

**CARBON SEQUESTRATION USING MICRO ALGAE AND
ESTIMATION OF BIOMOLECULES IN MICROALGAE**

PROJECT WORK

Submitted in partial fulfilment of the requirements for the award of Bachelor of
Science degree in B.Sc. Biochemistry

By

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**SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY**

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(DEEMED TO BE UNIVERSITY)**

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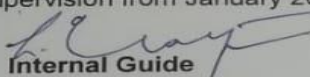
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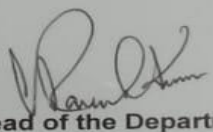
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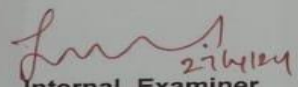
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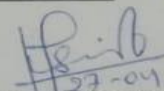

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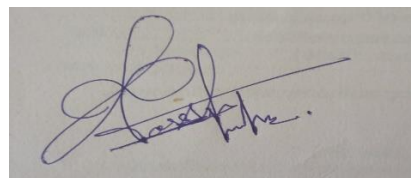

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ABSTRACT

Carbon dioxide sequestration by microalgae is receiving increased attention in alternative impact of increasing CO₂ in the atmosphere. For this study, we have chosen two different microalgae such as *Picochlorum* and *Chlorella* were cultured on with Conway medium. Parameters such as Total Chlorophyll, protein, Carbohydrate, and CO₂ fixation rate were determined. Based on the parameters *Picochlorum* and *Chlorella* is used for carbon sequestration study. Different concentrations of sodium bicarbonate (0.2%-1%) were supplemented to Conway medium for carbon utilization it can act as an alternative source of atmospheric CO₂. In this maximum amount of chlorophyll content were observed in 0.8% of Sodium bicarbonate. The uttermost amount of CO₂ fixation rate and lipid content was recorded at 0.4%, 0.6%, and 0.8% sodium bicarbonate concentration. Finally, the result indicated that *Picochlorum* could be used for CO₂ mitigation and having a rich source of polyunsaturated fatty acid.

Key words: Carbon sequestration, *picochlorum*, *chlorella*, carbondioxide, sodium carbonate

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LIST OF ABBREVIATIONS

KNO_3	Potassium nitrate
$\text{NaH}_2\text{PO}_2 \cdot \text{H}_2\text{O}$	Sodium hypophosphite
EDTA	Ethylenediaminetetraacetic acid
H_3BO_3	Boric acid
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	Manganese (II) Chloride
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	Ferric chloride hexahydrate.
ZnCl_2	Zinc Chloride
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	<i>Cobalt chloride hexahydrate</i>
$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	Ammonium heptamolybdate tetrahydrate
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	Copper sulphate pentahydrate
Conc. HCl	Hydro chloric acid
Vitamin B12	Cobalamin
Vitamin B1	Thiamine

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CARBON SEQUESTRATION USING MICROALGAE AND ESTIMATION OF BIOMOLECULES IN MICROALGAE

CHAPTER 1

INTRODUCTION

1.1 CARBON SEQUESTRATION

Globalization and industrialization had led to increased production of atmospheric carbon dioxide, an important greenhouse gas. As of right now, human activity alone has caused about 400 parts per million of carbon dioxide release into the atmosphere. Particularly, the shifting climate changing has given rise to a new aspect of deteriorating the environment worldwide and endangering all life, including human life. The largest issue the world facing now is global warming which is due to increased level of carbon dioxide emission as a result of human activities. Studies are being carried out to combat the effect of CO₂ released by absorbing or assimilating the CO₂ that is being released into atmosphere.

The process of removing CO₂ from the atmosphere is called carbon sequestration. A number of CO₂ collection and storage solutions are being considered as part of the global effort to prevent climate change. Variations in solar UV-B radiation have an impact on terrestrial ecosystems. Specifically, they have a direct effect on carbon fixation through effects on plants, and indirectly through effects on micro algae in aquatic systems, which are the major producers. Carbon oxide has been released in excess from industries that exacerbates the global problem of industrial gas exploitation adorns a micro algal cultivation technology. According to (Costa *et al.*, 2000) and (Langley *et al.* 2012), cyanobacteria and microalgae develop quickly and fix CO₂ at a rate that is 10 to 50 times higher. Microalgae can develop as little as 10% (v/v) of carbon dioxide.

To enhance global energy production with minimal carbon emissions, extensive research has been conducted to identify alternative energy sources and sustainable feedstocks. Geothermal energy, a resource abundant across the globe, offers various utilization methods. As of 2010, geothermal energy was directly utilized in 78 countries, contributing approximately 50 GW_{thermal} and 11 GW_{electric} to the global installed capacity of geothermal base-load power (Goldstein *et al.*, 2011).

