-less Petri dish seed germination, growth and yield parameters of potted plants and the physico-chemical analysis of potted soil.

2.15.1 Soil-less Petri dish seed germination

Soil-less Petri dish tomato seed germination was studied to evaluate the effect of biochar. Biochar was added at the rates of 0, 0.5, 1.0, 2.5, 5.0 g/Petri dish. All Petri dishes were covered with lids and incubated in the dark at 25°C for 72-96 hours when germination percentage, shoot and root length were assessed. The shoot and root length of germinated seeds were measured using a ruler (Solaiman et al., 2011).

2.15.2 Determination of potted plants

The pot experiment was conducted to investigate the growth of tomato plants as a result of addition of biochar produced from *M. aeruginosa*. The pot trial was conducted for a period of 30 days during November-December 2023 in an open air condition located in Aizawl, Mizoram, India as shown in Fig 2.5. The pot trial experiments were carried out in randomized complete block design with a total number of 12 pots with 4 treatments and 3 replications (Table 2.2).



0 day



10 days



20 days



30 days

Figure 2.5 Pot trial experiments during 30 days in an open air conditions.

Table 2.2 Details of pot experiment.

Design of experiment	Randomized complete block design
Number of treatments	4
Number of replications	3
Number of total pots	12
Pot volume	2562.93 cubic cm
Soil volume	804.8 cubic cm per pot

The plant used in this study was tomato plant and the details are shown in Table 2.3. 10 seeds of tomato were planted in each pot at a depth of 2 cm. The soil was watered with 100 ml tap water every morning at 7:00 am.

Table 2.3 Details of plant used in experiment.

Plants used in experiment	Tomato
Varieties	Selection-22
Germination	70% (min)
Physical Purity	99% (min)
Genetic Purity	98% (min)
Produced, Packed and Marketed	Durga Seed Farm

The soil was mixed with 10% coco peat acted as control and resulting in 4 treatments, soil with 2 % farm yard manure, soil with 2% biochar and soil with 5% biochar. The characteristics of coco peat (Table 2.4) and farm yard manure (Table 2.5) used in this experiment and the details of treatments studied in this experiments are shown in Table 2.6.

Table 2.4 Characteristics of coco peat used in this study.

Material	100% Coco peat
EC (ms/cm)	<0.5
Moisture	15%
pH	5.5 – 6.5
Manufactured and Marketed	Vaighai Agro Products Ltd

Table 2.5 Characteristics of farm yard manure used in this study.

Organic Carbon	14% (min)
Nitrogen	0.5% (min)
Phosphorus	0.5% (min)
Potassium	0.5% (min)
Moisture	25% (max)
Conductivity (dS/m)	<4
Particle size (through IS sieve)	90% (min)
pH	6.5 – 7.5

Manufactured and Marketed	Jaipur Bio Fertilizers
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Table 2.6 Details of treatments.

Treatment Number	Treatment details
T0	Control (10% coco peat)
T1	10% coco peat + 2% FYM
T2	10% coco peat + 2% Biochar
T3	10% coco peat + 5% Biochar

2.15.2.1 Environmental conditions

The environmental conditions including temperature and humidity of the study area were recorded according to Regional met center and India Meteorological Department (IMD) Guwahati. The climatic conditions of the study area were classified according to (Köppen et al., 2011).

2.15.2.2 Growth parameters

Plant height, root length and internode length were measured by using ruler. The plant from base of the stem at soil level to terminal bud of plants were recorded as plant height, the root of plant from soil level to the tip of tap root as root length, and the plant from the base near soil level to first node (where leaf appears) as internode length.

2.15.2.3 Biomass

The fresh weight and dried weight of biomass were measured using electronic weight balance. The whole fresh biomass, above ground and below ground biomass was

recorded as fresh weight biomass. For dried weight biomass, the plant was placed in oven at 70-80°C till the weight is constant and then weighed.

2.15.2.4 Leaf parameters

Leaf number, leaf length and leaf width were determined. The leaf number was recorded by counting the number of single leaf, the vertical distance across single leaf as leaf length and the horizontal distance across single leaf as leaf width.

2.15.2.5 Germination

Germination was determined by counting the number of germinated seeds and expressed in terms of percentage.

2.15.2.6 Physiological parameters

The water content of the plants was determined by based on the weight change between fresh and dried plants, and the water use efficiency was determined by the ratio between biomass yield and water consumed.

2.15.2.7 Pigments

Extraction and estimation of chlorophyll “a” and “b”, total chlorophyll and carotenoid were determined according to the method of (Arnon, 1949) and (Villanueva et al., 1985), and calculated by using the following formula:

$$\text{Chlorophyll a (mg g}^{-1} \text{ f.wt.)} = (10.1 \times A_{662} - 1.01 \times A_{644}) \times 0.2$$

$$\text{Chlorophyll b (mg g}^{-1} \text{ f.wt.)} = (16.4 \times A_{644} - 2.57 \times A_{662}) \times 0.2$$

$$\text{Total chlorophyll} = \text{chlorophyll a} + \text{chlorophyll b}$$

$$\text{Carotenoid (mg g}^{-1} \text{ f.wt.)} = (A_{470} - 1.28 \times \text{Ch a}) + (56.7 \times \text{Ch b}) / 256 \times 0.906$$

Where, Ch a is chlorophyll a, and Ch b is chlorophyll b.

2.15.3 Physico-chemical analysis of potted soil

2.15.3.1 Soil pH

Soil pH was determined by the method given by Schofield & Taylor (1955). 20 g of soil were mixed with the right amount of distilled water and the solution was agitated firmly for 5 min to make a 1:5 soil-water suspension. The mixture was allowed to stand for 1 h and then the pH was measured. Between each sample measurement, the electrode was cleaned with a stream of distilled water and dried with tissue paper.

2.15.3.2 Soil electrical conductivity

1 g of soil was mixed with the right amount of distilled water and the solution was agitated firmly for 5 min to make a 1:10 soil-water suspension. After 30 min, the electrical conductivity (EC) was measured using a pre-calibrated EC meter (Singh et al., 2017).

2.15.3.3 Soil cation exchange capacity

Soil cation exchange capacity was determined by using ammonium acetate method as the detail was described in 2.14.3.

2.15.3.4 Soil moisture content

Soil moisture content was determined by the method given by Mishra (1968). In moisture boxes with known weights, 20 g of soil samples were collected and then oven dried at 105°C for 24 h to obtained constant mass. The soil moisture content was calculated by the following formula:

$$\text{Soil moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

Where, W_1 is initial weight, and W_2 is final weight

2.15.3.5 Soil water holding capacity

Soil water holding capacity of was determined by using the keen box method as described by Piper (1945), and the detail was described in 2.14.5.

2.15.3.6 Soil organic carbon

Soil organic carbon was determined by using titration method given by Walkley & Black (1934), and the detail was described in 2.14.6.

2.15.3.7 Soil available phosphorus

Soil available phosphorus content was determined by ammonium molybdate method given by Olsen (1954), and the detail was described in 2.14.8.

2.15.3.8 Soil exchangeable potassium

Soil Potassium content was determined by the flame photometer method as described by Ghosh et al. (1983), and the detail was described in 2.14.9.

2.15.3.9 Soil available nitrogen

Available nitrogen of the soil sample was determined by the method given by Subbiah & Asija (1956). 20 g of soil sample was mixed with 20 ml of distilled water and 100 ml of 0.32% KMnO_4 solution. A 250 ml conical flask was filled with 20 ml of 0.02N H_2SO_4 , and two to three drops of methyl red indicator were added. The flask was set beneath the receiver tube, and the tip of receiver tube was thoroughly dipped into the H_2SO_4 solution. The distillation flask containing soil was filled with 100 ml of 0.32% KMnO_4 and 100 ml of 2.5% NaOH . The flask was then instantly attached to the distillation equipment and the distillation flask was heated. Excess ammonia in H_2SO_4 is titrated using 0.02N NaOH . The available nitrogen content was calculated by using the following formula:

$$\text{Soil available nitrogen (kg ha}^{-1}\text{)} = (X - Y) \times 0.00028/20 \times 2240000$$

Where, X is volume of N/50 H₂SO₄ taken in ml, and Y is volume of N/50 NaOH used in ml.

2.15.3.10 Soil microbial population

The soil microbial population was determined by serial dilution and plate counting method. 1 g of soil sample was mixed with 9 ml of distilled water in a sterile test tube and thoroughly vortex for 5 min. Serial dilution of soil suspension was made by transferring 1 ml of initial suspension to 9 ml of sterile distilled water in a series of test tube. Agar plates with suitable media suitable for fungi (potato dextrose agar) and bacteria (tryptic soy agar) was prepared. Once agar plate was solidified, 0.1 ml of each dilution was spread on the plate using glass spreader and incubated for 3 days. The number of colonies for each dilution was recorded and the number of colony-forming units per gram of soil (CFU/g) was calculated (Lee et al., 2021).

2.15.3.11 Soil acid phosphatase activity

Soil acid phosphatase activity was determined by using the protocol described by Tabatabai & Bremner (1970). 0.1 g of air-dried soil sample was mixed with 0.25 ml toluene and allowed to stand for 10 min, and then 4 ml of modified universal buffer (MUB pH-6.5) and 1 ml of 0.115M p-Nitrophenyl Phosphate (p-NP) solution was added. The mixture was agitated and incubated at 37°C for 1 h. 1 ml of 0.5M CaCl₂ and 4 ml of 0.5M NaOH were added to the mixture and then transferred to Whatman No.1 filter paper. The yellow-colored filtrate of phosphoric acid (p-Nitrophenol phosphate) was measured at 410 nm using spectrophotometer. For blank, 1 ml p-NP was added after CaCl₂ and NaOH were added into the mixture without soil just before filtration. The enzyme activity was expressed in terms of µg p-NP ml⁻¹ hr⁻¹.

2.15.3.12 Soil dehydrogenase activity

Soil dehydrogenase activity was determined by the method given by Casida Jr et al. (1964). 1 g of fresh soil was mixed with 0.1g of CaCO₃. 1 ml of 1% 2,3,5 Triphenyl

Tetrazolium Chloride (TTC) solution was added and agitated for 5 min and incubated at 30°C for 24 h. The resultant suspension was filtered using Whatman No. 1 filter paper. In a 50 ml volumetric flask, consecutive aliquots of concentrated methanol were used to extract triphenyl formazan (TPF). Pink colour was developed and measured at 485 nm by using spectrophotometer and methanol as a blank. The enzyme activity was expressed in terms of $\mu\text{gTPF ml}^{-1} \text{ h}^{-1}$.

2.16 Statistical Analysis

The statistical analysis was carried out using Microsoft excel 2010, Washington, USA. Experimental data was analyzed by one-way analysis of variance (ANOVA). A P value of <0.05 was considered statistically significant. The results were expressed as mean $\pm$ standard deviation of the mean.

CHAPTER 3

RESULTS

3.1 Test organism

The test organism used in this study was *Microcystis aeruginosa* and the photograph of the test organism is shown in Fig 3.1.

The morphological structure of test organism as characterized by Fritsch classification is stated as;

Microcystis aeruginosa

Order: Chroococcales

Class: Cyanophyceae

Characteristics: *Microcystis aeruginosa* is characterized by small cells with lacks of individual sheaths. Colonies are circular or slightly longer than broad and solid when young, as maturity, becomes clathrate with characteristic hyaline colonial mucilage. Cells spherical in shape, usually with gas vacuoles (Desikachary, T. V., 1959).

3.2 Carbon sequestration experiments by *M. aeruginosa* in laboratory conditions

The effect of bicarbonate concentrations on specific growth rate (μ) of *M. aeruginosa* was shown in Fig. 3.2A. Specific growth rate was significantly different within the concentrations of NaHCO_3 . Growth rate with 1 g L^{-1} bicarbonate retains the highest specific growth rate ($0.665 \pm 0.15 \text{ d}^{-1}$) at the end of 20 days culture period, indicating this concentration to be optimal for cell proliferation. In contrast, further increase in bicarbonate concentration to 5 g L^{-1} resulted in a marked decline in growth rate ($0.325 \pm 0.18 \text{ d}^{-1}$), suggesting possible inhibitory effects at higher bicarbonate levels.

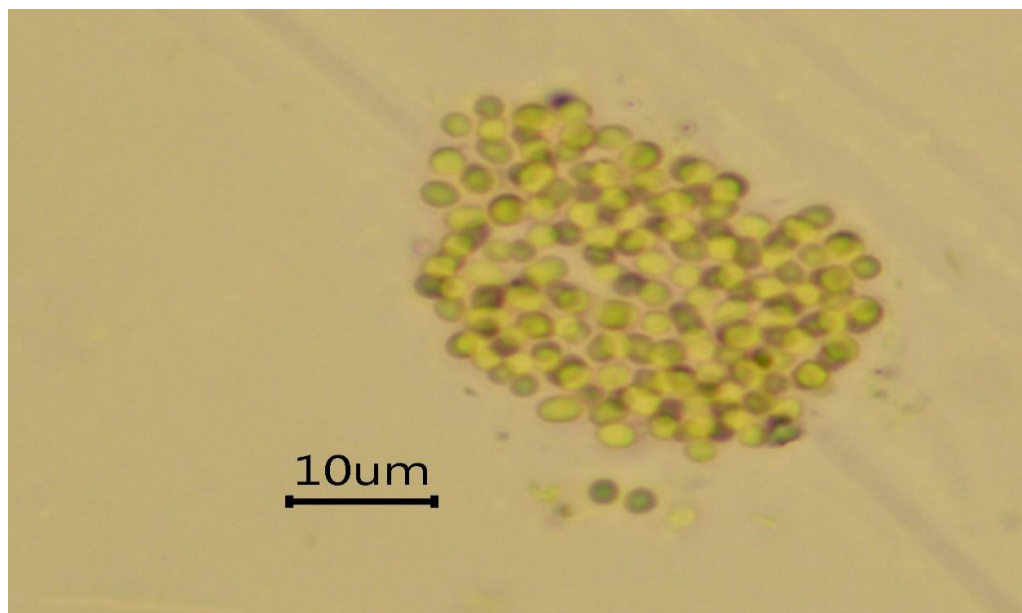
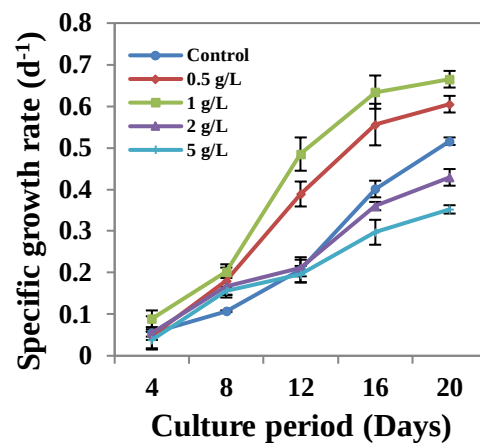
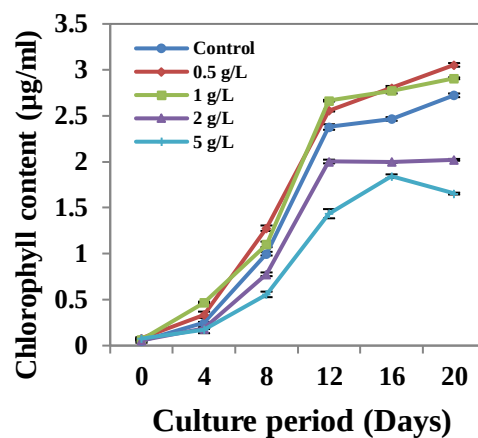


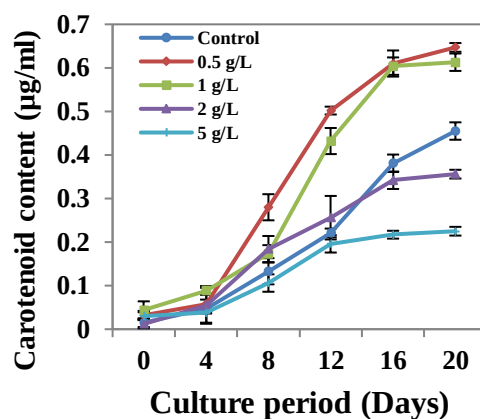
Figure 3.1 Photographs of test organism *M. aeruginosa*.



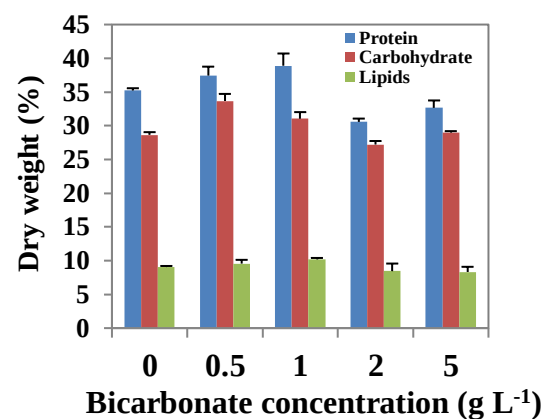
(A)



(B)



(C)



(D)

Figure 3.2 Effect of bicarbonate concentration on physiological and biochemical parameters of *M. aeruginosa*. (A) Specific growth rate. (B) Chlorophyll content. (C) Carotenoid content. (D) Protein, carbohydrate and lipid contents. Vertical bars represent the standard error of the mean ($n = 3$).

The addition of 0.5 g L⁻¹ bicarbonate had a significant positive impact on the levels of chlorophyll and carotenoid contents, approximately doubled when compared to treatments that received 5 g L⁻¹ (Fig. 3.2B & C).

The highest protein content (38.90 ± 1.81 %) was found at 1 g L⁻¹ bicarbonate treatment while the lowest (30.57 ± 0.49 %) at 2 g L⁻¹. At the same time, lipid content (10.19 ± 0.21 %) was found highest at 1 g L⁻¹ and carbohydrate content (33.67 ± 1.05 %) at 0.5 g L⁻¹ bicarbonate (Fig. 3.2D).

The biomass productivity of *M. aeruginosa* was significantly affected by the addition of bicarbonate, ranging from 0.250 ± 0.10 to 0.525 ± 0.06 g L⁻¹ d⁻¹. Biomass productivity at 1 g L⁻¹ d⁻¹ (0.525 ± 0.06 g L⁻¹ d⁻¹) was significantly higher ($p < 0.05$) than 5 g L⁻¹ d⁻¹ as depicted in Fig. 3.3A. Reduction of biomass productivity was observed with higher concentrations of sodium bicarbonate above 1 g L⁻¹ during the culture period.

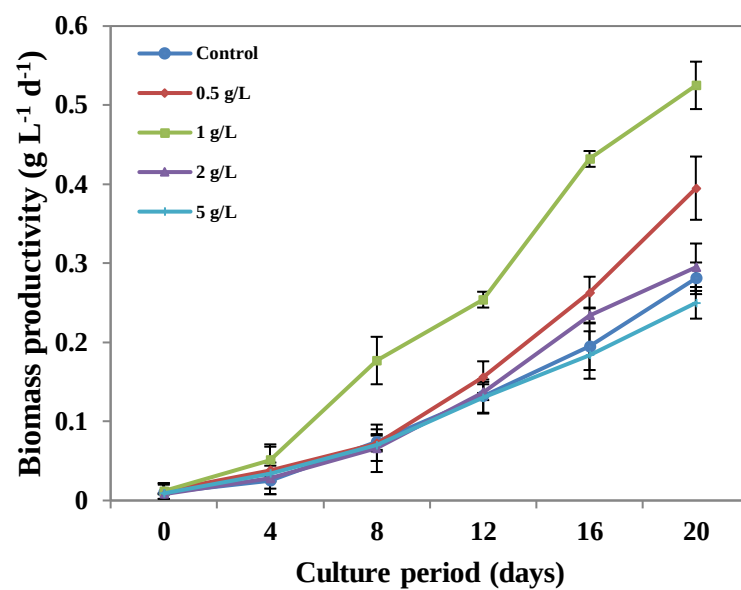
Bicarbonate concentration significantly influenced the CO₂ fixation rate of *Microcystis aeruginosa* during the 20 day culture period. The maximum CO₂ fixation rate (0.172 ± 0.02 g L⁻¹ d⁻¹) was obtained at 1 g L⁻¹ bicarbonate, whereas a sharp decline was observed at 5 g L⁻¹ (0.053 ± 0.06 g L⁻¹ d⁻¹). This suggests that moderate bicarbonate enrichment enhances inorganic carbon utilization, while higher concentrations exert an inhibitory effect on CO₂ fixation (Fig. 3.3B).

3.3 Biomass productivity of *M. aeruginosa* in semi continuous culture

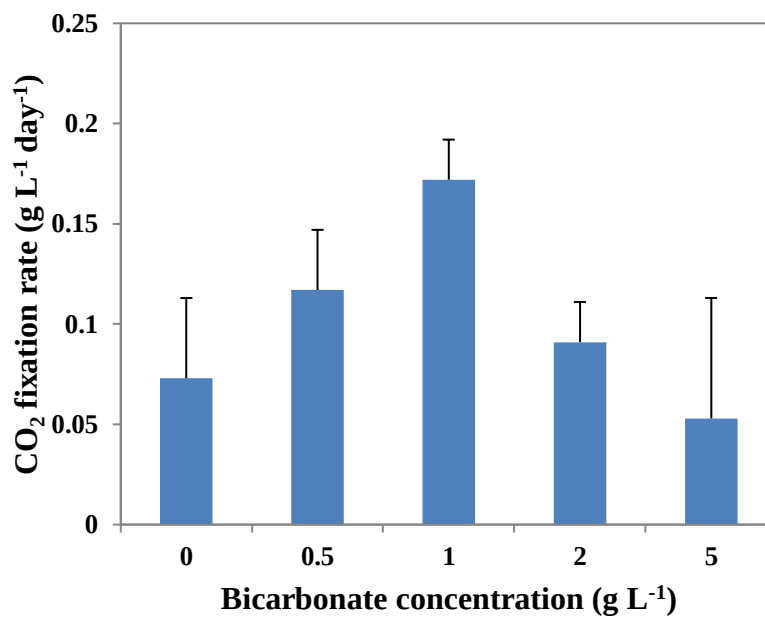
The biomass productivity at the end of first time interval (20 days) in semi-continuous culture was 0.954 ± 0.13 g L⁻¹ d⁻¹ (Fig. 3.4).

3.4 Production of biochar and bio oil produced from *M. aeruginosa*

Biochar production from biomass through pyrolysis is an effective way to convert organic material into a valuable soil amendment. The yield of biochar depends on various factors including the type of biomass and pyrolysis conditions (Lehmann & Joseph, 2015). The biochar yield from biomass of *M. aeruginosa* was found as 56% and the bio oil yield were 16.7%.



(A)



(B)

Figure 3.3 Effect of bicarbonate concentration on *M. aeruginosa*. (A) Biomass productivity. (B) CO₂ fixation rate. Vertical bars represent the standard error of the mean (n = 3).

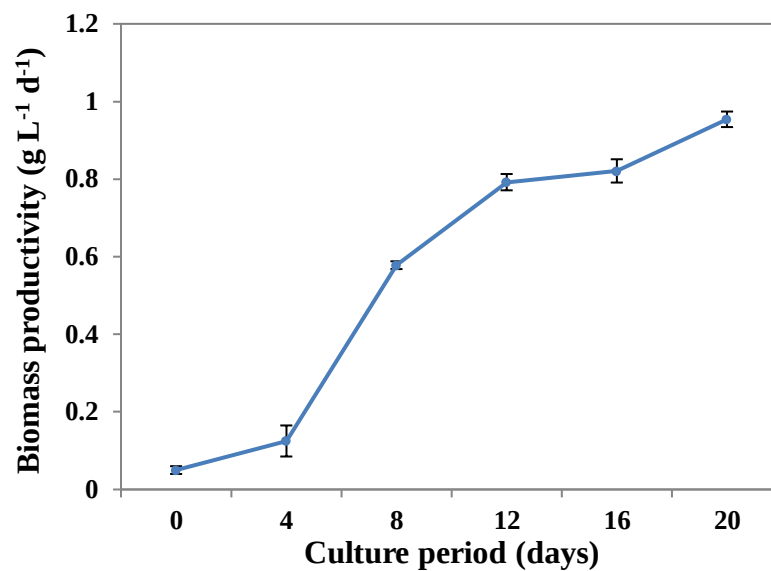


Figure 3.4 Biomass productivity of *M. aeruginosa* in semi-continuous culture, showing growth performance prior to biomass harvest. Vertical bars represent the standard error of the mean (n = 3).

3.5 Characteristics of bio oil

The component of organic compounds in the bio oil generated under traditional pyrolysis condition was identified using GC-MS analysis. From Fig 3.5, bio oil derived from *M. aeruginosa* was a complicated substance and more than 60 compounds were detected in the bio oil. When the data from the NIST library was compared with the mass spectrum, the results are presented in Table 3.1. *M. aeruginosa* pyrolysis produced bio-oil with a complex composition of organic molecules ranging from 2 to 30 carbons. It was evident that the primary constituents of the bio oil were alcohols, phenols, acids, furans, alkanes, ketones, and heterocyclic that included nitrogen (pyridines and indoles). The phenolic fragment derivatives may be originated from the heat degradation of proteins, which are the polyphenolic components found in aquatic biomass (Ross et al., 2008). Various kinds of alkanes and acids were identified, and most of these compounds evolved from the breakdown of carbohydrates. Nitrogen-containing heterocyclic substances in bio oils, including indoles and pyridines, were considered to be produced by protein breakdown. According to this study, bio-oil derived from *M. aeruginosa* cannot be utilized as biofuel, but it may be a source of compounds with additional value.

Table 3.1 Chemical compositions of bio oil from GC-MS.

S. No	Retenti on time	CAS	Compound name	Molecular formula	Molecular weight	Peak area (%)
1	6.667	503-74-2	Butanoic acid,3-methyl-	C ₅ H ₁₀ O ₂	102	0.96
2	6.820	116-53-0	Butanoic acid,2-methyl-	C ₅ H ₁₀ O ₂	102	0.51
3	6.98	79-05-	Propanamide	C ₃ H ₇ NO	73	0.28

		0				
4	7.361	96-48-0	Butyrolactone	C ₄ H ₆ O ₂	86	0.83
5	8.928	3658-80-8	Dimethyl trisulfide	C ₂ H ₆ S ₃	126	0.82
6	9.886	108-95-2	Phenol	C ₆ H ₆ O	94	0.61
7	10.564	626-90-4	Butanal, 3-methyl-,oxime	C ₅ H ₁₁ NO	101	3.57
8	11.208	100-51-6	Benzyl alcohol	C ₇ H ₈ O	108	0.26
9	12.401	108-52-1	2-Amino-4-methylpyrimidine	C ₅ H ₇ N ₃	109	0.31
10	12.808	616-45-5	2-Pyrrolidinone	C ₄ H ₇ NO	85	1.16
11	12.925	497-06-3	3-Butene-1,2-diol	C ₄ H ₈ O ₂	88	0.56
12	13.605	628-02-4	Hexanamide	C ₆ H ₁₃ NO	115	1.36
13	13.909	1119-29-5	Pentanamide, 4-methyl-	C ₆ H ₁₃ NO	115	3.65
14	14.386	106-31-0	Butanoic acid, anhydride	C ₈ H ₁₄ O ₃	158	3.74
15	14.901	5185-	2-Pentanone, 5-	C ₇ H ₁₂ O ₃	144	0.82

		97-7	(acetyloxy)-			
16	15.112	4030-18-6	Pyrrolidine, 1-acetyl-	C ₆ H ₁₁ NO	113	0.31
17	15.863	0-00-0	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	C ₆ H ₈ O ₄	144	3.52
18	16.043	675-20-7	2-Piperidinone	C ₅ H ₉ NO	99	3.92
19	17.887	1452-77-3	Picolinamide	C ₆ H ₆ N ₂ O	122	1.12
20	18.252	18158-32-5	Hexahydroindole	C ₈ H ₁₃ N	123	0.33
21	20.771	13482-23-0	Cyclohexanone, 4-methoxy-	C ₇ H ₁₂ O ₂	128	0.67
22	24.793	1852-17-1	N,N'-Trimethyleneurea	C ₄ H ₈ N ₂ O	100	1.26
23	25.228	0-00-0	5,6-Dihydrosolasodin-9.alpha.-ol	C ₂₇ H ₄₅ NO ₃	431	0.61
24	26.203	18418-22-9	Palmitic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester	C ₂₆ H ₄₂ O ₄	418	0.47
25	28.260	16935-34-5	5-Isopropyl-2,4-imidazolidinedione	C ₆ H ₁₀ N ₂ O ₂	142	3.10
26	28.848	52722-	1-(2-Hydroxyethyl)-2,2,6,6-tetramethyl-4-	C ₁₁ H ₂₃ NO ₂	201	1.17

		86-8	piperidinol				
27	29.309	61568-02-3	5-sec-Butyluracil		C ₈ H ₁₂ N ₂ O ₂	168	0.36
28	29.770	22160-42-5	2,5-Piperazinedione, 3-methyl-6-(1-methylethyl)-	3-	C ₈ H ₁₄ N ₂ O ₂	170	0.56
29	30.615	646-31-1	Tetracosane		C ₂₄ H ₅₀	338	0.27
30	30.705	0-00-0	Hexestrol, isopropoxyloxy-carbonyl-	O-	C ₂₂ H ₂₈ O ₄	356	0.43
31	31.066	65556-33-4	3-Methyl-2,3,6,7,8,8a-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione		C ₈ H ₁₂ N ₂ O ₂	168	3.11
32	31.356	5625-44-5	3,6-Diisopropylpiperazine-2,5-dione		C ₁₀ H ₁₈ N ₂ O ₂	198	0.47
33	31.973	19179-12-5	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-		C ₇ H ₁₀ N ₂ O ₂	154	2.26
34	32.366	0-00-0	2-Methyl-9-β-D-ribofuranosylhypoxanthine		C ₁₁ H ₁₄ N ₄ O ₅	282	0.38
35	32.594	2854-40-2	Cyclo(L-prolyl-L-valine)		C ₁₀ H ₁₆ N ₂ O ₂	196	0.35
36	32.876	5625-44-5	3,6-Diisopropylpiperazine-2,5-dione		C ₁₀ H ₁₈ N ₂ O ₂	198	1.24
37	33.891	112-39-0	Hexadecanoic acid, methyl ester		C ₁₇ H ₃₄ O ₂	270	0.65

38	34.164	0-00-0	Octahydrodipyrrolo[1,2-a:1',2'-d]pyrazine-5,10-dione-, (5aR,10aR) (isomer 2)	C ₁₀ H ₁₄ N ₂ O ₂	194	4.01
39	34.342	58917-56-9	Hexahydro-3-(1-methylpropyl)pyrrolo[1,2-a]pyrazine-1,4-dione	C ₁₁ H ₁₈ N ₂ O ₂	210	2.67
40	35.766	556-68-3	Cyclooctasiloxane, hexadecamethyl-	C ₁₆ H ₄₈ O ₈ Si ₈	592	0.16
41	36.729	112-61-8	Methyl stearate	C ₁₉ H ₃₈ O ₂	298	0.28
42	37.812	629-54-9	Hexadecanamide	C ₁₆ H ₃₃ NO	255	1.06
43	40.720	556-71-8	Cyclononasiloxane, octadecamethyl-	C ₁₈ H ₅₄ O ₉ Si ₉	666	0.43
44	42.489	559-70-6	.beta.-Amyrin	C ₃₀ H ₅₀ O	426	3.87
45	43.401	18919-94-3	Tetracosamethyl-cyclododecasiloxane	C ₂₄ H ₇₂ O ₁₂ Si ₁₂	888	0.84
46	45.181	112-84-5	13-Docosenamide, (Z)-	C ₂₂ H ₄₃ NO	337	1.32
47	45.489	111-02-4	Squalene	C ₃₀ H ₅₀	410	0.36

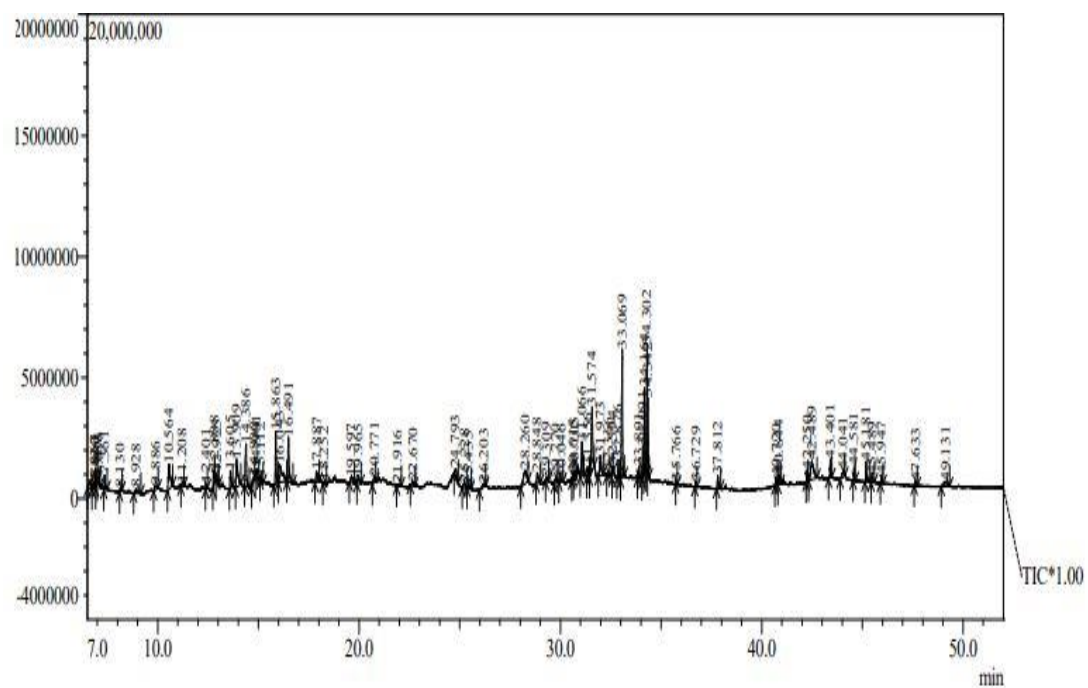


Figure 3.5 GC–MS chromatogram of bio-oil derived from traditional pyrolysis of *M. aeruginosa* biomass.

3.6 Characteristics of biochar

3.6.1 Proximate and ultimate analysis

The proximate and ultimate analysis of *M. aeruginosa* biochar is represented in Table 3.2. The yield of biochar derived from *M. aeruginosa* biomass (on dry weight basis) was observed as 56%. The pH, electrical conductivity and cation exchange capacity was found as 8.5, 0.63 dS m⁻¹ and 10.5 meq 100 g⁻¹ respectively. The moisture content (6.45%), ash (31.06%), volatile matter (18.23%) and water holding capacity (96.75%) were also observed. The carbon content and nitrogen content were 43.559% and 0.72% respectively while phosphorus content and potassium content were 0.748 mg kg⁻¹ and 15.59 mg kg⁻¹ respectively.

Table 3.2 Proximate and ultimate analysis of *M. aeruginosa* biochar.

S. No	Characteristics	Values
1	Yield (%)	56
2	pH	8.5
3	Electrical conductivity (dS m ⁻¹)	0.63
4	Cation exchange capacity (meq 100 g ⁻¹)	10.5
5	Moisture content (%)	6.45
6	Ash content (%)	31.06
7	Volatile matter (%)	18.23
8	Water holding capacity (%)	96.75
9	Carbon (%)	43.559
10	Nitrogen (%)	0.72

11	Phosphorus (mg kg ⁻¹)	0.748
12	Potassium (mg kg ⁻¹)	15.59

3.6.2 FTIR Spectroscopy

The FTIR spectra was studied to identify the functional groups that are present in biochar that had a role in stabilizing biochar synthesized from cyanobacterium *M. aeruginosa*. The FTIR spectrum was recorded in the range of 4000-400 cm⁻¹ wavelength and represented in Figure 3.6. The biochar produced from *M. aeruginosa* exhibits the variance in many functional groups and its vibration in biochar by showing its transmittance peak at various wavenumbers (3854.10 cm⁻¹, 3674.42 cm⁻¹, 3743.93 cm⁻¹, 2920.54 cm⁻¹, 2361.04 cm⁻¹, 1559.07 cm⁻¹, 1398.46 cm⁻¹, 1047.48 cm⁻¹, 562.70 cm⁻¹ and 442.65 cm⁻¹). The respective functional groups and vibrations for corresponding wavenumbers of biochar are mentioned in Table 3.3. The region of 3584–3700 cm⁻¹ exhibits distinct peaks that correlate to the stretching vibration of the hydroxyl group (O–H) in the activated biochar. The decrease in intensity of the -OH stretching vibration occurs due to a decrease in -OH content caused by hydration reaction, which disintegrates the OH bond (Qin et al., 2020). Additionally, this band confirms the existence of hydroxyl groups as well as their strong contact through hydrogen bonding. Distinct bands at around 2920 cm⁻¹ are assigned and C–H stretching vibration as well as alkyl chain (Murdock & Wetzel, 2009). The peaks at around 1559 cm⁻¹ corresponds to N-H bending of secondary amine. Furthermore, an additional little disturbance around 1398 cm⁻¹ signifies the stretching of O–H in the plane of free hydroxyl groups (Xu et al., 2020). The formation of strong peaks ranging from 1030-1060 cm⁻¹ confirms the presence of substantial stretching vibration of the C–C bond and C–OH (Bird et al., 2011). Peak around 500-600 cm⁻¹ represents C-I stretch of aliphatic iodo compounds and peak around 500-430 cm⁻¹ indicates S-S stretch of aryl disulfides.

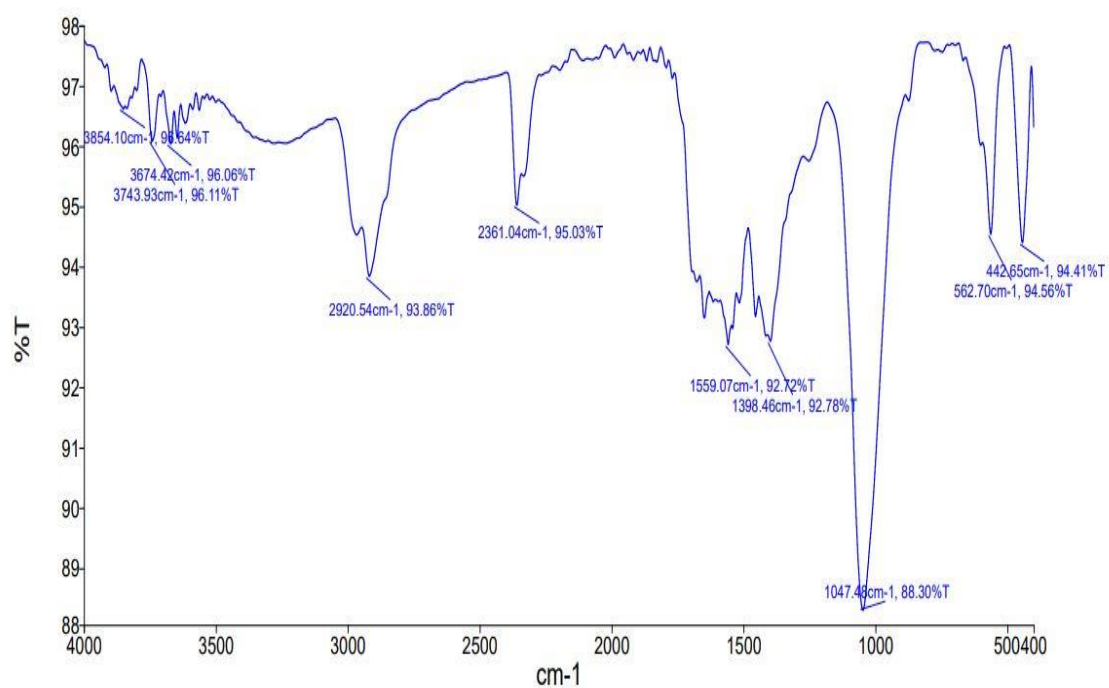


Figure 3.6 FTIR spectrum of biochar derived from *M. aeruginosa*.

Table 3.3 FTIR peaks and functional group assignments of biochar derived from *M. aeruginosa*.