Despite its potential, geothermal power plants pose environmental concerns due to the release of non-condensable gases (NCGs), primarily carbon dioxide (CO₂), into the atmosphere. This emission rate varies significantly based on the geographical location and the chemical composition of the ground, with Icelandic and U.S. plants releasing up to 100 g CO₂ per kWh. This emission rate significantly impacts the sustainability of geothermal energy as a future energy source. Currently, geothermal energy contributes a negligible fraction of the world's energy output, with only a few countries like Iceland, the Philippines, and New Zealand generating a significant portion of their electricity from geothermal resources.

As geothermal energy emerges as a sustainable technology, the global CO₂ release from geothermal power plants is expected to increase with further development. To mitigate CO₂ emissions, several strategies have been explored, including carbon capture and storage (CCS) and bio-mitigation of CO₂. CCS is a viable but costly technology for removing CO₂ from power plant flue gases (Chen *et al.*, 2012; Markewitz *et al.*, 2012). Bio-mitigation, which utilizes biomass from fixed CO₂, offers additional benefits such as food additives and cosmetics but faces challenges due to low efficiencies and CO₂ capture capacities (Chen *et al.*, 2012; Fu *et al.*, 2016; Kassim and Meng, 2017).

Further biotechnological advancements are necessary to make bio-mitigation a viable alternative for offsetting CO₂ emissions from geothermal power plants. Algae, particularly the green microalga *Chlorella vulgaris*, present an attractive biomass source due to their ability to grow on marginal lands without competing with food crops. They offer higher yields per unit area compared to land crops and are used in various applications, including food and feed production, wastewater treatment, and CO₂ sequestration [Fu *et al.*, 2017; Raheem *et al.*, 2018; Clarens *et al.*, 2010; Trentacoste *et al.*, 2013; Heredia-Arroyo *et al.*, 2011; Olguín, 2012; Zuniga *et al.*, 2016; Cordero *et al.*, 2011; Liang *et al.*, 2009). To enhance algal biomass production and reduce processing costs, indoor photobioreactor (PBR) systems have been developed for high-density culture (Choi *et al.*, 2013; Fu *et al.*, 2012).

LED-based PBRs facilitate algae cultivation and CO₂ capture by using geothermally produced electricity for photosynthesis and waste CO₂ as a carbon source for algal biomass and high-value products. However, light supply and delivery challenges limit the scalability of PBRs for photoautotrophic cultivation, necessitating the exploration of mixotrophic culture modes to improve biomass productivity and

reduce operating costs (Wichuk *et al.*, 2014; Liang *et al.*, 2009; Juntala *et al.*, 2015). The scalability of PBRs was assessed based on biomass productivity and yield on light energy, focusing on the mixotrophic growth of *C. vulgaris* with minimal sugar levels to stimulate light-driven photosynthetic growth. The addition of sugars was found to enhance biomass generation and stimulate photoautotrophic growth, leading to increased photosynthetically derived biomass generation and CO₂ sequestration.

A mixotrophic culture of *C. vulgaris* in LED-based PBRs was developed to improve the economic feasibility of PBR-based algal production systems, with a techno-economic analysis providing an in-depth understanding of the PBR system for algae cultivation and CO₂ sequestration. The escalating threat of global warming, exacerbated by the rising concentration of CO₂ in the atmosphere, necessitates urgent action. In 2011, the annual increase in atmospheric CO₂ was 1.94 ppm, a figure that is more than double the estimated value from 1959. Currently, CO₂ accounts for nearly 52% of total global warming [Velea *et al.*, 2009]. Biological CO₂ sequestration from flue gas, particularly through algae, is gaining traction due to its eco-friendly and cost-effective nature (Kumar *et al.*, 2013, 2011).

1.2 MICROALGAE

Microalgae, such as *Chlorella vulgaris*, exhibit photosynthetic efficiency nearly ten times higher than terrestrial plants, making them an attractive option for CO₂ sequestration (Skjanes *et al.*, 2007). These algae not only serve as a renewable energy source but also offer the potential for producing high-value products from their biomass. Algae can be cultivated in both open ponds and closed photobioreactors, with the latter offering advantages such as high productivity, ease of operation, better control over physiochemical parameters, and sterile operation. Airlift and bubble column photobioreactors are particularly favored for their simplicity in gas-liquid contacting applications (Kastanek *et al.*, 2010).

The airlift bioreactor is of particular interest due to its high mass transfer, regular light and dark cycle, and low and homogeneous shear stress to the cells [Kumar and Das, 2012; Vunjak-Novakovic *et al.*, 2005]. However, the application of biological CO₂ sequestration is currently limited to laboratory scales, primarily due to the challenges associated with using air-CO₂ gas mixtures. Recently, on-off

pulsing of flue gas has been adopted to mitigate the inhibitory effects of flue gas on algal cells, with a frequency of 10 seconds on-time and 5-9 minutes off-time reported as effective (He *et al.*, 2012; Jiang *et al.*, 2013; Chiu *et al.*, 2011). This strategy, however, requires continuous stirring of the culture for mixing and sophisticated instruments to control the frequency of flue gas injection.