Biochar	Wavenumber (cm ⁻¹)	Functional group	Vibration
<i>M. aeruginosa</i>	3674.42	Alcohol	O-H stretching
	2920.54	Alkane	C-H stretching
	2361.04	Carbon dioxide	O=C=O stretching
	1559.07	Secondary amine	>N-H bend
	1398.46	Carboxylic acid	O-H bending
	1047.48	Carboxylic and aromatic ester	C-O stretching
	562.70	Halo compound	C-I stretching
	442.65	Aryl disulfide	S-S Stretching

3.6.3 XRD characterization

The X-ray diffractograms of biochar is shown in Figure 3.7. A strong diffraction peak was observed at $2\theta = 29.16^\circ$ in the X-ray diffraction (XRD) analysis of the biochar derived from *M. aeruginosa*. The intensity (counts) versus 2θ graph suggests the presence of crystalline mineral phases, with the most prominent peak centered at 29.16° .

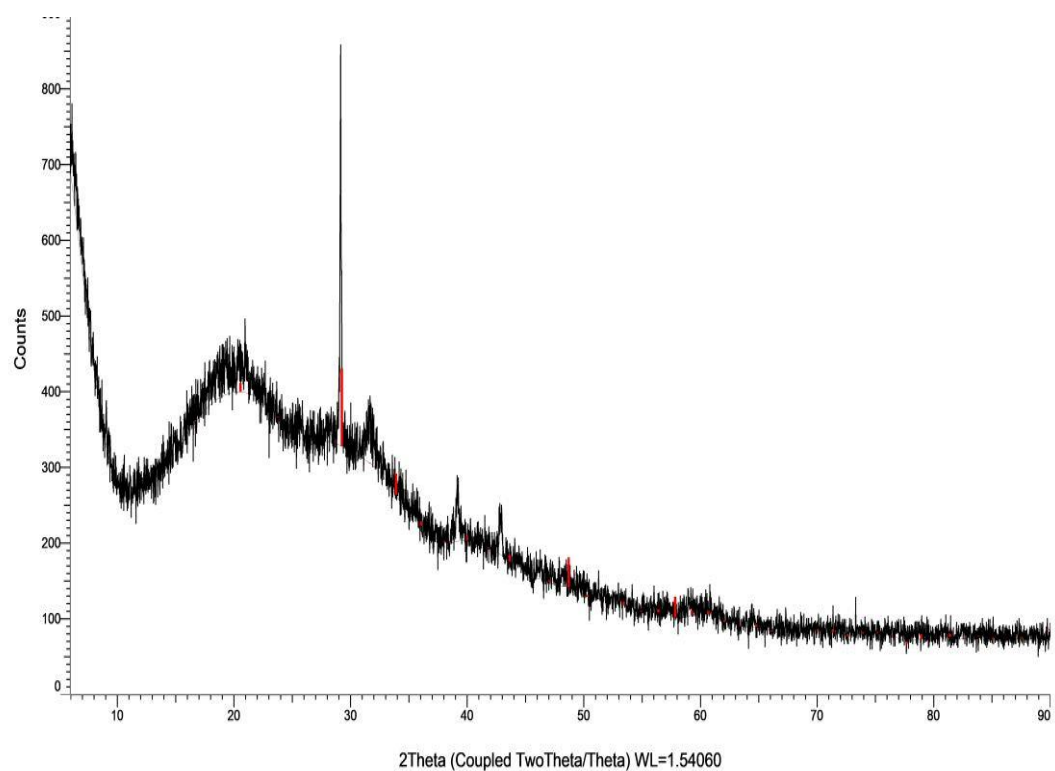


Figure 3.7 X-ray diffraction (XRD) pattern of biochar derived from *M. aeruginosa*.

3.6.4 SEM study

Scanning electron microscopy (SEM) has been used to study the morphology of biochar. The obtained SEM image of biochar is shown in Fig 3.8. Formation of ridges and surface undulations were visible from SEM image. The irregular, rough surface with cracks and channels formed as the biomass decomposes and restructures into carbon rich material.

3.6.5 TEM study

The TEM images of biochar derived from *M. aeruginosa* revealed the presence of mesopores on the surface. Additionally, well-defined lattice fringes were observed, indicating some degree of crystalline structure within the biochar matrix (Fig 3.9).

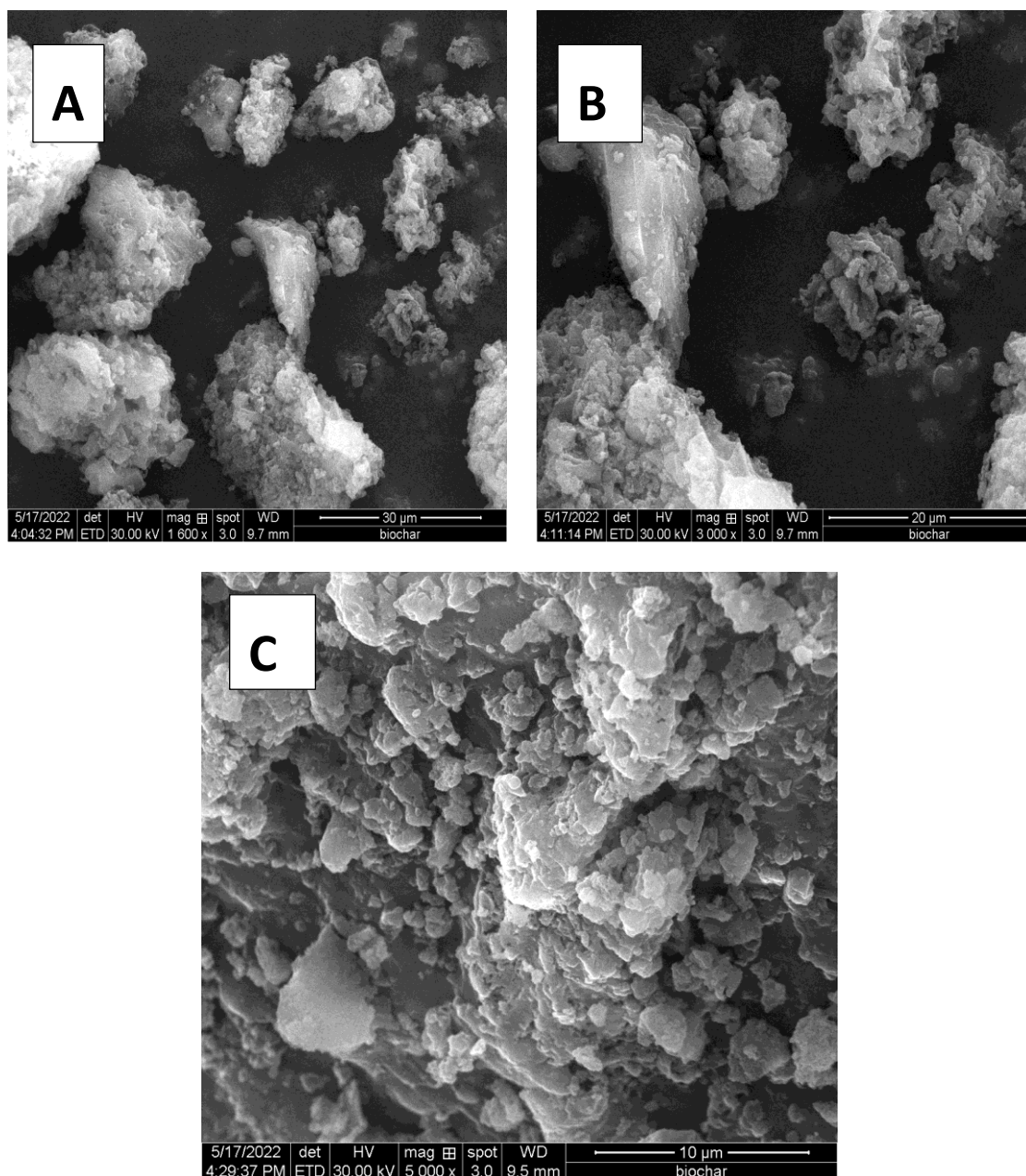


Figure 3.8 SEM image of biochar of *M. aeruginosa*. (A) 30 µm (B) 20 µm (C) 10 µm

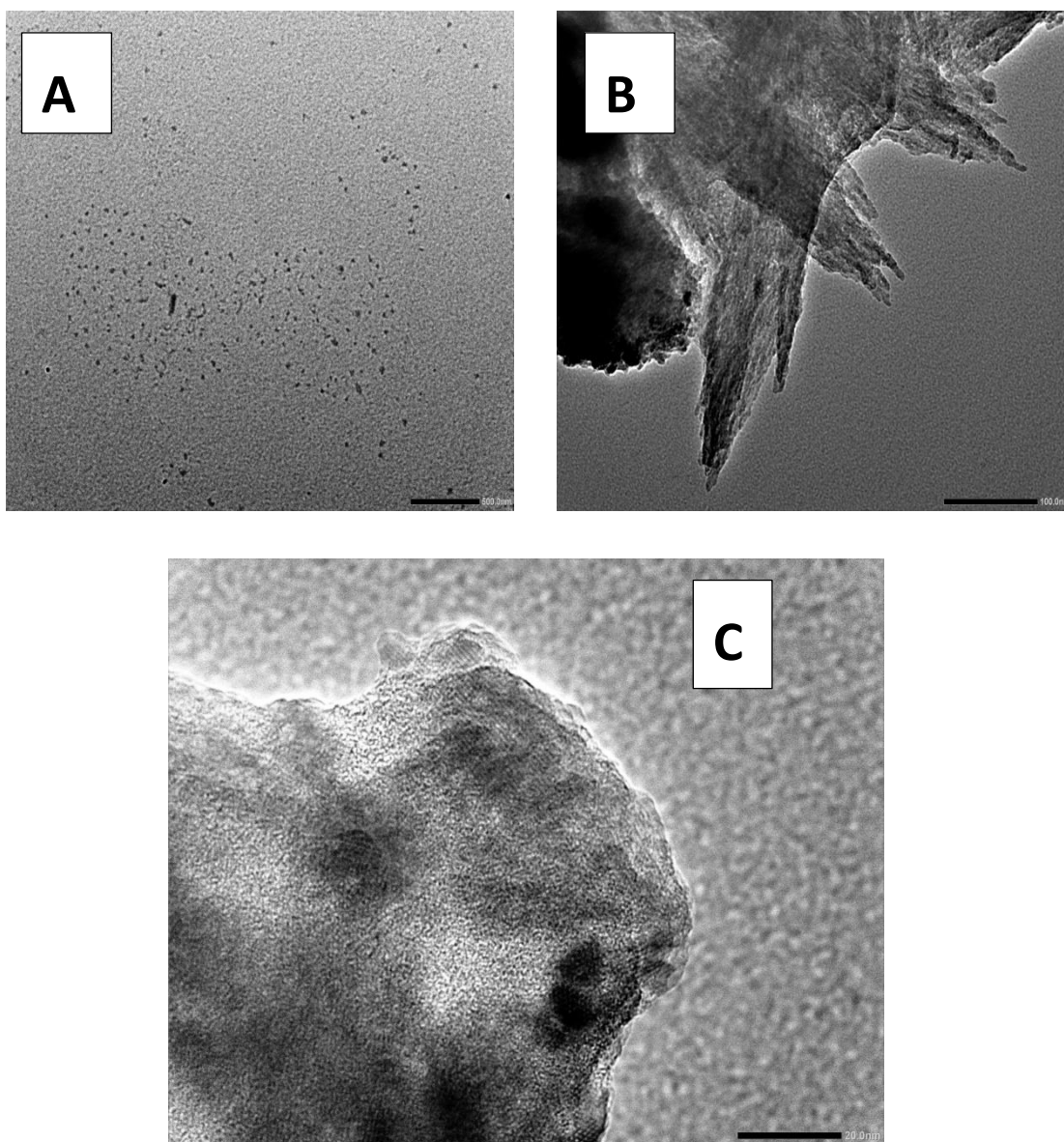


Figure 3.9 TEM image of biochar of *M. aeruginosa*. (A) 500 nm (B) 100 nm (C) 20 nm

3.6.6 Atomic absorption spectroscopy (AAS)

The trace elements present in the biochar was analyzed by using AAS. Results given in Table 3.4 showed high concentrations of Ca, Fe, Mg and Mn. Other elements such as Co, Cu, Mo, Se and Zn were present in the concentration ranging between 1-82.6 mg kg⁻¹ while Mo was present in traces (<1 mg kg⁻¹) in biochar.

Table 3.4 Elemental analysis of biochar through AAS.

S. No	Element	Content (mg kg ⁻¹)	% content
1	Iron	8789.55	44.3638
2	Magnesium	7652.15	38.6230
3	Calcium	2450.33	12.3676
4	Manganese	780.664	38.6230
5	Zinc	82.694	0.4173
6	Copper	42.169	0.2128
7	Cobalt	12.773	0.0644
8	Selenium	1.121	0.0056
9	Molybdenum	0.956	0.0048

3.7 Effect of biochar on applied agriculture

3.7.1 Effect of biochar on soil-less Petri dish seed germination

The effect of different biochar concentrations on seed germination, shoot and root length of tomato plants are shown in Fig. 3.10. The highest germination (%) was found in 1 g/petri dish biochar concentration with 90 ± 1.00 % and the lowest in 5 g/petri dish with 60 ± 1.00 %. The root length (4.65 ± 0.33 cm) and shoot length (4.77 ± 0.53 cm) of tomato plant at 0.5 g/petri dish treatment were significantly higher as compared to control (2.15 ± 1.06 cm) and 5 g/petri dish (2.70 ± 0.52 cm) respectively. The photograph of tomato plants is shown in Fig 3.11.

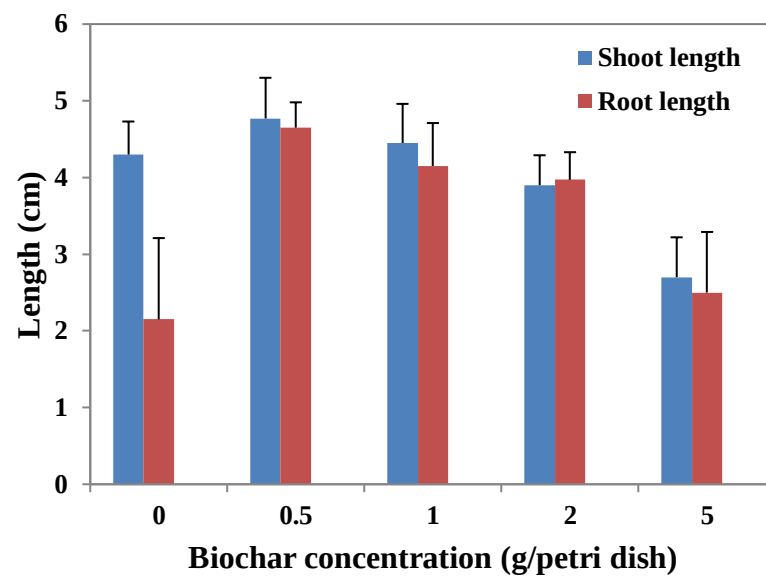
3.7.2 Effect of biochar on tomato plants: Pot experiment

The effect of biochar on tomato plants was studied after the end of experiments. The tomato plants were harvested and carefully washed with water to remove soil and dust particles. The significance difference between tomato plants from each treatment is shown in Fig 3.12.

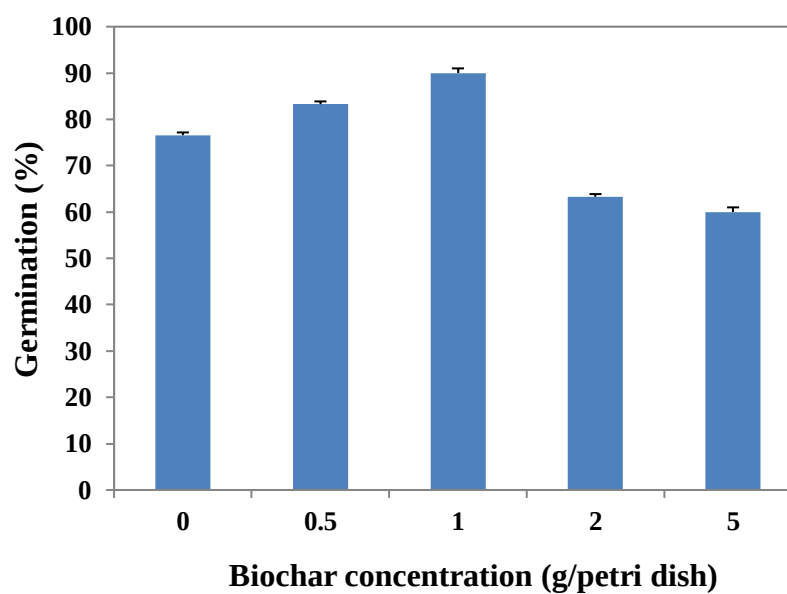
3.7.2.1 Environmental conditions

Aizawl has a mild, sub-tropical climate due to its location and elevation. Under the Koppen climate classification, Aizawl features a humid sub-tropical climate. In summer, temperatures are moderately warm, averaging around 20-30°C. In winter, daytime temperatures are cooler in comparison to the rest of the year, averaging around 11-21°C. The warmest month is April (33.98°C), coldest is January (5.19°C), wettest is August (705.11 mm) and driest is January (17.95 mm). The study period of experiment is in the month of November and December in the year 2023. The sea level of experimental site is 1027 m and the location of experimental site is 23°43'27.7284"N latitude and 92°43'7.1436"E longitude.

The variations of temperature during the experiment was $21.2 \pm 1.86^\circ\text{C}$ maximum and minimum of $15.9 \pm 2.16^\circ\text{C}$ (Fig 3.13A) and humidity was 74.35 ± 10.31 % maximum and 58.87 ± 8.65 % minimum (Fig 3.13B).



(A)



(B)

Figure 3.10 Effect of biochar on tomato plant growth parameters in soil-less Petri dish experiment. (A) Effect of biochar on shoot length and root length of tomato seedlings. (B) Effect of biochar on germination percentage of tomato seeds.



Figure 3.11 Photograph showing the effect of biochar at different application rates on tomato seedling germination and early growth under soil-less Petri dish conditions. (A) 0 g/petri dish, (B) 0.5 g/petri dish, (C) 1.0 g/petri dish, (D) 2.5 g/petri dish, (E) 5.0 g/petri dish.