Chlorella sp., known for its ability to survive in harsh and high oxidizing environmental conditions, is a promising candidate for CO₂ sequestration. It is not only a good source of carbohydrates, lipids, proteins, and vitamins but also produces metabolites and pigments with significant commercial importance, such as carotenoids, milbemycins and pregnenolone, which have applications in human consumption, aquaculture, and pharmaceuticals [Mahasneh, 1997; Hamasaki *et al.*, 1998; Siefermann-Harms, 1985; Prichard, 2005; Khisti *et al.*, 2003; Beyhan *et al.*, 2011; Agoramoorthy *et al.*, 2007].

The escalating levels of atmospheric CO₂ are recognized as a primary driver of global warming. The United Nations Framework Convention on Climate Change has emphasized the need for developed nations to reduce CO₂ emissions, with discussions on establishing carbon taxes or emission trading among its member nations. Industries are a significant source of CO₂ emissions, with the energy sector accounting for about 75% of total emissions. Several technologies aimed at CO₂ reduction are in development. Carbon Capture and Storage (CCS) involves capturing CO₂ from industrial exhaust gases, transporting it to a storage site, and depositing it underground. While the key technologies for CCS are available, cost reduction, improved CO₂ monitoring, and a detailed evaluation of the environmental impact of deposited CO₂ are crucial for its practical application. Carbon Capture and Utilization (CCU) is another strategy, which involves capturing CO₂ and converting it into commercially valuable products. However, the chemical conversion of CO₂ to valuable products in CCU lacks commercial viability due to high production costs and the need for highly selective catalysts.

Biological conversion of CO₂ to value-added products by microalgae presents an attractive alternative due to its low production cost, ability to directly fix CO₂ from exhaust gas without requiring catalysts, and the commercialization of microalgal mass cultures for aquaculture feed and nutraceuticals. However, these cultures are typically grown in atmospheric air containing only 0.04% CO₂, as excess CO₂ can lead to acidification and inhibit algal growth. Flue gas from coal-fired power

plants, a major CO₂ source, contains 12%-15% CO₂, making microalgae capable of growing in high CO₂ levels suitable for bioconversion of flue gas to valuable products. Research has identified cyanobacteria and eukaryotic microalgae that can tolerate high CO₂ concentrations (up to 10%), with some strains surviving even in 100% CO₂. These findings suggest a vestigial characteristic of ancestral photosynthetic microbes that lived on Early Earth. However, the optimal CO₂ concentration for their growth is generally lower than their maximum tolerance, making it important to evaluate the feasibility of culturing algal strains in high CO₂ concentrations. High-level-CO₂-preferring microalgae (HCP-microalgae) that show fast growth in high CO₂ concentrations (around 20% CO₂) are of particular interest for both paleontological and biotechnological potential. The focus was on isolating and characterizing HCP-microalgae that exhibit fast growth in 20% CO₂ concentration, with a particular emphasis on marine microalgae due to the ocean's vast habitat and the relatively underexplored ecology of CO₂-tolerant algae compared to freshwater ones.

1.3 APPLICATION OF MICROALGAE

Microalgae used skin care product and more in biological field in current day's scientist are firmly obscure on biotechnology route to decrease the CO₂ emissions in the air in to ground and ocean. When compare to all accessible alternatives like switching to renewable energy sources, enhancing energy efficiency, curbing emissions from agriculture, forestry and deploying. Carbon Sequestration and Storage (CSS) paradigm will solve the energy and climate challenge. In present study declare that growth of algae is ne natively influenced by increasing CO₂ (Ferrell and Sarisky-Reed, 2010). And these micro algae have been used to clear outgas from power-plant (Zeiler *et al.*, 1995). In this context, present chapter discusses about the scope of reduction in carbon dioxide emission using microalgae in indoor condition with sodium bicarbonate as a sole carbon source. in the presence lipid content (PUFA).

CHAPTER 2

LITERATURE SURVEY

In this sense, microalgae are garnering interest globally due to their remarkable rate of growth, ability to withstand extreme conditions, and affordability of production. The biochemical underpinnings of microalgae-CO₂ fixation and the development of the strains of microalgae utilized in CO₂ fixation research are examined and described in this article. A study of the advancements in techniques to enhance microalgae-CO₂ fixation is included, as well as the most recent developments in the co-development of a microalgae carbon sequestration business and other industries. Last but not least, the issues facing the sector and its projected growth paths (Zhang, *et al.*, 2021).

A thorough grasp of the cutting-edge materials and techniques that could enable carbon sequestration and storage in concrete was the goal of the study. Since there were no comparable review studies, the investigation's main focus was on cementitious materials' capability to absorb carbon, rather than how carbon curing affected the strength and durability of the concrete. In order to do this, the pertinent carbonation reactions were examined, and then the factors that have the most effects on the carbonation reactions' efficiency were assessed. After analyzing how different binder compositions affected cementitious materials' ability to sequester carbon, the possibility of substituting aggregates was investigated in order to optimize the concrete's ability to do so. The following are the study's primary conclusions (Kazemian *et al.*, 2023).

The carbon sequestration using microalgae and biochemical analysis reveals that microalgae have the potential to capture and recycle carbondioxide (CO₂) emissions from various sources, such as power plants and I, through photosynthesis. This technology offers a promising way to reduce greenhouse gas emissions and combat climate change. Microalgae can convert CO₂ into biomass, which can be further processed into biofuels, nutraceuticals, animal feed, and other valuable products (Li, *et al.*, (2023).

Different sources of CO₂ emissions and capturing technology including microalgae. Details about the methods of cultivating and separating the algal biomass followed by thermochemical and biochemical conversion to fuels/chemicals, microalgae-derived bioplastics, and microalgae biomass blended plastic are outlined in this review. Commercially viable continuous and semicontinuous biorefining processes such as hydrothermal flash hydrolysis and environmental benefits of using microalgae as carbon sinks and wastewater treatment are also provided (Balan, *et al.*, 2023).

Microalgae are a diverse group of photosynthetic organisms that can be exploited for the production of different compounds, ranging from crude biomass and biofuels to high value-added biochemicals and synthetic proteins algae can be improved in terms of photosynthetic efficiency, biofuel and high value-added compound production. Finally, we critically evaluate developments over recent years and explore future potential in the field (Kselíková, *et al.*, (2022)

These devices present a viable and encouraging alternative to the carbon mitigation techniques now in use. The main component of microalgae is a wide variety of swiftly growing microorganism groups that are able to flourish in photoautotrophic, heterotrophic, and mixotrophic environments. Because these microalgae can fix carbon dioxide at a rate 10-50 times higher per unit than terrestrial plants, they can be grown in barren environments. Furthermore, the numerous advantages of employing microalgae for carbon dioxide fixation are demonstrated by the production of food, feed, fine chemicals, and biofuels from this biomass of microalgae. A few important details regarding the many forms of carbons that microalgae incorporate, together with a precise description of recent advancements in the deft application of microalgae for According to Sarwer *et al.*

(2020), using microalgal (Banerjee *et al.*, 2020).

Investigating the storage of carbon dioxide by microalgae. Three microalgae species—*Chlorococcum humicola*, *Chlorella vulgaris*, and *Scenedesmus quadricauda*—are subjected to UV-B radiation as part of this investigation. The study investigates how large-scale, year-round cultivation of microalgal strains with high

growth and CO₂ fixation rates can produce both long-chain unsaturated fatty acids and saturated fatty acids. The possibility of substituting expensive feed materials like fishmeal with non-traditional feed sources like algae. In the current investigation, three distinct microalgal cultures were employed, and it was found that *Scenedesmus quadricauda* produced more PUFA content when exposed to UV-B light with sodium bicarbonate serving as the only inorganic carbon source (Maryshamya *et al.*, 2019).

Microalgae have the ability to lower CO₂ emissions from flue gas and can be used in industrial settings as a photosynthetic cell factory. To create a successful CO₂ fixation system, microalgae strains with high growth rates and the ability to adapt to severe conditions and concentrated carbon sources are essential. The foundation for strategic methods to enhance microalgae strains for industrial applications is laid by this review (Watanabe *et al.*, 2016).

Biological CO₂ sequestration using algal reactors is one of the most promising and environmentally benign technologies to sequester CO₂. This research alleviate certain limitations associated with the technology such as low CO₂ sequestration efficiency and low biomass yields. The demonstrate an increase in CO₂ sequestration efficiency by maneuvering chemically aided biological sequestration of CO₂. *Chlorella* sp. and *Spirulina platensis* showed 46% and 39% mean fixation efficiency, respectively, at input CO₂ concentration of 10%. When the input CO₂ concentration was 10%, the mean fixation efficiency for *Chlorella* sp. and *Spirulina platensis* was 46% and 39%, respectively (Ramanan *et al.*, 2010).

The purpose of this study was to determine whether *Chlorella sorokiniana* might be used to sequester CO₂ from industrial flue gas. Various strategies, including diluted flue gas, flue gas after passing through a scrubber, flue gas traveling through serially connected photobioreactors, and two separate reactors, were employed in an attempt to maximize the production of algal biomass. The inlet flue gas's CO₂ content decreased by the largest amount, 4.1% (v/v). The total lipid's fatty acid content was established in order to assess its viability for use as food, feed, and biofuel (Kumar *et al.*, 2014)

Shown how to cultivate a *Chlorella* colony utilizing low doses of carbohydrates at stimulating levels to encourage CO₂ sequestration and photosynthetic efficiency. *Chlorella vulgaris*, a fast-growing species of green microalgae, was cultivated in photobioreactors (PBRs) of various sizes, and a method for boosting CO₂ sequestration under light-emitting diode illumination was created utilizing tiny dosages of sugars. Low levels of glucose supplementation increased biomass production and CO₂ capture by 10% as a result of photoautotrophic growth, and this rise was correlated with an increase in provided photon flux. Additionally, a techno-economic model was created, which shows that LED-based PBRs are a workable method of turning CO₂ into algal biomass with additional value (Fu *et al.*, 2019)