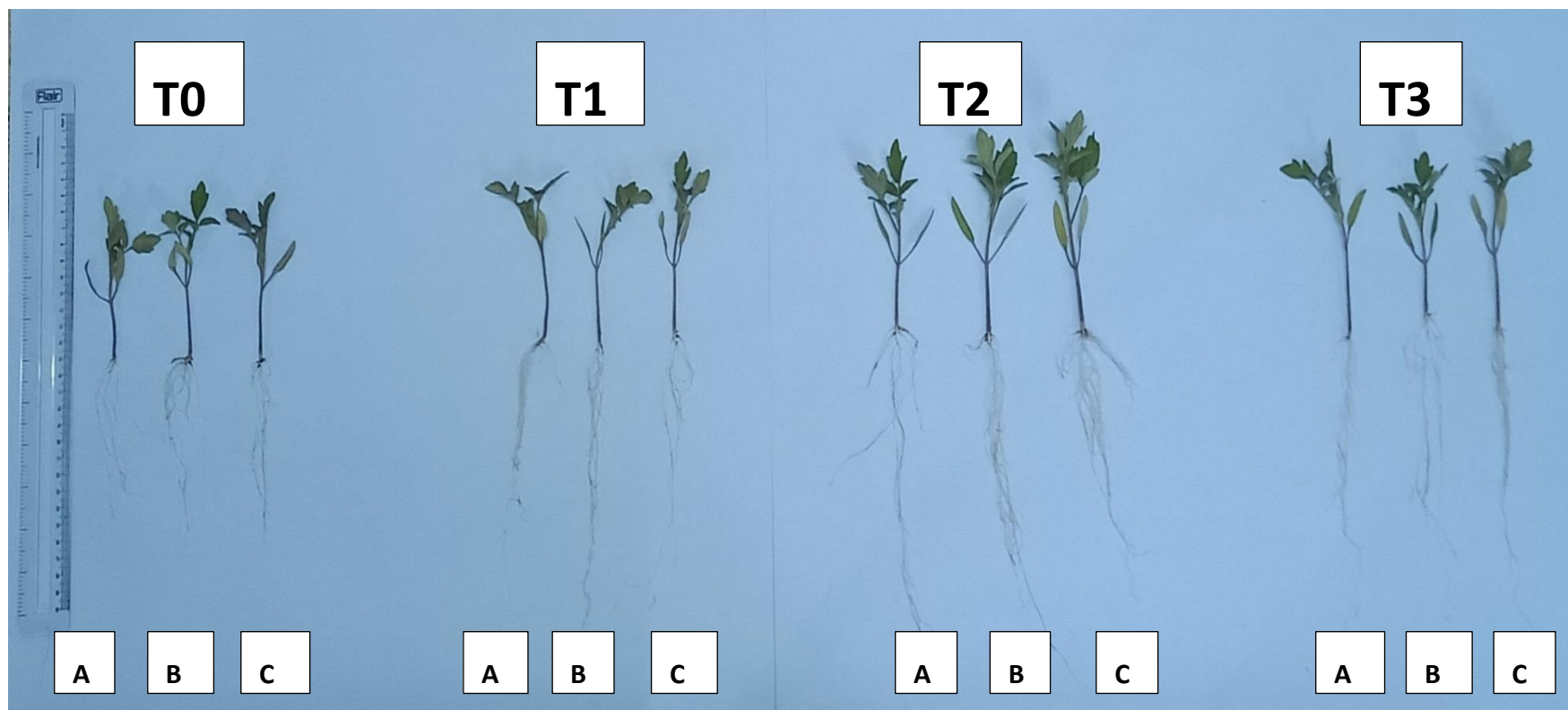
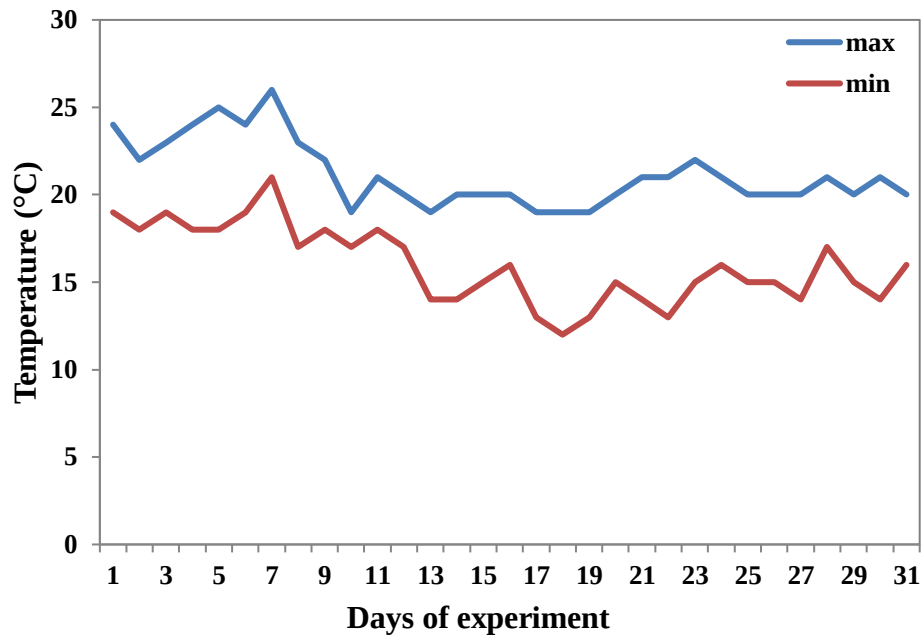
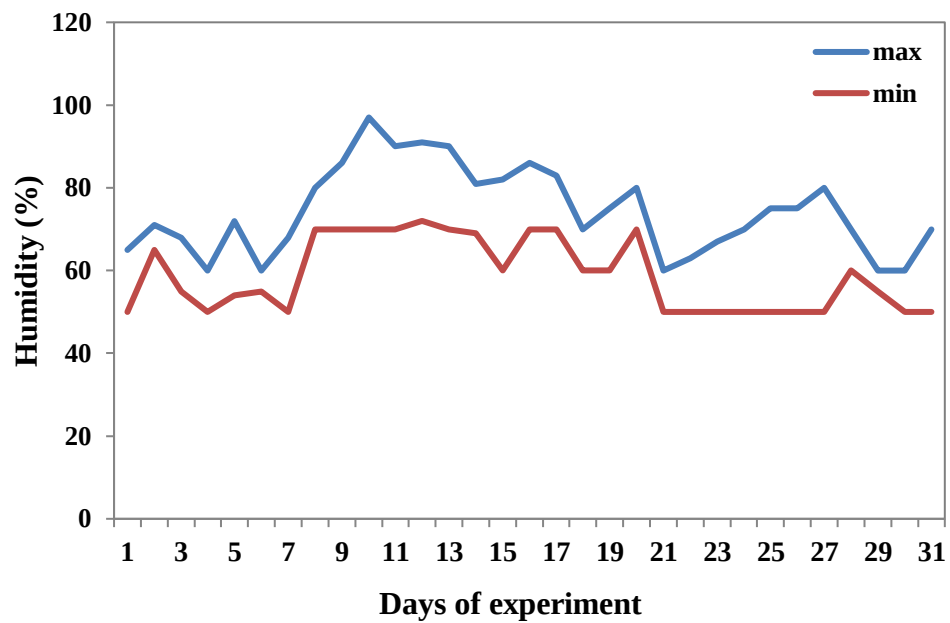


Figure 3.12 The healthiest and longest tomato plants from each treatment in the pot experiment. (T0) Control (10% coco peat) treatment of soil, (T1) 2% FYM treatment of soil, (T2) 2% Biochar treatment of soil, (T3) 5% Biochar treatment of soil. (A) (B) (C) each replicate.



(A)



(B)

Figure 3.13 Environmental conditions during the pot experiment. (A) Variation in temperature during pot experiment. (B) Variation in humidity during pot experiment.

3.7.2.2 Effect of biochar on growth of tomato plants

The highest shoot length was observed in T2 treatment with 13.60 ± 0.88 cm and lowest by T0 treatment with 10.40 ± 0.50 cm. The root length of tomato plant was found highest in T2 treatment with 23.53 ± 2.77 cm and the lowest in T0 treatment with 11.03 ± 0.77 cm. Plants from T1 treatment shows 4.73 ± 0.20 cm internode length while T0 treatment shows 3.76 ± 0.28 cm (Fig 3.14A).

3.7.2.3 Effect of biochar on biomass of tomato plants

T2 treatment shows significant positive effect on fresh weight biomass of tomato plant with 1.32 ± 0.08 g as compared to T0 treatment with 0.47 ± 0.03 g (Fig 3.14B). The dried weight biomass of T2 treatment (0.11 ± 0.01 g) was found doubled as compared to T0 treatment with 0.05 ± 0.01 g (Fig 3.14C).

3.7.2.4 Effect of biochar on leaf parameters of tomato plants

T2 treatment shows 3.46 ± 0.12 cm of leaf length on tomato plant while T0 shows 2.43 ± 0.12 cm. T2 shows 2.13 ± 0.12 cm leaf width while T0 shows 1.50 ± 0.08 cm (Fig 3.15A). The leaf numbers on tomato plant were 18.66 ± 1.24 on T2 treatment while 11.66 ± 0.47 on T0 (Fig 3.15B).

3.7.2.5 Effect of biochar on germination of tomato plants

The addition of biochar on soil has significantly effect on germination of tomato plants. Tomato plants on T2 treatment shows 93.33 ± 4.71 %, which is significantly higher as compared to soil without biochar T0 treatment (60.00 ± 8.16 %). The significant difference is shown in Fig 3.15C.

3.7.2.6 Effect of biochar on water content and water use efficiency of tomato

The highest water content of tomato plant was found on T3 treatment were 92.92 ± 1.13 %, while the lowest water content was found on T1 treatment with 85.23 ± 1.02 % (Fig 3.16A). The water use efficiency was found highest in T2 (0.43 ± 0.01 g L⁻¹) while lowest in T0 with 0.15 ± 0.01 g L⁻¹ (Fig 3.16B).

3.7.2.7 Effect of biochar on pigment content of tomato plants

The chlorophyll “a”, chlorophyll “b”, total chlorophyll and carotenoid contents was found highest in tomato plants of T2 treatment with $2.099 \pm 0.05 \text{ mg g}^{-1} \text{ f. wt.}$, $1.796 \pm 0.19 \text{ mg g}^{-1} \text{ f. wt.}$, and $3.896 \pm 0.22 \text{ mg g}^{-1} \text{ f. wt.}$, $0.482 \pm 0.02 \text{ mg g}^{-1} \text{ f. wt.}$ respectively (Fig 3.16C).

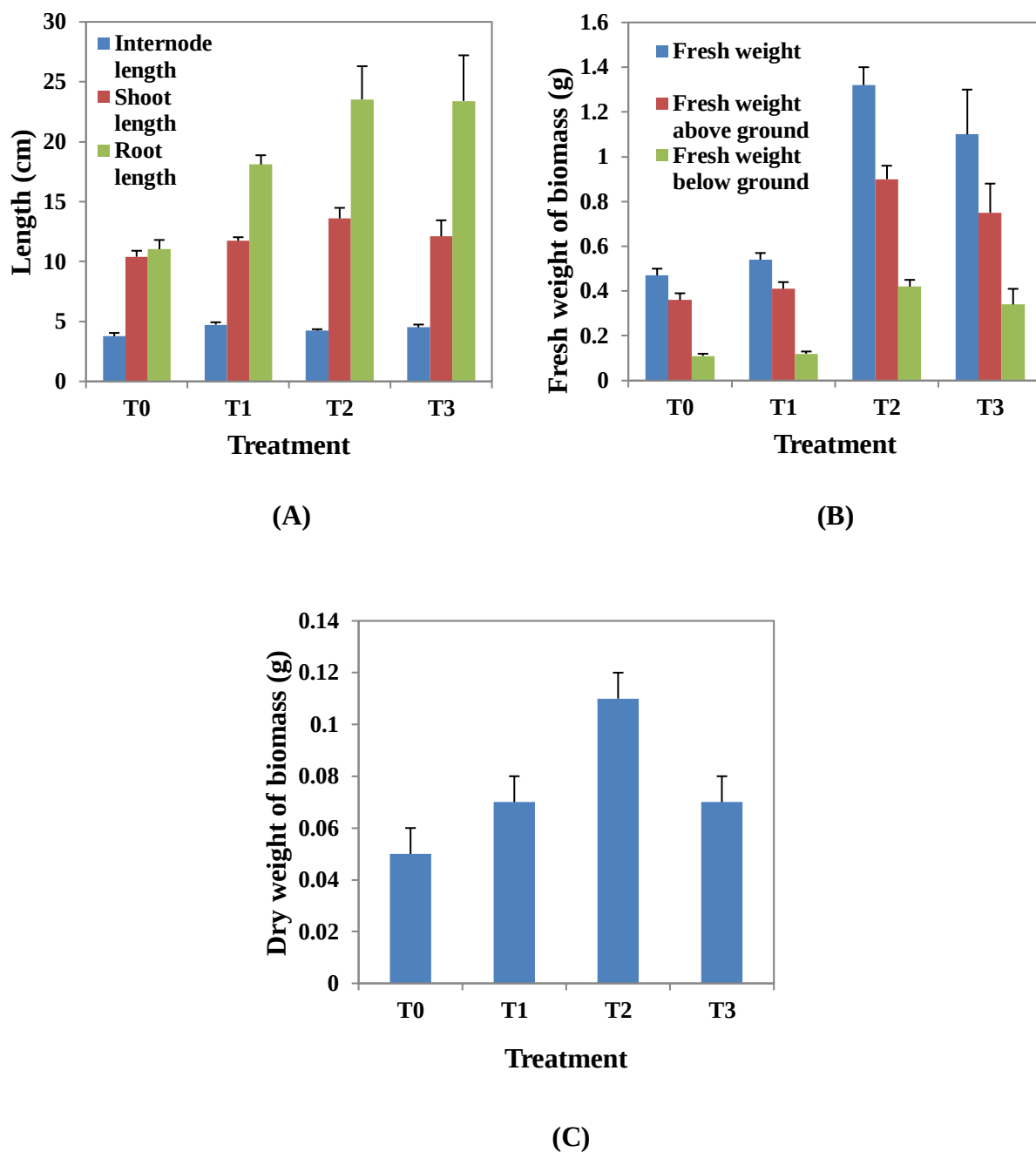
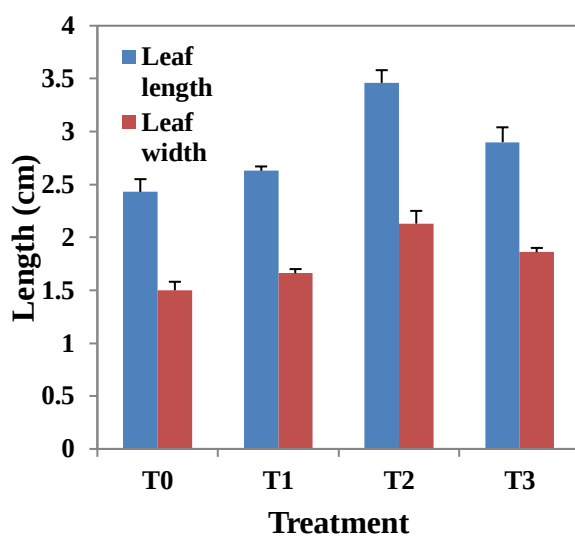
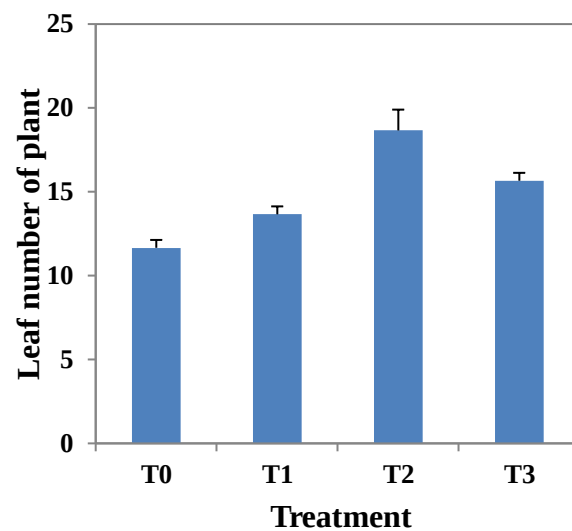


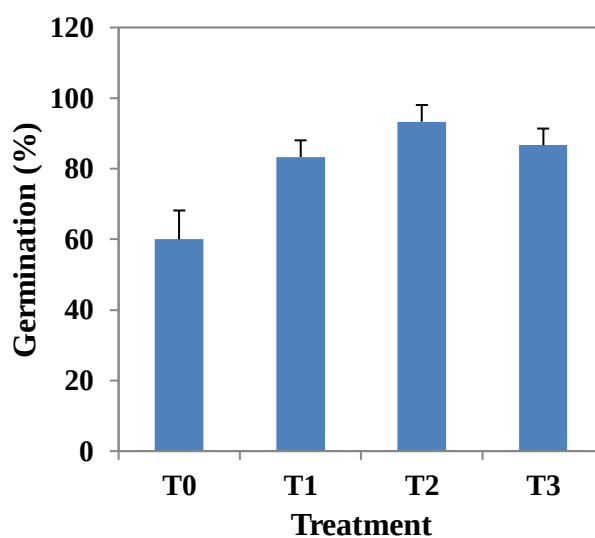
Figure 3.14 Effect of different treatments of soil on various parameters of tomato plants. T0: soil without biochar (control), T1: soil with 2% farm yard manure, T2: soil with 2% biochar, T3: soil with 5% biochar. (A) Effect of biochar on shoot, root and internode length of tomato plants. (B) Effect of biochar on fresh weight biomass of tomato plants. (C) Effect of biochar on dry weight biomass of tomato plants.



(A)



(B)



(C)

Figure 3.15 Effect of different treatments of soil on various parameters of tomato plants. T0: soil without biochar (control), T1: soil with 2% farm yard manure, T2: soil with 2% biochar, T3: soil with 5% biochar. (A) Effect of biochar on leaf length and leaf width of tomato plants. (B) Effect of biochar on leaf number of tomato plants. (C) Effect of biochar on germination of tomato plants.

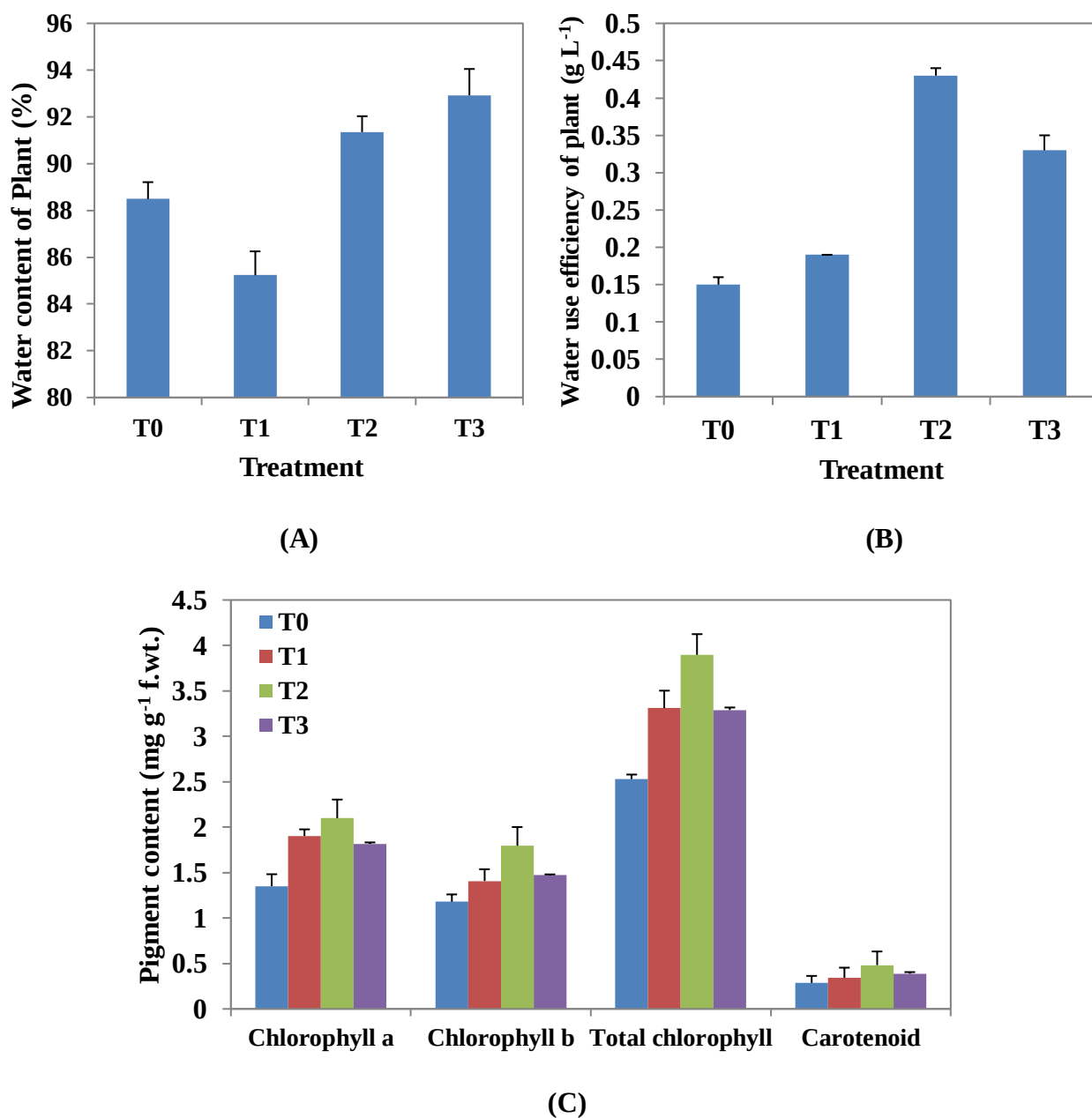


Figure 3.16 Effect of different treatments of soil on various parameters of tomato plants. T0: soil without biochar (control), T1: soil with 2% farm yard manure, T2: soil with 2% biochar, T3: soil with 5% biochar. (A) Effect of biochar on water content of tomato plants. (B) Effect of biochar on water use efficiency of tomato plants. (C) Effect of biochar on pigment content of tomato plants.

3.7.3 Effect of biochar on physico-chemical analysis of potted soil

The effect of biochar on soil amendments were analyzed on initial stage of the experiments and the final stage of experiments. The soils with different treatments were first oven dried, pulverized and sieve (< 2 mm) and the physico-chemical properties of soil were analyzed. The same procedure was repeated after harvest.

3.7.3.1 Effect of biochar on soil pH

The addition of biochar on soil increases soil pH around 7 which were conducted during the experiment. T3 treatment shows highest increase with 9.78 ± 0.16 % increase in soil pH. T1 treatment shows lowest increase with 3.73 ± 0.53 % at the end of experiment as shown in Fig. 3.17.