Genetic engineering, adaptive laboratory evolution, and random mutagenesis techniques were assessed in order to choose microalgae with high biomass, high growth rate, robust tolerance, and high CO₂ fixation efficiency. Additionally, the viability of integrating wastewater and flue gas treatment with the use of highvalue-added products was examined in order to better achieve the industrialization of microalgae CO₂ fixation technology. The use of microalgae CO₂ fixation technology in the aforementioned aspects is anticipated to produce a more optimal mechanism of microalgae carbon sequestration in the future, given the current hurdles facing the technology. Simultaneously, it offers a stable and advantageous framework for the complete implementation of sustainable development, the gradual advancement of the carbon peak and carbon neutrality, and the realization of clean, green, low-carbon, and efficient (Tarafdar, *et al.*, 2023)

The uptake of carbon dioxide by microalgae can be enhanced through the application of several techniques such as modified photobioreactors, membranes, chemical approaches, solvents, genetically edited strains, omics, and nanotechnology. We also talk about the economic feasibility of producing bioenergy and capturing carbon dioxide using microalgae. Over 300 tons of biomass may be produced by microalgal farming on an average of 13 million acres, which can store about 0.5 gigatons of CO₂ (Tripathi, *et al.*, 2023).

Article provides a brief yet comprehensive review of the present carbon sequestration and utilization technologies, focusing primarily on biological CO₂ capture by microalgae in the context of bio-refinery. The paper discusses various products of microalgal biorefinery and aims to assess the opportunities, challenges and current

state-of-the-art of microalgae-based CO₂ bioconversion, which are essential to the sustainability of this approach in terms of the environment as well as the economy (Singh, *et al.*, 2019).

As a greenhouse gas (GHG), carbon dioxide is one of the main pollutants that warms the planet. The amount of carbon dioxide in the atmosphere has increased to 400 parts per million (ppm) in the last 150 years due to human activity. This is more than it has been in hundreds of thousands of years. A number of CO₂ collection and storage solutions are being considered as part of the global effort to prevent climate change. Microalgae with the capacity to photosynthesize, including *Chlorella vulgaris*, *Scenedesmus* sp., *Chroococcus* sp., and *Chlamydomonas* sp., have been actively employed in closed and open photobioreactors for the purpose of mitigating CO₂ emissions throughout the world. A broad range of organisms, including microalgae, macroalgae (seaweed), and cyanobacteria (previously known as "blue-green algae"), are referred to as algae as feed sources for bioenergy (Chakrabarti, *et al.*, (2018).

Biochemical analysis in microalgae is a crucial process for understanding the composition and characteristics of these organisms, which are widely used in various fields, including food, feed, pharmaceuticals, and bioenergy. The biochemical composition of microalgae varies widely depending on the species and growth conditions, and it includes proteins, carbohydrates, lipids, pigments, and other compounds. (Brown, *et al.*, 1992).

Growing interest has been shown in algae-based nutritional supplements, whether in the form of complete biomass (e.g., *Chlorella* and *Arthrospira*) or refined extracts that contain carotenoids and omega-3 fatty acids. Microalgae are a possible source of renewable nutrition. The biorefinery technique now poses a hurdle to the commercial generation of bioactive chemicals from microalgae. The biochemical makeup of microalgae, the difficulties of mass cultivation, and possible medicinal uses are the main topics of this paper. The benefits of both open and closed growth systems are examined, along with typical issues that arise with extensive growth systems. Barkia, *et al.*, (2019).

The fact that microalgae are among the most prolific biological systems for producing biomass and storing carbon is becoming increasingly acknowledged. Harvesting all of the biomass results in significantly greater efficiency than are achievable in

terrestrial biomass production systems. Micro-algae are a good choice for carbon capture since they can transfer bicarbonate into cells. In open ponds, carbon dioxide—or bicarbonate—capturing efficiency of up to 90% have been documented. As a result, microalgae may be used for CO₂ capture and sequestration. The size of the microalgal production facilities required to capture carbon-dioxide (CO₂) emissions from stationary point sources like power plants and cement kilns is likewise manageable. In this post, I go over potential methods for sequestering CO₂ with less negative environmental effects utilizing microalgae (Sayre, R. 2010).

The fact that microalgae are among the most prolific biological systems for producing biomass and storing carbon is becoming increasingly acknowledged. Harvesting all of the biomass results in significantly greater efficiency than are achievable in terrestrial biomass production systems. Micro-algae are a good choice for carbon capture since they can transfer bicarbonate into cells. In open ponds, carbon dioxide—or bicarbonate—capturing efficiency of up to 90% have been documented. As a result, microalgae may be used for CO₂ capture and sequestration. The size of the microalgal production facilities required to capture carbon-dioxide (CO₂) emissions from stationary point sources like power plants and cement kilns is likewise manageable. This article discusses the use of microalgae to sequester CO₂ with less negative environmental effects (Sayre, R. 2010).

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

To determine the carbon sequestration using microalgae and estimation of biomolecules in microalgae (*Picochlorum* and *Chlorella*).

3.2 OBJECTIVES

1. To collect and mass cultivate microalgae (*Picochlorum* and *Chlorella*).
2. To analyse the carbon sequestration of microalgae (*Picochlorum* and *Chlorella*).
3. To determine the biochemical content
4. To determine the carbon content and carbon fixation rate.

CHAPTER 4

4. MATERIALS AND METHODS

4.1 COLLECTION AND CULTIVATION OF MICROALGAE

Micro algae *Picochlorum* and *Chlorella* culture was gifted from Dr.G.Muralidaran from Bharathidasan University, Tiruchirappalli, Tamil Nadu 620024 was grown in Conway medium under laboratory condition in 12/12 light dark cycle at 27 ± 1 °C. The study was carried out for a period of 30 days and all the growth parameters were analyzed and recorded at every 3 days interval.

CONWAY MEDIUM

Nutrient Solution-A

Table 4.1.1 Conway medium composition

Chemical name	Chemical formula	Concentration
Pottasium nitrate	KNO ₃	100.0g
Sodium phosphate	NaH ₂ PO ₄ .	20.0g
Sodium salt	EDTA	45.0g
Boric acid	H ₃ BO ₃	33.6g
Manganese(II)chloride tetrahydrate	MnCl ₂	0.36g

Iron (III) chloride	FeCl ₃	1.3g
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This all the chemicals dissolved in distilled water make up to 1liter.

Trace metal (solution)

Zinc chloride	ZnCl ₂	2.19g
Cobalt(II) chloride	CoCl ₂ · 6H ₂ O	2.0g
Ammonium heptamolybdate	(NH ₄) ₆ Mo ₇ O ₂₄	0.9g
Copper(II) sulfate	CuSO ₄	2.0g
Concentrated HCL	H ₂ SO ₄	Few drops

Dissolved in distilled water make up to 1liter.

Vitamin solution

Vitamin B1	Thiamine HCL	200mg
Vitamin B12	Cyanobalsmin	10mg

These two vitamins add to distilled water make up to 100ml.

Then take 1liter sea water add 1 ml of nutrient solution, 0.5 ml of trace metal solution and 0.1 ml of vitamin solution. Then the sea water used for micro algae cultivation

4.2 SODIUM BICARBONATE AS CARBON SOURCE

Sodium bicarbonate was added to the algal culture medium (Conway medium) as the only source of carbon in concentrations ranging from 0.2, 0.4, 0.6, 0.8 and 1%. It can function as a substitute carbon source indoors customs. Each experiment in the study

used to 250 ml white bottle with 100 ml of Conway medium. Sample one is represent the control for 100ml medium (Conway medium), sample two represent the 100ml medium with 0.20% concentration of sodium bicarbonate, sample three represent the 100ml medium with 0.40% concentration of sodium bicarbonate, sample four represent the 100ml medium with 0.60% concentration of sodium bicarbonate, sample five represent the 100ml medium with 0.80% concentration of sodium bicarbonate, sample six represent the 100ml medium with 1.00% concentration of sodium bicarbonate. This variables occurs in *Picochlorum* and *Chlorella* this two species. (Sodium bicarbonate, and inoculum size). After the algae was carry out for up to 30 days. Then the carbohydrate and chlorophyll content was analyzed by 0th day, 4th day, 8th day, 12th day, 16th day, 20th day, 24th day, 28th and 30th day. This analysis carry out in three days intervals. And then chlorophyll content, carbohydrate, protein, and lipid was analysed by 0th day, 15th day, and 30th day. After that carbonate and bicarbonate fixation rate was analyzed by after 30th day.

4.3 DETERMINATION OF BIOCHEMICAL CONTENT

4.3.1 Extraction and Estimation of Pigment (*Lichtenthaler*)

Five ml of *Picochlorum* and *Chlorella* different varies cultures sample was taken and centrifuged at 5000 rpm for 10 minutes and the supernatant was discarded. The algal pellet was then added with 5mL of 80% acetone and macerated by sonication. Then it was covered with black paper and kept overnight at 4°C. The sample was then centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and the optical density was measured at 644.8 nm, 661.6 nm and 470 nm Milton Roy UV - Visible spectrophotometer.