Table 3.5 One-way analysis of variance (ANOVA) of pH among different soil treatments such as Control (10% coco peat) T0, T1 (2% FYM), T2 (2% biochar) and T3 (5% biochar). Marked effects are significant at $p \leq 0.05$.

S.No.	Source of variation	F-Value	P-Value
1.	T0 x T1 x T2 x T3	27.191	0.000*
2.	T0 x T1	9.108	0.039*
3.	T0 x T2	8.204	0.045*
4.	T0 x T3	14.393	0.019*
5.	T1 x T2	119.667	0.000*
6.	T1 x T3	77.594	0.000*
7.	T2 x T3	5.976	0.070

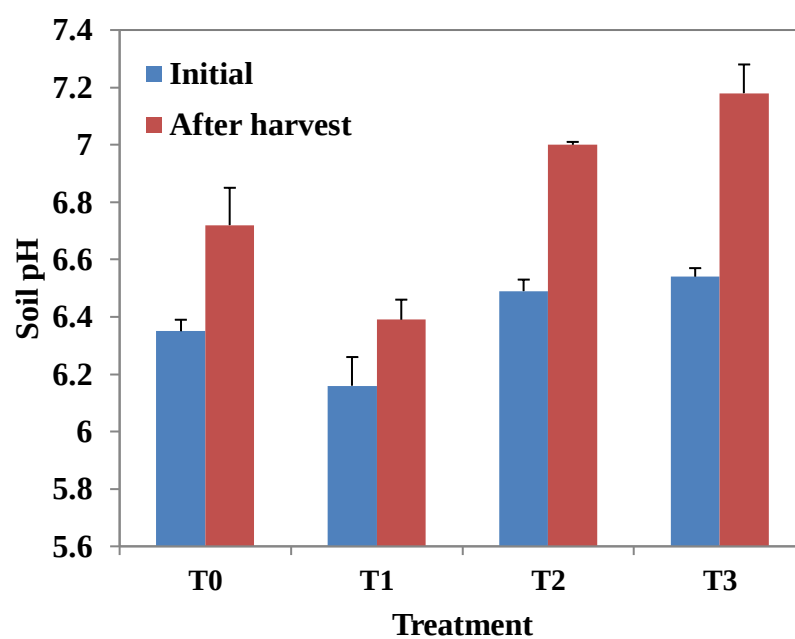


Figure 3.17 Effect of biochar on pH of soil. The experiment was conducted during 30 days. The soil pH was analyzed at initial and after harvest of tomato plants in different treatments of soil T0: Control (10% coco peat), T1: 10% coco peat + 2% FYM, T2: 10% coco peat + 2% Biochar and T3: 10% coco peat + 5% Biochar.

3.7.3.2 Effect of biochar on soil electrical conductivity

T3 shows highest increase in electrical conductivity of soil at the end of experiment (82.14 ± 0.07 %) while T0 shows lowest increase with 60.86 ± 0.10 % (Fig 3.18).

Table 3.6 One-way analysis of variance (ANOVA) of electrical conductivity among different soil treatments such as Control (10% coco peat) T0, T1 (2% FYM), T2 (2% biochar) and T3 (5% biochar). Marked effects are significant at $p \leq 0.05$.

S.No.	Source of variation	F-Value	P-Value
1.	T0 x T1 x T2 x T3	24.264	0.000*
2.	T0 x T1	8.476	0.043*
3.	T0 x T2	9.090	0.039*
4.	T0 x T3	84.210	0.000*
5.	T1 x T2	19.043	0.012*
6.	T1 x T3	49.329	0.002*
7.	T2 x T3	8.163	0.046*

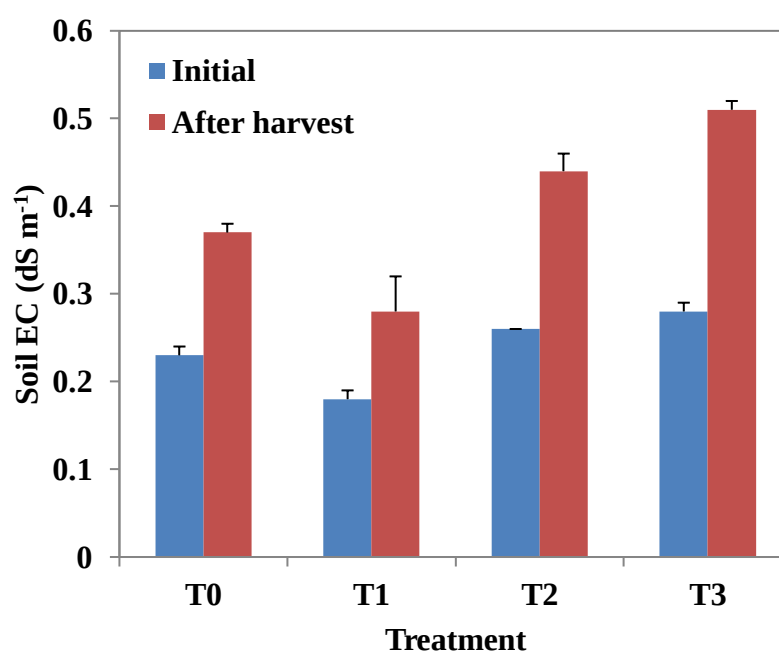


Figure 3.18 Effect of biochar on electrical conductivity of soil. The experiment was conducted during 30 days. The soil electrical conductivity was analyzed at initial and after harvest of tomato plants in different treatments of soil T0: Control (10% coco peat), T1: 10% coco peat + 2% FYM, T2: 10% coco peat + 2% Biochar and T3: 10% coco peat + 5% Biochar

3.7.3.3 Effect of biochar on soil cation exchange capacity

The biochar addition with T2 treatment shows highest increase in cation exchange capacity at the end of experiment with 9.04 ± 0.23 % as compared to the lowest increase on T1 treatment with 1.34 ± 1.62 % (Fig 3.19).

Table 3.7 One-way analysis of variance (ANOVA) of soil cation exchange capacity among different soil treatments such as Control (10% coco peat) T0, T1 (2% FYM), T2 (2% biochar) and T3 (5% biochar). Marked effects are significant at $p \leq 0.05$.

S.No.	Source of variation	F-Value	P-Value
1.	T0 x T1 x T2 x T3	53.470	0.000*
2.	T0 x T1	13.152	0.022*
3.	T0 x T2	19.737	0.011*
4.	T0 x T3	17.827	0.013*
5.	T1 x T2	386.496	0.000*
6.	T1 x T3	358.951	0.000*
7.	T2 x T3	8	0.047*

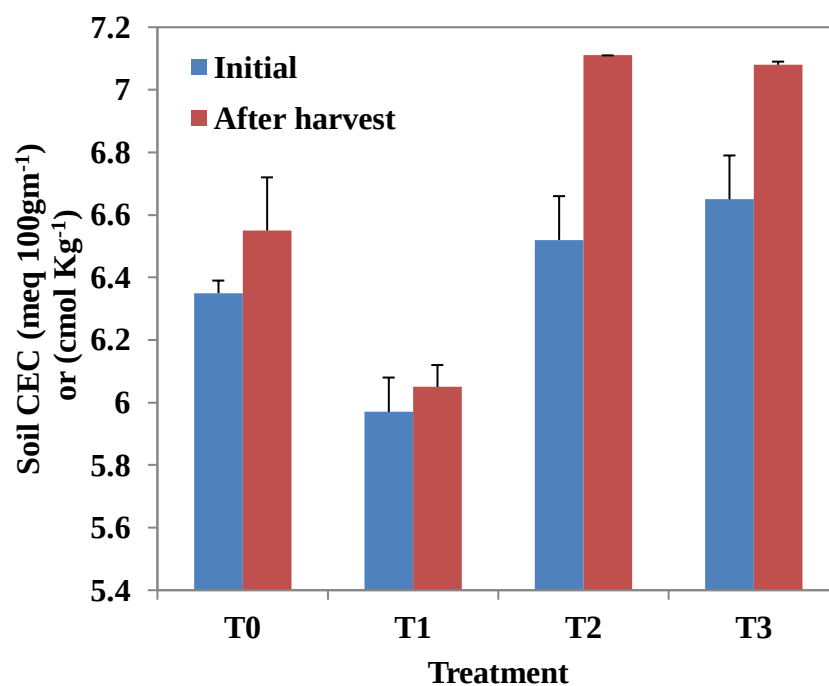


Figure 3.19 Effect of biochar on cation exchange capacity of soil. The experiment was conducted during 30 days. The soil cation exchange capacity was analyzed at initial and after harvest of tomato plants in different treatments of soil T0: Control (10% coco peat), T1: 10% coco peat + 2% FYM, T2: 10% coco peat + 2% Biochar and T3: 10% coco peat + 5% Biochar

3.7.3.4 Effect of biochar on soil moisture content

The biochar addition treatment on soil T3 shows highest increase with 54.43 ± 0.04 % of soil moisture content as compared to control (without biochar addition) as lowest with 25.94 ± 0.09 % as shown in Fig 3.20.

Table 3.8 One-way analysis of variance (ANOVA) of soil moisture content among different soil treatments such as Control (10% coco peat) T0, T1 (2% FYM), T2 (2% biochar) and T3 (5% biochar). Marked effects are significant at $p \leq 0.05$.

S.No.	Source of variation	F-Value	P-Value
1.	T0 x T1 x T2 x T3	35.893	0.000*
2.	T0 x T1	56.805	0.001*
3.	T0 x T2	107.820	0.000*
4.	T0 x T3	23.182	0.008*
5.	T1 x T2	17.005	0.014*
6.	T1 x T3	28.337	0.005*
7.	T2 x T3	16.432	0.015*

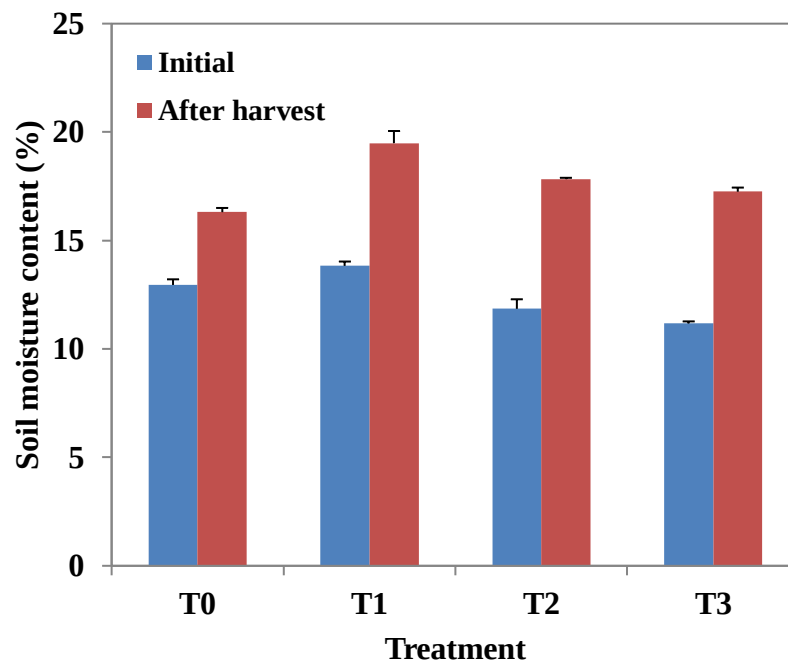


Figure 3.20 Effect of biochar on moisture content of soil. The experiment was conducted during 30 days. The soil moisture content was analyzed at initial and after harvest of tomato plants in different treatments of soil T0: Control (10% coco peat), T1: 10% coco peat + 2% FYM, T2: 10% coco peat + 2% Biochar and T3: 10% coco peat + 5% Biochar

3.7.3.5 Effect of biochar on soil water holding capacity

T3 shows highest increase in soil water holding capacity at the end of experiment with 4.68 ± 0.10 % while T1 shows lowest with 1.16 ± 0.49 % as shown in Fig 3.21.

Table 3.9 One-way analysis of variance (ANOVA) of soil water holding capacity among different soil treatments such as Control (10% coco peat) T0, T1 (2% FYM), T2 (2% biochar) and T3 (5% biochar). Marked effects are significant at $p \leq 0.05$.

S.No.	Source of variation	F-Value	P-Value
1.	T0 x T1 x T2 x T3	362.600	0.000*
2.	T0 x T1	8.113	0.046*
3.	T0 x T2	566.201	0.000*
4.	T0 x T3	387.200	0.000*
5.	T1 x T2	832.468	0.000*
6.	T1 x T3	510.034	0.000*
7.	T2 x T3	9.221	0.038*

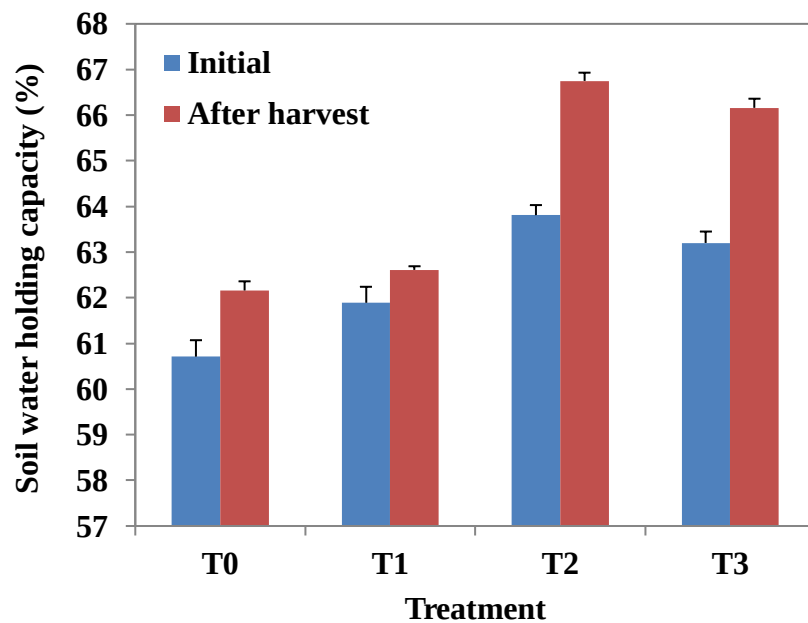


Figure 3.21 Effect of biochar on water holding capacity of soil. The experiment was conducted during 30 days. The soil water holding capacity was analyzed at initial and after harvest of tomato plants in different treatments of soil T0: Control (10% coco peat), T1: 10% coco peat + 2% FYM, T2: 10% coco peat + 2% Biochar and T3: 10% coco peat + 5% Biochar

3.7.3.6 Effect of biochar on soil organic carbon

The addition of biochar increases soil organic carbon, 5% biochar treatment increase with 69.43 ± 0.05 % and 2% biochar treatment increase with 67.75 ± 0.05 % as shown in Fig 3.22.

Table 3.10 One-way analysis of variance (ANOVA) of soil organic carbon among different soil treatments such as Control (10% coco peat) T0, T1 (2% FYM), T2 (2% biochar) and T3 (5% biochar). Marked effects are significant at $p \leq 0.05$.

S.No.	Source of variation	F-Value	P-Value
1.	T0 x T1 x T2 x T3	30.791	0.000*
2.	T0 x T1	10.319	0.032*
3.	T0 x T2	60.071	0.001*
4.	T0 x T3	38.200	0.003*
5.	T1 x T2	35.392	0.004*
6.	T1 x T3	14.364	0.019*
7.	T2 x T3	8.319	0.044*

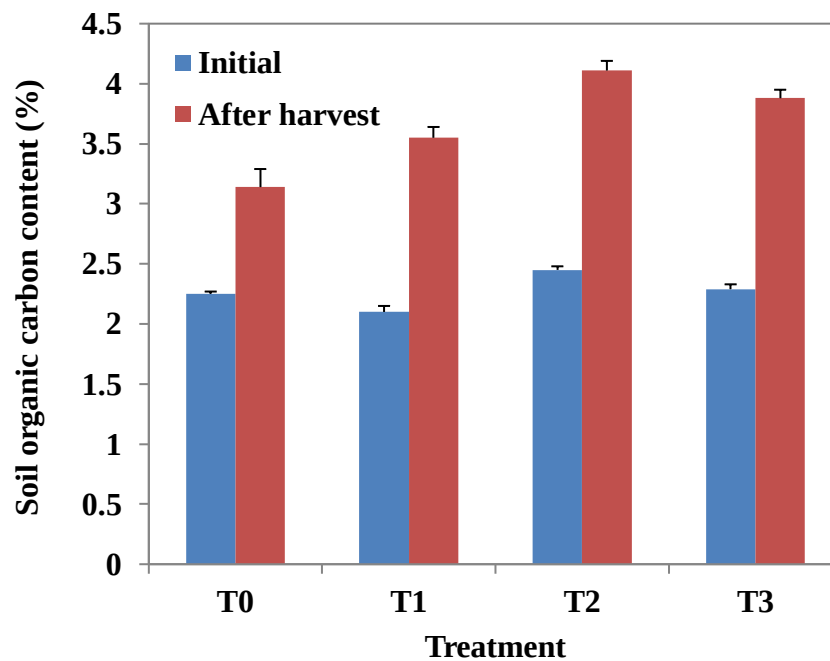


Figure 3.22 Effect of biochar on organic carbon content of soil. The experiment was conducted during 30 days. The soil organic carbon content was analyzed at initial and after harvest of tomato plants in different treatments of soil T0: Control (10% coco peat), T1: 10% coco peat + 2% FYM, T2: 10% coco peat + 2% Biochar and T3: 10% coco peat + 5% Biochar

3.7.3.7 Effect of biochar on soil available phosphorus

T2 shows increase in soil available phosphorus with 32.30 ± 0.22 % while T1 shows decrease in soil available phosphorus at the end of experiment with 5.51 ± 1.44 % (Fig 3.23).

Table 3.11 One-way analysis of variance (ANOVA) of soil available phosphorus among different soil treatments such as Control (10% coco peat) T0, T1 (2% FYM), T2 (2% biochar) and T3 (5% biochar). Marked effects are significant at $p \leq 0.05$.

S.No.	Source of variation	F-Value	P-Value
1.	T0 x T1 x T2 x T3	23.006	0.000*
2.	T0 x T1	8.556	0.043*
3.	T0 x T2	35.266	0.004*
4.	T0 x T3	40.298	0.003*
5.	T1 x T2	1.792	0.026*
6.	T1 x T3	19.372	0.011*
7.	T2 x T3	8.34	0.044*

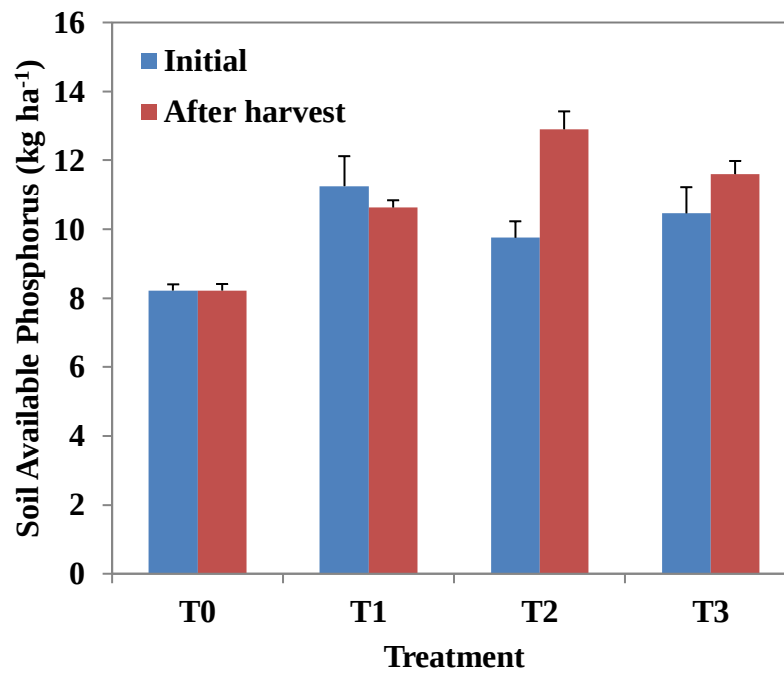


Figure 3.23 Effect of biochar on available phosphorus of soil. The experiment was conducted during 30 days. The soil available phosphorus was analyzed at initial and after harvest of tomato plants in different treatments of soil T0: Control (10% coco peat), T1: 10% coco peat + 2% FYM, T2: 10% coco peat + 2% Biochar and T3: 10% coco peat + 5% Biochar

3.7.3.8 Effect of biochar on soil exchangeable potassium

T3 shows 6.04 ± 0.24 % increase in soil exchangeable potassium followed by T2 with 4.66 ± 0.16 % while T0 shows 0.42 ± 2.79 % (Fig 3.24).