Estimation of pigments

The chlorophyll pigment was quantified by using the following formula,

$$\begin{aligned} \text{Chlorophyll } a \text{ (mg/L)} &= 11.24 \times A_{661.6} - 2.404 \times A_{644.8} \\ \text{Chlorophyll } b \text{ (mg/L)} &= 20.13 \times A_{644.8} - 4.19 \times A_{661.6} \\ \text{Total carotenoids (mg/L)} &= 1000 \times A_{470} - 1.9 \times \text{Chl } a - 63.14 \times \text{Chl } b \end{aligned}$$

<i>Picochlorum</i>	<i>Chlorella</i>
Control (normal medium)	Control (normal medium)
0.20% NaHCO ₃	0.20% NaHCO ₃
0.40% NaHCO ₃	0.40% NaHCO ₃
0.60% NaHCO ₃	0.60% NaHCO ₃
0.80% NaHCO ₃	0.80% NaHCO ₃
1.00% NaHCO ₃	1.00% NaHCO ₃

4.3.2 Extraction and estimation of carbohydrate (Dubois et al., 1956)

Reagents

Phosphate buffer 0.1M (pH 6.8)

Sodium phosphate buffer of 0.1M, pH 6.8 was prepared by mixing 49ml of 0.2N Na₂HPO₄ and 51 ml of 0.2N NaH₂PO₄ and made up to 100 ml with glass distilled water.

5% Phenol

Five gram Phenol crystals was dissolved in 100 ml of glass distilled water and refrigerated

Extraction of carbohydrate

Five ml of *Picochlorum* and *Chlorella* algal cultures was taken and centrifuged at 5000 rpm for 15 minutes. The algal pellet was then sonicated using Phosphate buffer then centrifuged at 5000 rpm for 10 minutes. The supernatant was taken for the estimation of total protein.

Estimation of carbohydrate;

To the 0.2 ml of sample, 1ml of 5% phenol and 5ml of H₂SO₄, was added mixed thoroughly. The solution was allowed to stand at room temperature for 30 minutes. The optical density was read at 490nm. Standard graph was prepared with different concentrations of D-glucose ranging from 10 to 100µg/ml.

4.3.3 Extraction and Estimation of Total Protein (Bradford, 1976)

Reagents:

0.1 M Phosphate buffer (pH 7.0)

Sixty one ml of 0.2 M Na_2HPO_4 was added to 39 ml of 0.2M NaH_2PO_4 and diluted to 200 ml with glass distilled water.

Coomassie Brilliant Blue reagent

One hundred mg of CBBG - 250 was dissolved in 50ml of 93% ethanol. To this 100 ml of 85% Phosphoric acid was added and diluted to 1000 ml with glass distilled water.

Extraction of Protein

Five ml of *Picochlorum* and *Chlorella* algal culture samples was taken and centrifuged at 5000 rpm for 10 minutes and the supernatant was discarded. The algal pellets were then sonicated using Phosphate buffer then the cells were centrifuged at 5000 rpm for 10 minutes. The supernatant was taken for the estimation of total protein.

Estimation of Protein

To 0.2 ml of sample protein 5ml of protein reagent was added and mixed thoroughly. The absorbance was read at 595 nm against a reagent blank. The amount of protein was calculated by using a standard graph with Bovine Serum Albumin ranging from 10 to 100 μg /ml.

4.3.4 Extraction and estimation of lipid (Folch et al., 1956)

Reagents

Chloroform: Methanol (2:1V/V)

Fifty ml of chloroform was mixed with 25ml methanol.

Physiological saline (0.9%)

NaCl of 900mg was dissolved in glass distilled water and made up to 100ml.

Phosphovanillin reagent

Two hundred mg of vanillin was added to the mixture of 80 ml Orthophosphoric acid and 20ml of distilled water.

Extraction of lipid (Folch *et al.*, 1956)

Five ml of *Picochlorum* and *Chlorella* culture samples was taken and centrifuged at 5000rpm for 5minutes. The pellet was homogenized in a Sonicator with 6ml of Chloroform: Methanol (2:1). It was then transferred to a separating funnel and added with 2ml of 0.9ml NaCl solution and mixed well. This mixture was left undisturbed for overnight. Then from the lower chloroform phase containing lipid 0.5ml was collected in a clean vial and the solvent was allowed to evaporate at room temperature and the pellet was collected.

Estimation of lipid

To the pellet 0.5ml of concentrated sulphuric acid was added and mixed well. The tubes were closed with glass marbles kept in a boiling water bath for 10min and allowed to cool at room temperature. To 0.2ml of sample 5ml of vanillin reagent was added and mixed well. Allowed to stand for 30minutes and the colour developed was read at 520nm. Standard graph was prepared using cholesterol ranging from 5.0 to 50 µg/ml and the values are expressed as µg/ml.

4.4 CARBON FIXATION RATE ALGAE:

4.4.1 Estimation of carbonate and bicarbonate rate

REAGENTS

Sulphuric acid 0.02N preparation

Dilute 0.72ml of concentrated Sulphuric acid is mixed with 1 litre of sterile distilled water.

Sodium carbonate solution (standard)

0.27 g of Sodium carbonate was dissolved in to distilled water and made up to 250 ml.