Table 3.12 One-way analysis of variance (ANOVA) of soil exchangeable potassium among different soil treatments such as Control (10% coco peat) T0, T1 (2% FYM), T2 (2% biochar) and T3 (5% biochar). Marked effects are significant at $p \leq 0.05$.

S.No.	Source of variation	F-Value	P-Value
1.	T0 x T1 x T2 x T3	85.801	0.000*
2.	T0 x T1	16.9	0.014*
3.	T0 x T2	870.25	0.000*
4.	T0 x T3	1024	0.000*
5.	T1 x T2	28.657	0.005*
6.	T1 x T3	38	0.003*
7.	T2 x T3	12.5	0.024*

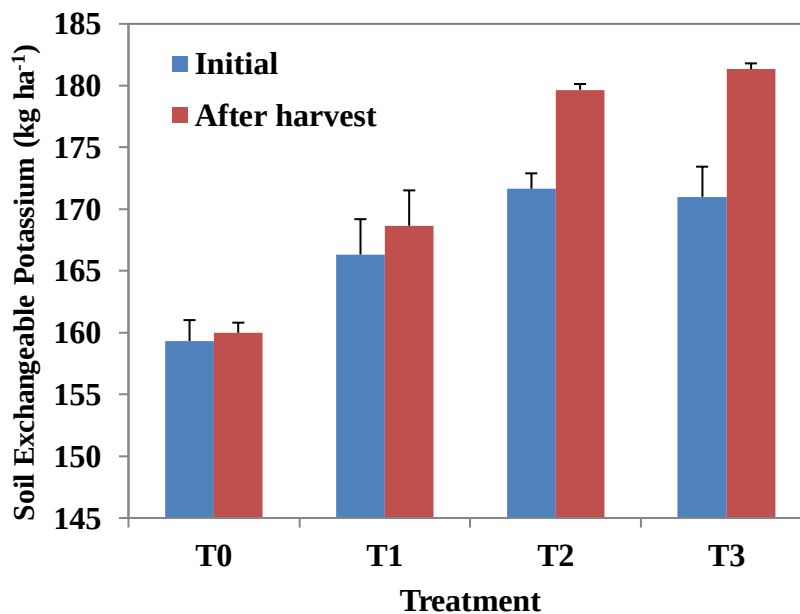


Figure 3.24 Effect of biochar on exchangeable potassium of soil. The experiment was conducted during 30 days. The soil exchangeable potassium was analyzed at initial and after harvest of tomato plants in different treatments of soil T0: Control (10% coco peat), T1: 10% coco peat + 2% FYM, T2: 10% coco peat + 2% Biochar and T3: 10% coco peat + 5% Biochar

3.7.3.9 Effect of biochar on soil available nitrogen

The soil available nitrogen increases at the end of experiment with 11.64 ± 0.12 % by T2 treatment while decrease in soil available nitrogen was observed in T0 with 0.64 ± 5.46 % (Fig 3.25).

Table 3.13 One-way analysis of variance (ANOVA) of soil available nitrogen among different soil treatments such as Control (10% coco peat) T0, T1 (2% FYM), T2 (2% biochar) and T3 (5% biochar). Marked effects are significant at $p \leq 0.05$.

S.No.	Source of variation	F-Value	P-Value
1.	T0 x T1 x T2 x T3	91.271	0.000*
2.	T0 x T1	66.393	0.001*
3.	T0 x T2	294	0.000*
4.	T0 x T3	280.9	0.000*
5.	T1 x T2	23.5	0.008*
6.	T1 x T3	9.112	0.039*
7.	T2 x T3	10	0.034*

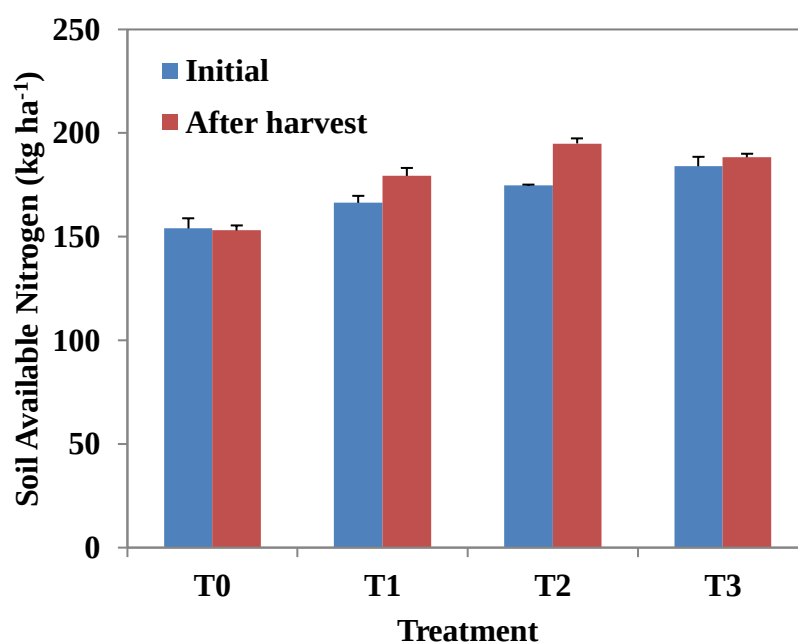


Figure 3.25 Effect of biochar on available nitrogen of soil. The experiment was conducted during 30 days. The soil available nitrogen was analyzed at initial and after harvest of tomato plants in different treatments of soil T0: Control (10% coco peat), T1: 10% coco peat + 2% FYM, T2: 10% coco peat + 2% Biochar and T3: 10% coco peat + 5% Biochar

3.7.3.10 Effect of biochar on soil microbial population

Both addition of biochar treatment T2 (19.50 ± 0.36 %) and T3 (9.80 ± 0.39 %) on soil increases soil fungal population while both treatment without biochar T0 (2.37 ± 3.47 %) and T1 (14.07 ± 0.27 %) decreases soil fungal population (Fig 3.26).

Table 3.14 One-way analysis of variance (ANOVA) of soil fungal population among different soil treatments such as Control (10% coco peat) T0, T1 (2% FYM), T2 (2% biochar) and T3 (5% biochar). Marked effects are significant at $p \leq 0.05$.

S.No.	Source of variation	F-Value	P-Value
1.	T0 x T1 x T2 x T3	20.488	0.000*
2.	T0 x T1	11.25	0.028*
3.	T0 x T2	4.9	0.091
4.	T0 x T3	14	0.020*
5.	T1 x T2	30.25	0.005*
6.	T1 x T3	42.05	0.002*
7.	T2 x T3	4.9	0.091

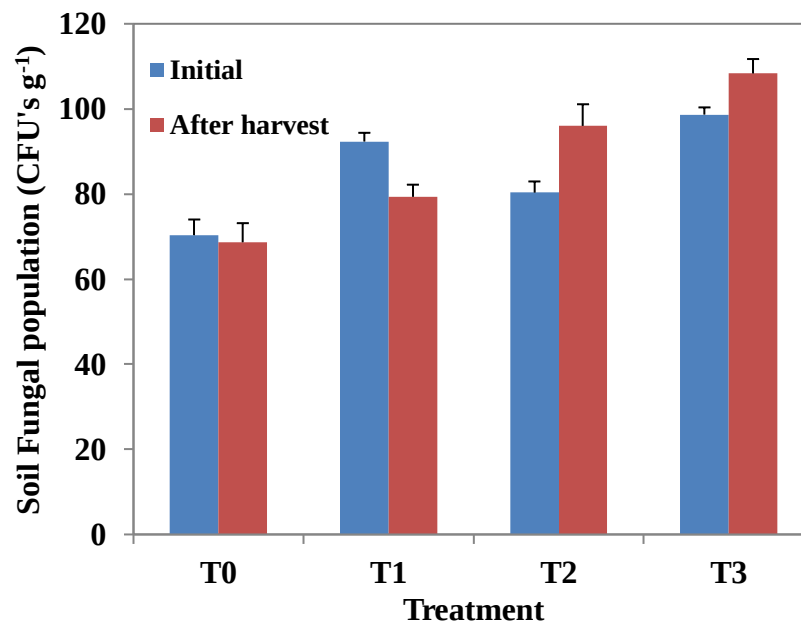


Figure 3.26 Effect of biochar on fungal population of soil. The experiment was conducted during 30 days. The soil fungal population was analyzed at initial and after harvest of tomato plants in different treatments of soil T0: Control (10% coco peat), T1: 10% coco peat + 2% FYM, T2: 10% coco peat + 2% Biochar and T3: 10% coco peat + 5% Biochar

T0 treatment shows highest increase in bacterial population with 24.79 ± 0.05 % while the lowest increase was shown by T1 treatment with 1.06 ± 3.54 % (Fig 3.27).

Table 3.15 One-way analysis of variance (ANOVA) of soil bacterial population among different soil treatments such as Control (10% coco peat) T0, T1 (2% FYM), T2 (2% biochar) and T3 (5% biochar). Marked effects are significant at $p \leq 0.05$.

S.No.	Source of variation	F-Value	P-Value
1.	T0 x T1 x T2 x T3	71.854	0.000*
2.	T0 x T1	236.454	0.000*
3.	T0 x T2	38.241	0.003*
4.	T0 x T3	22.909	0.008*
5.	T1 x T2	33.373	0.004*
6.	T1 x T3	133.218	0.000*
7.	T2 x T3	10.929	0.029*

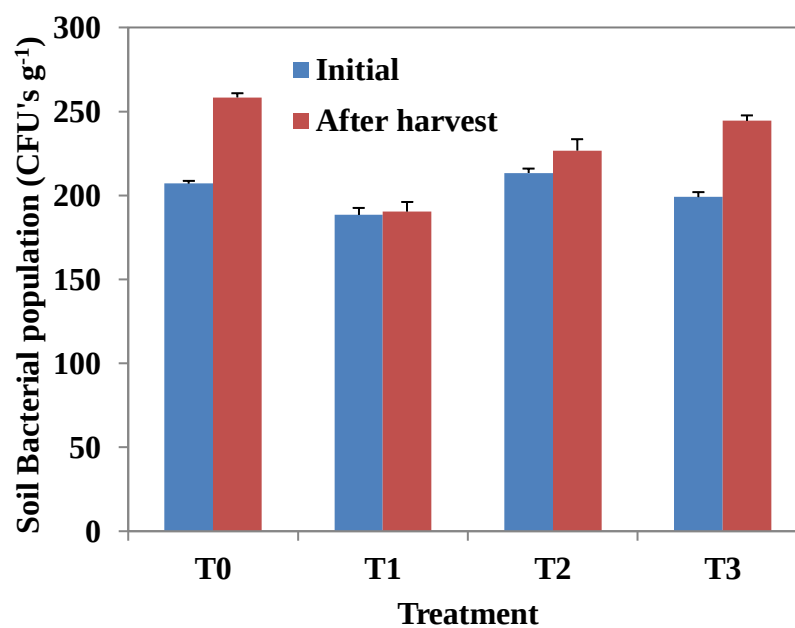


Figure 3.27 Effect of biochar on bacterial population of soil. The experiment was conducted during 30 days. The soil bacterial population was analyzed at initial and after harvest of tomato plants in different treatments of soil T0: Control (10% coco peat), T1: 10% coco peat + 2% FYM, T2: 10% coco peat + 2% Biochar and T3: 10% coco peat + 5% Biochar

3.7.3.11 Effect of biochar on soil acid phosphatase activity

The soil acid phosphatase activity was significantly increase by T2 treatment with 33.29 ± 0.02 % as compared to T1 treatment with 1.34 ± 1.36 % (Fig 3.28)

Table 3.16 One-way analysis of variance (ANOVA) of soil acid phosphatase activity among different soil treatments such as Control (10% coco peat) T0, T1 (2% FYM), T2 (2% biochar) and T3 (5% biochar). Marked effects are significant at $p \leq 0.05$.

S.No.	Source of variation	F-Value	P-Value
1.	T0 x T1 x T2 x T3	649.269	0.000*
2.	T0 x T1	15.921	0.016*
3.	T0 x T2	889.910	0.000*
4.	T0 x T3	827.668	0.000*
5.	T1 x T2	1185.307	0.000*
6.	T1 x T3	814.088	0.000*
7.	T2 x T3	9.1125	0.039*

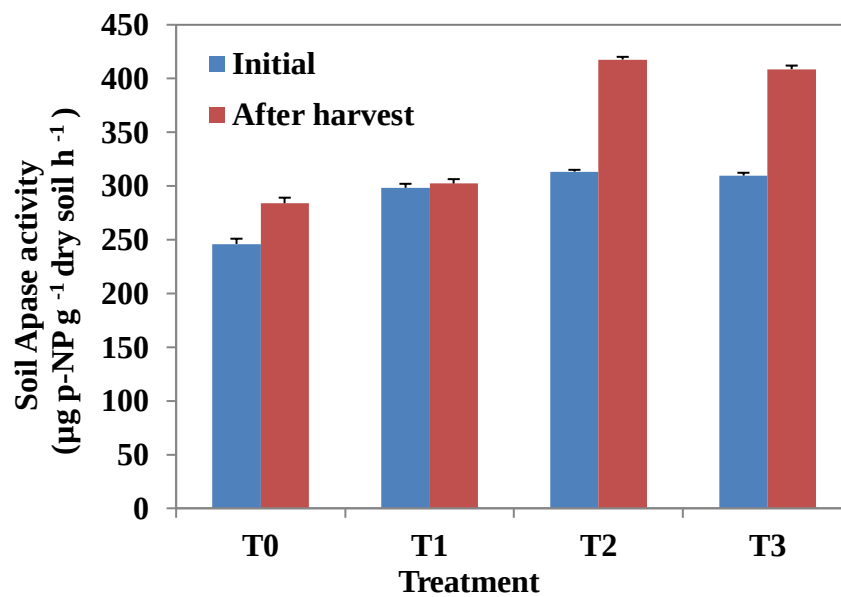


Figure 3.28 Effect of biochar on acid phosphatase activity of soil. The experiment was conducted during 30 days. The soil acid phosphatase activity was analyzed at initial and after harvest of tomato plants in different treatments of soil T0: Control (10% coco peat), T1: 10% coco peat + 2% FYM, T2: 10% coco peat + 2% Biochar and T3: 10% coco peat + 5% Biochar

3.7.3.12 Effect of biochar on soil dehydrogenase activity

Biochar addition treatment T3 shows significantly increase in soil dehydrogenase activity at the end of experiment with 126.47 ± 0.02 % as compared to treatment with farm yard manure (2.56 ± 1.00 %) as shown in Fig 3.29.

Table 3.17 One-way analysis of variance (ANOVA) of soil dehydrogenase activity among different soil treatments such as Control (10% coco peat) T0, T1 (2% FYM), T2 (2% biochar) and T3 (5% biochar). Marked effects are significant at $p \leq 0.05$.

S.No.	Source of variation	F-Value	P-Value
1.	T0 x T1 x T2 x T3	21.623	0.000*
2.	T0 x T1	28.9	0.005*
3.	T0 x T2	6.25	0.066
4.	T0 x T3	0.818	0.416
5.	T1 x T2	60.75	0.001*
6.	T1 x T3	28	0.006*
7.	T2 x T3	13	0.022*

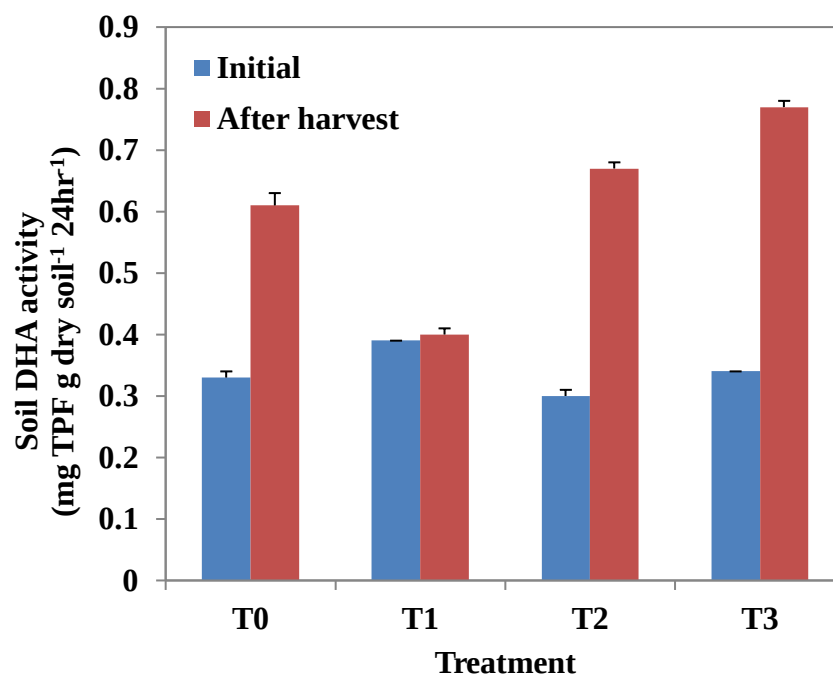


Figure 3.29 Effect of biochar on dehydrogenase activity of soil. The experiment was conducted during 30 days. The soil dehydrogenase activity was analyzed at initial and after harvest of tomato plants in different treatments of soil T0: Control (10% coco peat), T1: 10% coco peat + 2% FYM, T2: 10% coco peat + 2% Biochar and T3: 10% coco peat + 5% Biochar

CHAPTER 4

DISCUSSION

The ability to store gaseous CO₂ produced by point sources in ponds as bicarbonate is one of the more attractive aspects of producing algal biomass. Bicarbonate is a source of carbon dioxide that is transported and used by cyanobacteria and eukaryotic algae (Maheshwari et al., 2020). Bicarbonate, a non gaseous form of CO₂, is the predominant (>50%) chemical component of CO₂ in water at pH values between 6.4 and 10.3. Bicarbonate transporters, which are found in both the plasma membrane and the chloroplast envelope of eukaryotic algae, are responsible for pumping this momentarily trapped carbon into algal cells. Bicarbonate is transformed into CO₂ inside the chloroplast, fixed by the enzyme rubisco (ribulose biphosphate carboxylase oxygenase) to produce two molecules of 3-phosphoglycerate.

The present study investigated the impact of varying concentrations of sodium bicarbonate (NaHCO₃) on the specific growth rate of *M. aeruginosa* over a 20-day culture period. The results demonstrated a significant difference in growth rates across different NaHCO₃ concentrations, with the highest specific growth rate observed at 1 g L⁻¹ and the lowest at 5 g L⁻¹. The specific growth rate of 0.665 ± 0.15 d⁻¹ at 1 g L⁻¹ NaHCO₃ indicates that this concentration provides optimal conditions for the proliferation of *M. aeruginosa*. Sodium bicarbonate serves as a source of inorganic carbon, which is essential for the photosynthetic process in cyanobacteria. At this optimal concentration, the availability of inorganic carbon is sufficient to maximize photosynthetic efficiency and, consequently, cell division and growth. Conversely, the lowest specific growth rate of 0.325 ± 0.18 d⁻¹ at 5 g L⁻¹ NaHCO₃ suggests that higher concentrations of bicarbonate may exert inhibitory effects on the growth of *M. aeruginosa*. High bicarbonate concentrations can lead to elevated pH levels in the culture medium, potentially resulting in an alkaline environment that is detrimental to cyanobacterial growth. Excessive alkalinity can disrupt cellular homeostasis and impair metabolic processes (Geddie & Hall, 2019). Additionally, high bicarbonate levels might also interfere with the uptake of other essential

nutrients, thereby limiting growth. These findings have significant implications for the cultivation practices of *M. aeruginosa*, particularly in biotechnological applications where optimizing growth conditions are crucial. The present study suggests that maintaining bicarbonate concentrations at or around 1 g L⁻¹ can enhance the productivity of *M. aeruginosa* cultures, making it an effective strategy for biomass production. This concentration ensures adequate inorganic carbon availability without the adverse effects associated with higher bicarbonate levels. The observed specific growth rates in this study are consistent with those reported in the literature. Yang et al., (2024) reported that moderate bicarbonate concentrations could enhance the growth of *Spirulina platensis*, another cyanobacterium, by providing sufficient inorganic carbon while avoiding the inhibitory effects of high alkalinity. The addition of 0.5 g L⁻¹ bicarbonate significantly boosted chlorophyll and carotenoid levels, highlighting the importance of optimal inorganic carbon availability for photosynthetic efficiency. Chlorophylls are essential for capturing light energy, while carotenoids play crucial roles in photoprotection and light harvesting. The enhanced pigment production suggests that 0.5 g L⁻¹ NaHCO₃ provides a favorable environment for *M. aeruginosa*, promoting efficient photosynthesis and cellular metabolism. The observed increase in pigment content at 0.5 g L⁻¹ NaHCO₃ aligns with findings from other studies on cyanobacteria and microalgae. For instance, Magwell et al., (2023) reported that moderate bicarbonate levels significantly enhanced chlorophyll and carotenoid content in *Spirulina platensis*. Similarly, Zhou et al., (2018) found that optimal bicarbonate concentrations promoted pigment synthesis in *Chlorella vulgaris*, leading to increased photosynthetic efficiency.

The highest protein content (38.90 ± 1.81 %) was observed at 1 g L⁻¹ NaHCO₃, indicating that this concentration optimally supports protein synthesis in *M. aeruginosa*. Proteins are crucial for cellular functions and structural components, and their synthesis is heavily dependent on nitrogen availability and overall metabolic activity. The lipid content peaked at 10.19 ± 0.21 % with 1 g L⁻¹ bicarbonate, suggesting that this concentration also optimally supports lipid synthesis. Lipids in microalgae serve as energy reserves and are crucial for

membrane structure. The highest carbohydrate content (33.67 ± 1.05 %) was observed at 0.5 g L^{-1} bicarbonate. Carbohydrates serve as primary storage compounds and structural components in microalgae. At lower bicarbonate levels, *M. aeruginosa* might prioritize carbohydrate synthesis as a form of carbon storage, especially when protein and lipid synthesis are not maximized.

The CO_2 fixation by *M. aeruginosa* at different concentrations of sodium bicarbonate was observed at a range of $0.053 \pm 0.06 \text{ g L}^{-1} \text{ d}^{-1}$ to $0.172 \pm 0.02 \text{ g L}^{-1} \text{ d}^{-1}$. The maximum CO_2 fixation rate was observed at $1 \text{ g L}^{-1} \text{ NaHCO}_3$ suggests that this concentration provides the most favorable conditions for photosynthetic activity in *M. aeruginosa*. Sodium bicarbonate serves as an important inorganic carbon source, enhancing the availability of CO_2 for the Calvin cycle, which is essential for photosynthesis. Several microalgae species have been studied for CO_2 reduction, such as *Chlorella kessleri*, *Scenedesmus obliquus* (de Morais & Costa, 2007), *Chlorocuccum littorale*, *Dunaliella tertiolecta*, *Chlorella vulgaris*, *Spirulina platensis*, *Botryococcus braunii* (Sydney et al., 2010), *Nannochloropsis oculata* (Chiu et al., 2015) and *Chlorella sp.*

Semi-continuous culture systems generally offer higher productivity compared to batch cultures due to the continuous removal of a portion of the culture and the addition of fresh medium. This process maintains the culture in an exponential growth phase, thereby optimizing conditions for sustained high productivity (Lalrinkimi & Kant Mehta, 2022). In contrast, batch cultures often experience a decline in growth rates as nutrients become depleted and waste products accumulate. The high biomass productivity observed in this study suggests significant potential for biochar and bio oil production. Efficient biomass generation is a critical factor in the economic feasibility of biochar production from microalgae. The productivity rate of $0.954 \pm 0.13 \text{ g L}^{-1} \text{ d}^{-1}$ indicates that semi-continuous cultures could provide a reliable and scalable method for producing large quantities of algal biomass, which can be further processed into biochar and bio oil. A biochar yield of 56% from *M. aeruginosa* biomass is notably high compared to many terrestrial biomass sources. This high yield can be attributed to the composition and structure of *M. aeruginosa*, which typically contains a high proportion of recalcitrant compounds

such as carbohydrates and proteins that contribute to the solid residue after pyrolysis (Lehmann & Joseph, 2015). The cellular composition of *M. aeruginosa* includes significant amounts of polysaccharides and proteins, which, upon pyrolysis, contribute to the formation of a stable carbon-rich biochar. The presence of minerals and ash content also plays a role in the biochar yield, acting as catalysts during the pyrolysis process (Wang et al., 2024). The yield of biochar is also influenced by pyrolysis conditions such as temperature, heating rate, and residence time. Lower temperatures typically favor higher biochar yields, whereas higher temperatures tend to increase bio-oil and gas production. The bio-oil yield of 16.7% is within the expected range for algal biomass but lower compared to some terrestrial biomass. Bio-oil yield depends on the volatile content of the biomass and the pyrolysis conditions. Cyanobacteria, including *M. aeruginosa*, generally have lower lignin content compared to terrestrial plants but higher lipid and protein content, which can influence the yield and composition of bio-oil. Lipids contribute significantly to bio-oil production, whereas proteins can produce nitrogenous compounds that may complicate bio-oil refinement (Hossain et al., 2022). The yield of bio-oil can potentially be increased by optimizing pyrolysis parameters such as temperature and heating rate. Higher temperatures tend to enhance the thermal cracking of biomass, increasing the production of bio-oil and gases at the expense of biochar.

The pH of the biochar was found to be 8.5, indicating its alkaline nature. This alkalinity can be beneficial for ameliorating acidic soils, thereby enhancing soil health and promoting microbial activity which thrives better in neutral to slightly alkaline conditions. The addition of biochar with a high pH can help in neutralizing acidic soils, leading to improved crop yields and soil fertility. The electrical conductivity (EC) of 0.63 dS m⁻¹ indicates a moderate level of soluble salts. While excessive salts can be detrimental to plant growth, moderate levels can enhance nutrient availability and uptake. This property is crucial in ensuring that plants receive essential nutrients without experiencing the adverse effects of salinity. A cation exchange capacity (CEC) of 10.5 meq 100 g⁻¹ suggests that the biochar has a moderate ability to retain and supply cations such as calcium, magnesium, and potassium. High CEC is beneficial for soil fertility as it helps in nutrient retention

and reduces nutrient leaching, thereby improving the overall nutrient profile of the soil. The moisture content of the biochar was 6.45%, indicating its ability to retain some water. Although relatively low, this property contributes to the biochar's role in improving soil moisture retention, especially in sandy soils which typically have poor water holding capacity. Enhanced moisture retention can lead to better plant growth and resilience during drought conditions. Literature indicates that biochar may retain over 2.0 to 2.7 times its weight (Yu et al., 2017). A 31.06% ash indicates a notable amount of inorganic material present. A high ash level in biochar suggests that vital minerals are present, which may help plants flourish. It also suggests that there may be less carbon-rich organic material in the biochar, which is important for soil amendment reasons. There are readily decomposable organic molecules present, as evidenced by the volatile matter level of 18.23% in biochar. This can increase soil microbial activity because microorganisms use these substances as a food supply. Therefore, the presence of volatile materials can encourage nutrient cycling and biological activity in the soil. Among its many advantages is a high water-holding capacity of 96.75% in this biochar. This trait is very crucial for strengthening the structure of the soil, lowering soil erosion, and increasing plant resilience to drought. It makes sure the soil stays wet for extended periods of time, which lowers the requirement for frequent watering. Because of its distinct porosity, range of particle sizes, large surface area, and mulching effect, adding biochar to soil enhances its capacity to store water (Kurniawan et al., 2023). The carbon content of 43.559% is noteworthy for soil carbon sequestration, augmenting soil organic matter and aiding in the decrease of atmospheric CO₂ levels. The carbon content of biochar is mostly in an aromatic form from high-temperature pyrolysis, rendering it resistant to degradation and disintegration when incorporated into soil (Tan et al., 2021). Even though the nitrogen level of 0.72% is not very high, it nonetheless adds to the soil's nitrogen pool. Plant growth and development depend on nitrogen, and biochar can serve as a slow-release supply of nitrogen to gradually increase soil fertility. The phosphorus content of 0.748 mg kg⁻¹, though low, provides a vital nutrient for plant root development and energy transfer. Phosphorus availability from biochar can help in sustaining plant growth, especially in phosphorus-deficient soils. For plants to

perform physiological processes including water control, enzyme activation, and photosynthesis, a potassium level of 15.59 mg kg⁻¹ is essential. Plant stress tolerance and increased crop yields can result from biochar-amended soils with an adequate potassium content.

The FTIR analysis of biochar derived from *M. aeruginosa* reveals a variety of functional groups that can significantly influence its effectiveness and utility as a soil amendment. These functional groups are characteristics of cellulose which are principle polymers of biochar (Wang et al., 2023) and are recalcitrant and locked into biochar even after pyrolysis. The strong absorption band at 3674.42 cm⁻¹, corresponding to O-H stretching vibrations, indicates the presence of hydroxyl groups. These alcohol groups can enhance the hydrophilicity of biochar, promoting its interaction with water and soil moisture. This can improve soil water retention, making it beneficial in drought-prone areas by enhancing the soil's ability to retain moisture and support plant growth. C-H stretching vibrations of alkanes are linked to the peak at 2920.54 cm⁻¹. The presence of these aliphatic hydrocarbons suggests that the biochar retains some organic characteristics from the original biomass. This can contribute to the organic matter content of the soil, improving its structure and fertility over time. The band at 2361.04 cm⁻¹ indicates the presence of carbon dioxide, suggesting potential CO₂ adsorption capabilities. Biochar's ability to sequester CO₂ can contribute to reducing greenhouse gas emissions from the soil, making it an environmentally friendly amendment. Additionally, this property can help in maintaining soil pH levels, benefiting plant growth. The bending vibrations of secondary amines correlate to the absorption band at 1559.07 cm⁻¹. Nitrogen-containing functional groups such as amines can enhance the biochar's ability to improve soil nitrogen content. Incorporating biochar with secondary amines into soil can help in slow-release nitrogen fertilization, reducing the need for chemical fertilizers. The absorption at 1398.46 cm⁻¹, associated with O-H bending vibrations of carboxylic acids, suggests the presence of these highly reactive groups. Carboxylic acids can participate in ion exchange processes in the soil, enhancing nutrient availability to plants. C-O stretching vibrations of carboxylic and aromatic esters are shown by the peak at 1047.48 cm⁻¹. The peak at 1047.48 cm⁻¹ indicates C-O

stretching vibrations of carboxylic and aromatic esters. These ester groups can contribute to the biochar's chemical reactivity, facilitating the binding of soil nutrients and organic matter. The absorption at 562.70 cm^{-1} corresponds to C-I stretching vibrations, indicating the presence of halo compounds. These compounds could imply that the biochar has retained some halogenated compounds from the original biomass, which might influence its reactivity in soil. The band at 442.65 cm^{-1} is attributed to S-S stretching vibrations of aryl disulfides. Disulfide bonds can add to the mechanical strength and stability of biochar, making it a durable soil amendment.

The prominent XRD peak at $2\theta = 29.16^\circ$ typically corresponds to the (104) lattice plane of calcite (CaCO_3), indicating that calcite is a major crystalline component in the biochar obtained from *M. aeruginosa* pyrolysis. This result is consistent with previous studies, where calcite formation is frequently reported in pyrolyzed microalgal biomass. The presence of calcite or related carbonates could influence the physicochemical properties of the biochar, such as its alkalinity, cation exchange capacity, and potential for environmental applications.

The observed ridges, surface undulations, cracks, and channels in the SEM images indicate a highly porous and heterogeneous surface morphology of the biochar. Such features are typical of biochars derived from pyrolysis and contribute to increased surface area and porosity, which can enhance adsorption capacity and reactivity in environmental applications. The surface roughness and irregularity likely facilitate interaction sites for sorption or catalysis, consistent with reports on biochar morphology from cyanobacterial biomass.

The presence of mesopores visible in the TEM images of biochar suggests a porous structure favorable for adsorption and catalytic applications. The observed lattice fringes indicate partial crystallinity within the biochar, which may contribute to its physicochemical properties and stability. Such features are consistent with pyrolyzed cyanobacterial biochars and enhance their utility in environmental remediation and soil applications.

The AAS analysis revealed that the biochar derived from *Microcystis aeruginosa* contains significant concentrations of metal elements, with iron being the most abundant at 8789.55 mg/kg, followed by magnesium (7652.15 mg/kg) and calcium (2450.33 mg/kg). Other metals detected include manganese, zinc, copper, cobalt, selenium, and molybdenum in decreasing concentrations. The high iron and magnesium content suggests the potential of this biochar to influence nutrient availability and catalytic properties. The presence of micronutrients such as zinc, copper, and cobalt may enhance biochar's role in soil amendment and plant nutrition when applied environmentally. Trace amounts of selenium and molybdenum are notable for their biological importance but require careful consideration regarding their environmental impact. Overall, the diverse metal profile reflects the biochar's complex mineral composition, which may contribute to its multifunctional applications, including adsorption, catalysis, and nutrient management in ecological and agricultural systems.

The impact of various biochar treatments on the growth parameters of tomato plants, specifically plant height, root length, and internode length, reveals significant differences among the treatments. The highest plant height observed in the biochar treatment (13.60 ± 0.88 cm) in soil compared to the control (without biochar treatment) with 10.40 ± 0.50 cm indicates that biochar provides a more favorable environment for tomato plant growth. This can be attributed to the enhanced nutrient availability and improved soil structure provided by biochar treatment. The increase in plant height suggests that biochar treatment may contain a balanced mix of essential nutrients that promote cell division and elongation, leading to taller plants. The root length of tomato plants was significantly higher in the biochar treatment in soil (23.53 ± 2.77 cm) compared to the control (11.03 ± 0.77 cm). Root development is a crucial factor for water and nutrient uptake, and a robust root system enhances the plant's ability to access soil resources. It was reported that when compared to the control, data pertaining to the root demonstrated that the application of wood biochar increased the root's dry weight and total biomass. In order to measure a plant's root lifespan and its capacity to absorb water and nutrients, root development is crucial (Liang et al., 2023). The internode length of tomato plants in farm yard manure

treatment of soil measured 4.73 ± 0.20 cm, whereas the control treatment exhibited 3.76 ± 0.28 cm. An essential growth factor that affects the plant's overall design is internode length. Longer internodes in the farm yard manure treatment might be a sign of improved photosynthesis and light penetration (Poorter et al., 2012).

The application of biochar as a soil amendment has demonstrated a significant positive impact on the biomass of tomato plants, both in terms of fresh weight and dried weight. These findings align with the growing body of literature that highlights the benefits of biochar in enhancing soil fertility and plant growth. Tomato plants treated with biochar had a substantially larger fresh weight biomass (1.32 ± 0.08 g) than plants that were not treated with biochar (0.47 ± 0.03 g). Several essential characteristics of biochar are responsible for this considerable rise in fresh biomass. It was discovered that the dry weight biomass of tomato plants treated with biochar (0.11 ± 0.01 g) was double that of plants not treated with biochar (0.05 ± 0.01 g). The increase in dried biomass provides more evidence that biochar is a useful tool for encouraging plant growth. Since it does not account for fluctuations in water content, dried biomass represents the real build-up of organic matter within the plant and is therefore a more reliable indicator of plant development (Carillo et al., 2020). The germination rate of tomato seeds was markedly higher in biochar treatment (T2), where 93.33 ± 4.71 % of seeds germinated successfully. In contrast, the germination rate in the control group without biochar (T0) was substantially lower, with only 60.00 ± 8.16 % of seeds germinating. This finding suggests that the presence of biochar in the soil promotes and enhances the germination process of tomato seeds. Our results are consistent with previous studies that have demonstrated the positive effects of biochar on seed germination across various plant species (Carril et al., 2023). The water content of tomato plants varied significantly among the different treatments. The highest water content was observed in plants grown in soil amended with biochar, with a mean water content of 92.92 ± 1.13 %. In contrast, plants grown in soil treated with farmyard manure exhibited the lowest water content, with a mean value of 85.23 ± 1.02 %. Water use efficiency, measured as the amount of biomass produced per unit of water consumed, also varied across the treatments. The highest water use efficiency was observed in the treatment with 2%

biochar amendment, where plants exhibited a water use efficiency of $0.43 \pm 0.01 \text{ g L}^{-1}$. In contrast, the control treatment without any soil amendment showed the lowest water use efficiency, with a value of $0.15 \pm 0.01 \text{ g L}^{-1}$. These results indicate that biochar amendment improves the efficient utilization of water by plants, resulting in higher biomass production per unit of water consumed. The comparison between biochar and farmyard manure treatments highlights the superiority of biochar in enhancing water content and water use efficiency in tomato plants. While both amendments contribute to soil moisture retention, biochar appears to offer greater benefits in terms of water conservation and plant performance.

In the present study, the pH of the soil increased significantly as a result of the addition of biochar; among the treatments, the 5% biochar treatment showed the greatest increase. This rise in pH toward neutral values in the soil is a sign of the alkalizing action of biochar, which can decrease soil acidity and increase nutrient availability. Biochar was reported to increase soil pH in various studies (Chan et al., 2007; Khan et al., 2022). The presence of basic cations in biochar and their transformation into alkaline compounds including oxides, carbonates, and hydroxides during pyrolysis may be the cause of increase in pH. In contrast to biochar made at moderate temperatures, high pyrolysis biochar is alkaline in nature (Wang et al., 2022). Additionally, the electrical conductivity increased most with the 5% biochar treatment, suggesting higher soil salinity and enhanced ion mobility. In the present study, the addition of biochar had a favorable effect on the cation exchange capacity (CEC) of the soil; the maximum increase was observed in the 2% biochar treatment. When biochar was added to soil, CEC increases due to the presence of oxygen containing functional groups like hydroxyl, carbonyl and carboxyl (Domingues et al., 2020).

The outcomes of this investigation showed that biochar improves soil gravimetric water content. It was well documented in literature that biochar application on soil could improve water holding capacities of soil (Basso et al., 2013). Furthermore, 5% biochar treatment in particular showed increased moisture content and water-holding capacity in the biochar-amended soils, underscoring the benefit of biochar in enhancing soil water retention and drought resilience.

Both the 5% and 2% biochar treatments demonstrated notable increases in soil organic carbon content as a result of the addition of biochar. This increase in soil organic carbon can improve the fertility, microbial activity, and soil structure, all of which are factors in the general health of the soil. Additionally, the use of biochar treatments improved the availability of soil nutrients, especially potassium and phosphorus, which are critical for the growth and development of plants. Higher fungal population densities in 2% and 5% treatments indicate that the addition of biochar increased soil microbial populations, especially fungal communities. A climate that is conducive to microbial activity and nutrient cycling is suggested by this increase in soil microbial abundance, which may have a domino effect on soil health and ecosystem functioning. Interestingly, the control group exhibited the highest increase in bacterial population compared to other treatments. This unexpected finding suggests that certain conditions in the control group may have favored bacterial proliferation. Conversely, the farmyard manure treatment showed the lowest increase in bacterial population, indicating potential limitations or adverse effects associated with this organic amendment. Further investigation is warranted to elucidate the underlying mechanisms driving these contrasting microbial responses to different soil treatments.

In the present study, Biochar addition, particularly the 2% treatment, significantly enhanced soil acid phosphatase activity compared to farmyard manure treatment. Acid phosphatase enzymes play a crucial role in phosphorus mineralization and nutrient cycling in soils. The observed increase in acid phosphatase activity suggests that biochar amendment may promote the release of organic phosphorus compounds, thereby enhancing phosphorus availability for plant uptake. Moreover, the 5% biochar treatment demonstrated a substantial increase in soil dehydrogenase activity compared to farmyard manure treatment. Dehydrogenase enzymes are involved in the oxidation of organic matter and are considered indicators of soil microbial activity and overall soil health. The elevated dehydrogenase activity observed in biochar-amended soils implies enhanced microbial activity and metabolic processes, which can contribute to improved soil fertility and nutrient cycling. This was supported by various studies (Zhang et al.,

2021; Pokharel et al., 2020; Garbuz et al., 2022), who reported the positive effect of biochar on enzyme activities. However at the same time, no effect or negligible of biochar addition on soil enzyme activity was also reported (Futa et al., 2020).

Comparative analysis revealed that biochar addition exerted greater stimulatory effects on soil enzyme activities compared to farmyard manure treatment. This present study suggests that biochar might be a more effective soil amendment for enhancing microbial activity and enzymatic functions in agricultural soils. The specific mechanisms underlying these differential responses warrant further investigation and could be influenced by factors such as biochar properties, microbial community composition, and soil physicochemical characteristics.

SUMMARY

The ability to store gaseous CO₂ produced by point sources in ponds as bicarbonate presents an attractive avenue for producing algal biomass. Bicarbonate serves as a source of carbon dioxide utilized by cyanobacteria and eukaryotic algae. Bicarbonate transporters in these organisms are responsible for pumping trapped carbon into algal cells, where it is transformed into CO₂ and fixed by the enzyme rubisco to produce 3-phosphoglycerate.

A study investigated the impact of varying sodium bicarbonate (NaHCO₃) concentrations on the specific growth rate of *Microcystis aeruginosa* over a 20-day culture period. Results showed a significant difference in growth rates across different NaHCO₃ concentrations, with the highest specific growth rate observed at 1 g L⁻¹ and the lowest at 5 g L⁻¹. This suggests that maintaining bicarbonate concentrations around 1 g L⁻¹ can enhance productivity while avoiding inhibitory effects associated with higher levels.

Furthermore, the study examined the composition of algal biomass and its potential for biochar and bio oil production. Semi-continuous culture systems were found to offer higher productivity compared to batch cultures due to continuous nutrient supply and waste removal. This productivity, combined with the high biochar yield from *Microcystis sp.* biomass, suggests the potential for scalable biochar production.

The composition of the produced biochar indicates its potential for soil amendment. With its alkaline pH, moderate soluble salts, and significant cation exchange capacity, biochar can enhance soil fertility and microbial activity. Its ability to retain water and nutrients, along with its high carbon content, makes it valuable for improving soil structure and promoting plant growth.

In a separate experiment, the impact of biochar on tomato plant growth was studied. Biochar-treated soil showed significant improvements in plant height, root length, internode length, and biomass compared to untreated soil. Additionally,

biochar treatment increased the germination rate and water use efficiency of tomato plants, highlighting its positive effects on plant development and water conservation.

Analysis of soil properties following biochar addition revealed increases in pH, electrical conductivity, cation exchange capacity, moisture content, and soil organic carbon. These improvements, coupled with enhanced microbial activity and enzymatic functions, indicate the potential of biochar as a superior soil amendment compared to traditional organic fertilizers like farmyard manure.

Overall, these studies underscore the multifaceted benefits of utilizing bicarbonate for algal biomass production and employing biochar as a sustainable soil amendment. They provide valuable insights into optimizing growth conditions for algal cultures and enhancing soil fertility and plant growth through biochar application, thereby contributing to sustainable agricultural practices and environmental stewardship.

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<https://doi.org/10.1016/j.biortech.2018.08.131>

BIO-DATA

Name : R. Lalremdika
Father's Name : R. Lalnunmawia
Mother's Name : Zoramthangi
Nationality : Indian
Date of Birth : 18-07-1992
Sex : Male
Marital Status : Married
Contact Number : 9615510149
Email Address : lalremdikaralte@gmail.com

ACADEMIC QUALIFICATIONS

Name of the degree/exam	Board/ University	Year of passing	Division	(%/Grade)
HSLC	Mizoram Board of School Education (MBSE)	2007	Distinction	77
HSSLC	Mizoram Board of School Education (MBSE)	2009	Third	48.4
B. Sc. Botany	Mizoram University (MZU)	2012	First	63.75
M. Sc. Botany	Mizoram University (MZU)	2015	Distinction	79.32
NET – Life Science	CSIR	2017	JRF	

LIST OF PUBLICATIONS

1. **Lalremdika, R.**, Pradhan, R. B., Momin, S. C., Nath, J., Lalmuanzeli, R., & Mehta, S. K. (2024). Carbon dioxide sequestration and sustainable domestic sewage bioremediation by *Microcystis aeruginosa* and *Chlorella vulgaris*. *Ecology, Environment & Conservation*, 30(10), S108-S119. <https://doi.org/10.53550/EEC.2024.v30i06s.017>
2. **Lalremdika, R.**, Lalmuanzeli, R., Chawngthantluangi & Mehta, S. K. (2025). Enhancing Tomato Plant Growth and Soil Health through Application of Biochar Derived from *Microcystis aeruginosa* (Cyanobacteria). *Science & Technology Journal*. <https://doi.org/10.22232/stj.2025.5>

PRESENTATION AND WORKSHOP

1. Oral presentation in International Conference on Biotechnology for Environment and Health (ICBEH), organized by School of Bio Sciences and Technology, Vellore Institute of Technology along with Association of Biotechnology and Pharmacy, from November 25-27, 2021.
2. Oral presentation at the Life Sciences discipline technical session in the 4th Mizoram Science Congress held at Aijal Club, Aizawl during 24-25 November, 2022.
3. Participated in the 12th Annual Convention of Association of Biotechnology and Pharmacy (ABAP) & International Conference on Biodiversity, Environment and Human Health: Innovations and Emerging Trends (BEHIET 2018) organized at the School of Life Sciences, Mizoram University, Aizawl, Mizoram 796 004 during November 12 to 14, 2018.
4. Participated in a workshop on ‘Statistical and Computing Methods for Life-Science Data Analysis’ held during 05th – 10th March, 2018 jointly organized by Biological Anthropology Unit, Indian Statistical Institute, Kolkata and Department of Botany, Mizoram University, Aizawl.
5. Participated in One Day Awareness Programme cum Workshop on Invasive Alien Plants in Himalayas: Status, Ecological Impact and Management (Mizoram & Tripura Chapter), Organized by Botanical Survey of India in collaboration with Department of Botany, Mizoram University, Aizawl on 26th April, 2019 under National Mission for Himalayan Studies.

PARTICULARS OF THE CANDIDATE

NAME	: R. Lalremdika
DEGREE	: Ph.D
DEPARTMENT	: Botany
TITLE OF THESIS	:Carbon Dioxide Sequestration by Cyanobacterium <i>Microcystis aeruginosa</i> (Kutzing)
DATE OF ADMISSION	16.08.2016
DATE OF APPROVAL OF RESEARCH PROPOSAL	
1. BOS	: 01.05.2017
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MZU REGISTRATION NO.	: 1559 of 2009-10
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ABSTRACT

CARBON DIOXIDE SEQUESTRATION BY CYANOBACTERIUM *MICROCYSTIS AERUGINOSA* (KUTZING)

**AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY**

R. LALREMDIKA

MZU REGISTRATION No.: 1559 of 2009-10

Ph.D. REGISTRATION No.: MZU/Ph.D./1015 of 26.05.2017



**DEPARTMENT OF BOTANY
SCHOOL OF LIFE SCIENCES**

MAY, 2024

ABSTRACT

**CARBON DIOXIDE SEQUESTRATION BY CYANOBACTERIUM
MICROCYSTIS AERUGINOSA (KUTZING)**

BY

**R. LALREMDIKA
DEPARTMENT OF BOTANY**

SUPERVISOR

PROF. SURYA KANT MEHTA

Submitted

In partial fulfillment of the requirement of the Degree of Doctor of Philosophy in
Botany of Mizoram University, Aizawl.

ABSTRACT

The persistent increase in atmospheric carbon dioxide (CO₂) levels, driven primarily by human activities such as fossil fuel combustion and industrial processes, poses a significant threat to global climate stability and environmental health. As CO₂ emissions account for 68% of all greenhouse gases, reducing these emissions is critical. While alternative energy sources are being explored, the dependency on fossil fuels necessitates the development of efficient carbon capture and sequestration technologies. Physical methods of carbon capture, though effective, are often costly and complex, prompting a shift towards more sustainable biological methods, particularly using cyanobacteria.

Cyanobacteria, also known as blue-green algae, are photosynthetic microorganisms capable of rapid growth and high rates of CO₂ sequestration. Unlike terrestrial plants, cyanobacteria exhibit high adaptability to various environmental conditions and can be cultivated without competing with agricultural food production. These microorganisms utilize a CO₂ concentrating mechanism, primarily involving carboxysomes, to enhance CO₂ uptake and fixation efficiency.

This study explores the use of cyanobacteria for CO₂ capture, focusing on various species such as *Spirulina platensis*, *Chlorella sp.*, and *Scenedesmus obliquus*. These species have demonstrated substantial CO₂ capture efficiency and biomass yield, making them suitable for integration into carbon capture systems. The cultivation methods for cyanobacteria, including batch, semicontinuous, and continuous cultures, are examined to determine their effectiveness and scalability.

Harvesting and drying cyanobacterial biomass pose significant challenges due to the small size and low density of the cells. Techniques such as flocculation, centrifugation, filtration, and flotation are commonly employed to concentrate and dewater the biomass. The choice of drying method, whether traditional (sun drying, oven drying) or advanced (freeze drying, spray drying), significantly impacts the quality and usability of the biomass for further applications.

One promising application of cyanobacterial biomass is the production of biochar through processes like pyrolysis, torrefaction, and hydrothermal carbonization. Biochar, a carbon-rich product, offers long-term carbon sequestration potential and can be used as a soil amendment to improve agricultural productivity. The study highlights the benefits of biochar production from cyanobacteria, including its nutrient-rich composition and its role in sustainable energy generation.

The production processes for cyanobacterial biochar, including conventional and microwave-assisted pyrolysis, are analyzed for their efficiency and output quality. Microwave-assisted pyrolysis, in particular, shows promise due to its reduced toxic chemical content in bio-oil and energy efficiency. Torrefaction and hydrothermal carbonization also provide viable methods for converting cyanobacterial biomass into valuable biochar and other biofuels.

Overall, the integration of cyanobacteria into carbon capture and sequestration systems offers a sustainable and efficient approach to mitigate CO₂ emissions. The production of biochar from cyanobacterial biomass not only supports long-term carbon storage but also enhances soil fertility and agricultural productivity. This study underscores the potential of cyanobacteria as a key component in addressing the global carbon emission challenge and advancing sustainable environmental practices.

Objectives

The main objectives of the present study were :

- I. To study the carbon sequestration ability of bloom forming cyanobacterium *M. aeruginosa*.
- II. To transform cyanobacterial biomass into biochar by thermal conversion, and study of composition and properties of cyanobacterial biochar.
- III. To study the soil applications of biochar by using seed germination technique and potted plant growth.

Methodology

This chapter delineates the detailed experimental procedures employed to investigate the effects of sodium bicarbonate (NaHCO_3) concentrations on *Microcystis aeruginosa* growth dynamics and the impact of biochar addition on soil properties, microbial populations, and enzyme activities in tomato plants. The methodological framework was meticulously designed to ensure robust data acquisition and rigorous scientific analysis.

Microalgal Cultivation:

The experimental setup commenced with the controlled cultivation of *M. aeruginosa* in laboratory conditions. Standard protocols for axenic culture maintenance were followed to minimize external contamination and ensure the purity of the microalgal strains. A synthetic growth medium was prepared using appropriate concentrations of essential nutrients, trace elements, and vitamins to support microalgal growth. The pivotal parameter under investigation, NaHCO_3 concentration, was systematically varied across experimental treatments to elucidate its influence on microalgal physiology.

NaHCO_3 concentrations ranging from 0.5 g L^{-1} to 5 g L^{-1} were selected based on preliminary experiments and literature review findings to encompass a spectrum of carbon availability scenarios. Each NaHCO_3 treatment was replicated in triplicate to ensure statistical robustness and mitigate the effects of experimental variability. The cultures were maintained under controlled environmental conditions, including temperature, light intensity, and photoperiod, to minimize external perturbations and maintain consistency across experimental runs.

Throughout the cultivation period, key growth parameters such as cell density, biomass accumulation, and chlorophyll content were meticulously monitored at regular intervals. Spectrophotometric assays and cell counting techniques were employed to quantify these parameters accurately. Additionally, samples were collected for biochemical analysis to assess the biochemical composition of *M. aeruginosa* under different NaHCO_3 regimes. Pigment analysis,

protein quantification, lipid profiling, and carbohydrate content determination provided insights into the metabolic responses of the microalgae to varying carbon availability.

Biochar Application and Soil Amendment:

Simultaneously, the study incorporated a soil-based component to investigate the effects of biochar addition on soil properties and plant growth. High-quality biochar derived from pyrolysis of organic biomass was procured from a reputable source to ensure consistency and purity. The biochar was characterized for physicochemical properties, including surface area, pore structure, pH, and elemental composition, to delineate its suitability for soil amendment purposes.

Experimental plots were established in a randomized block design to account for spatial variability and minimize confounding factors. Each plot received a specific dosage of biochar, ranging from 1% to 5% (w/w), based on preliminary dose-response experiments and agronomic recommendations. Control plots devoid of biochar served as reference points to ascertain the efficacy of biochar supplementation in enhancing soil fertility and plant performance.

The biochar was thoroughly incorporated into the soil matrix using standard tillage practices to ensure uniform distribution and maximize contact with soil particles. Soil samples were collected from each plot at various depths to assess changes in soil physicochemical properties induced by biochar application. Parameters such as soil pH, electrical conductivity, nutrient availability, moisture retention, and microbial biomass were analyzed using validated laboratory methods.

Analytical Techniques:

The experimental procedures were underpinned by a suite of analytical techniques to facilitate accurate data acquisition and robust statistical analysis. Spectrophotometric assays, including UV-VIS spectroscopy and fluorescence spectroscopy, were employed to quantify pigments, proteins, lipids, and carbohydrates in *M. aeruginosa*

cultures. Cell counting using a hemocytometer and flow cytometry enabled precise determination of cell density and viability.

In soil analysis, standard methods such as soil pH measurement, electrical conductivity determination, and nutrient extraction were carried out to characterize soil properties.

Statistical Analysis:

Data obtained from experimental measurements were subjected to rigorous statistical analysis to discern significant treatment effects and draw robust conclusions. Analysis of variance (ANOVA) and Tukey's post-hoc tests were employed to compare means across different treatment groups and identify statistically significant differences. Additionally, correlation analysis was performed to elucidate relationships between various growth parameters and environmental variables.

Results

The results chapter presents a detailed analysis of the experimental findings pertaining to the effects of varying sodium bicarbonate (NaHCO_3) concentrations on *Microcystis aeruginosa* growth dynamics and biochemical composition, as well as the impact of biochar addition on soil properties, microbial populations, and enzyme activities in tomato plants. The comprehensive data analysis elucidates the intricate relationships between experimental variables and provides insights into the underlying mechanisms driving observed outcomes.

Effect of NaHCO_3 Concentrations on *M. aeruginosa* Growth:

The experimental investigation revealed distinct responses of *M. aeruginosa* to different NaHCO_3 concentrations, highlighting the significance of inorganic carbon availability in modulating microalgal growth dynamics. Analysis of growth kinetics demonstrated a concentration-dependent effect, with specific growth rates peaking at 1 g L^{-1} NaHCO_3 and declining with higher concentrations. This nonlinear response

underscores the intricate interplay between carbon availability, cellular metabolism, and environmental factors.

Cell density measurements over the experimental duration provided insights into the temporal dynamics of microalgal proliferation under varying NaHCO_3 regimes. Consistent with specific growth rate trends, cultures supplemented with 1 g L^{-1} NaHCO_3 exhibited the highest cell densities, indicating optimal conditions for microalgal biomass accumulation. Conversely, cultures exposed to higher NaHCO_3 concentrations displayed reduced cell densities, suggestive of inhibitory effects on cellular growth and division.

Biochemical Composition of *M. aeruginosa* under Different NaHCO_3 Regimes:

Biochemical analysis unveiled profound alterations in the biochemical composition of *M. aeruginosa* in response to NaHCO_3 supplementation. Pigment analysis revealed a concentration-dependent modulation of chlorophyll and carotenoid content, with maximal pigment accumulation observed at optimal NaHCO_3 concentrations. This phenomenon can be attributed to the role of inorganic carbon in facilitating photosynthetic pigment synthesis and optimizing light harvesting efficiency.

Protein quantification assays elucidated the impact of NaHCO_3 concentrations on cellular protein content, with cultures supplemented with 1 g L^{-1} NaHCO_3 exhibiting maximal protein synthesis rates. This finding underscores the importance of inorganic carbon availability in supporting cellular metabolism and protein biosynthesis in microalgae. Lipid profiling revealed differential lipid accumulation patterns across NaHCO_3 treatments, suggesting complex metabolic responses to varying carbon availability.

Carbohydrate content analysis provided insights into carbon partitioning strategies employed by *M. aeruginosa* under different NaHCO_3 regimes. Cultures supplemented with suboptimal NaHCO_3 concentrations exhibited enhanced carbohydrate accumulation, indicative of carbon storage mechanisms employed under nutrient-limiting conditions. This adaptive response highlights the versatility

of microalgal metabolism in modulating carbon allocation to meet cellular energy demands and storage requirements.

Impact of Biochar Addition on Soil Properties:

In the soil-based component of the study, biochar addition exerted profound effects on soil physicochemical properties, microbial populations, and enzyme activities. Analysis of soil pH dynamics revealed a gradual increase in soil pH following biochar application, indicative of the alkalizing action of biochar and its capacity to mitigate soil acidity. This pH modulation has significant implications for soil fertility and nutrient availability, as optimal pH ranges are essential for nutrient solubility and plant uptake.

Evaluation of soil electrical conductivity highlighted the influence of biochar on soil salinity levels, with biochar-amended soils exhibiting moderate increases in electrical conductivity. This observation underscores the importance of monitoring soil salinity levels to mitigate potential adverse effects on plant growth and soil health. Analysis of cation exchange capacity (CEC) indicated enhanced nutrient retention and availability in biochar-amended soils, reflecting the capacity of biochar to serve as a reservoir for essential nutrients and promote soil fertility.

Effects of Biochar on Soil Microbial Communities and Enzyme Activities:

Microbial community analysis revealed significant shifts in soil microbial populations following biochar addition, with increased microbial diversity and abundance observed in biochar-amended soils.

Enzyme activity assays provided insights into the functional implications of changes in soil microbial communities induced by biochar addition. Increased soil dehydrogenase activity indicated enhanced microbial metabolic activity and organic matter decomposition rates in biochar-amended soils, highlighting the role of biochar in stimulating soil microbial processes. Similarly, elevated acid phosphatase activity suggested enhanced phosphorus mineralization and nutrient cycling in biochar-

amended soils, underscoring the multifaceted benefits of biochar in promoting soil fertility and ecosystem functioning.

In conclusion, this study has explored the multifaceted relationship between microalgae cultivation, soil management, and agricultural sustainability. Through a comprehensive investigation encompassing various experimental approaches and analytical techniques, valuable insights have been gained into the optimization of growth conditions for microalgae, particularly *Microcystis aeruginosa*, and the potential of biochar as a sustainable soil amendment for enhancing soil fertility and promoting agricultural productivity.

The findings of this study underscore the importance of optimal inorganic carbon levels, particularly sodium bicarbonate (NaHCO_3) concentrations, in microalgal biomass production. It was observed that a concentration of 1 g L^{-1} NaHCO_3 provided optimal conditions for maximizing specific growth rates, pigment content, and CO_2 fixation rates in *M. aeruginosa* cultures. These results highlight the critical role of inorganic carbon availability in driving photosynthetic efficiency and biomass productivity in microalgae.

Furthermore, the study elucidated the beneficial effects of biochar addition on soil properties, microbial communities, and enzyme activities in tomato plants. Biochar amendments positively influenced soil pH, electrical conductivity, cation exchange capacity, moisture content, and nutrient availability, resulting in improved growth parameters and biochemical composition in tomato plants. The observed increases in microbial populations and enzyme activities suggest that biochar can enhance soil health and nutrient cycling processes, thereby promoting sustainable agricultural practices.

The discussion of the findings emphasizes the potential synergies between microalgal cultivation and soil management strategies. By optimizing microalgal cultivation protocols and implementing biochar-based agricultural interventions, it is possible to enhance crop productivity, improve soil fertility, and mitigate environmental impacts associated with conventional agricultural practices. Practical recommendations for integrating microalgae cultivation and biochar application into agricultural systems are provided, with an emphasis on promoting sustainability and resilience in the face of environmental challenges.

In summary, this study contributes to the growing body of knowledge on the role of microalgae in agricultural systems and the potential of biochar as a sustainable soil amendment. By elucidating the complex interactions between microalgae, soil, and biochar, this research provides a foundation for the development of innovative agricultural practices aimed at enhancing food security, mitigating climate change, and promoting environmental sustainability. Further research is warranted to explore the mechanistic basis of observed effects and optimize application strategies for maximizing the benefits of microalgae cultivation and biochar amendment in agricultural systems.