Indicator

Phenolphthalein

Methyl Orange

Estimation of carbonate and bicarbonate

Twenty ml of algal culture was taken and centrifuged at 5000 rpm for 10 minutes and the supernatant were taken for the estimation process. Twenty ml of the supernatant was taken in a conical flask and add 2 drops of Phenolphthalein

indicator. The development of pink colour indicates the presence of carbonates and it was titrated against 0.02 N of H₂SO₄ until the pink colour disappears. The titrated value was noted to that sample, add 2 drops of Methyl orange indicator it is then titrated against 0.02 N of H₂SO₄ appearance of pinkish red indicates the end point.

$$\text{Carbonate} = \frac{2X \times 0.02 \times 30 \times 1000}{\text{Volume of sample}}$$

$$\text{Bicarbonate} = \frac{(Y - 2X) \times 0.02 \times 61 \times 1000}{\text{Volume of sample}}$$

Y = Burette reading of bicarbonate

X = Burette reading of carbonate

The values are expressed as mg/L.

4.4.2 Estimation of the carbon dioxide biofixation rate

The carbon fixation rate was calculated from the elemental analysis of the biomass, as shown in equation

$$P_{\text{CO}_2} = C_c P \left(\frac{M_{\text{CO}_2}}{M_c} \right)$$

C_c is the carbon content (w/w) in the cell and was calculated from the elemental composition of the cell. MCO₂ is the molecular weight of CO₂ 44.01 g/mol, and M_c is the molecular weight of carbon.

Determination of carbon content via TOC Analyser

The carbon content of the dried algae biomass was determined using a model Analytikjena, Multi N/C,2100,Germany-solid module and calibrated using a calcium carbonate standard to represent the carbon. CO₂ fixation by algae was calculated from the dried biomass carbon content values. CO₂ is calculated based on the

principle of combustion temperature (1150°C); here oxygen is used as a carrier gas (99.9%).

CHAPTER 5

RESULTS AND DISCUSSION

5.1 CULTIVATION OF MICROALGA

Micro algae inoculation. *Picochlorum* and *Chlorella* grown in Conway medium.

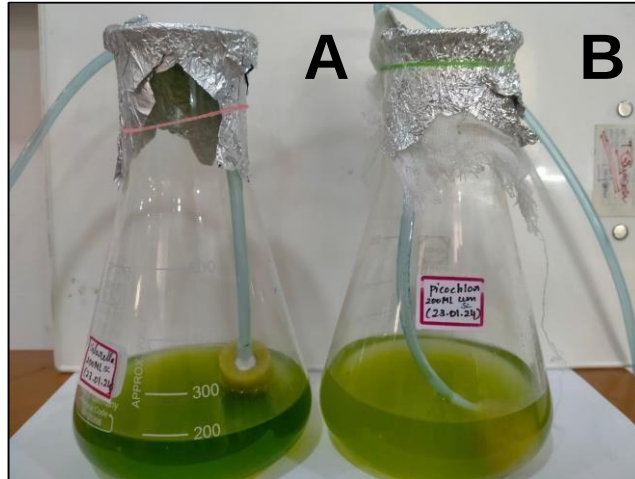


Figure 5.1.1 *Chlorella* and *Picochlorum* sample



Figure 5.1.2 (A) *Picochlorum* inoculation

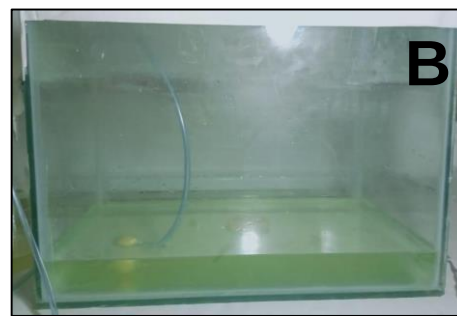


Figure 5.1.3 (B) *Chlorella* inoculation

5.2 SUPPLEMENTED WITH SODIUM BICARBONATE AS CARBON SOURCE

Different concentration of sodium bicarbonate give the carbon source of microalgae. It was mention in the Fig 5.2.1. The control using for Conway medium with microalgae *PICOCHLORUM*, *CHLORELLA*



Figure 5.2.1 Control (A), sample (B) 0.20% NaHCO₃, sample (C) 0.40% NaHCO₃ Sample (D) 0.60% NaHCO₃, Sample (E) 0.80% NaHCO₃, Sample (F) 1.00% NaHCO₃. *PICOCHLORUM*, *CHLORELLA*

5.3 DETERMINATION OF BIOCHEMICAL CONTENT

5.3.1 Estimation of chlorophyll pigment *Picochlorum*

Micro algae growth was analysed using the pigment levels. it was measured at 644.8 λ , 661.6 λ and 470 λ in Milton Roy UV-vis spectrophotometer (Lichtenthaler, 1987). The reading was mention in Table 5.3.1 Absorbance of chlorophyll pigment *Picochlorum*.

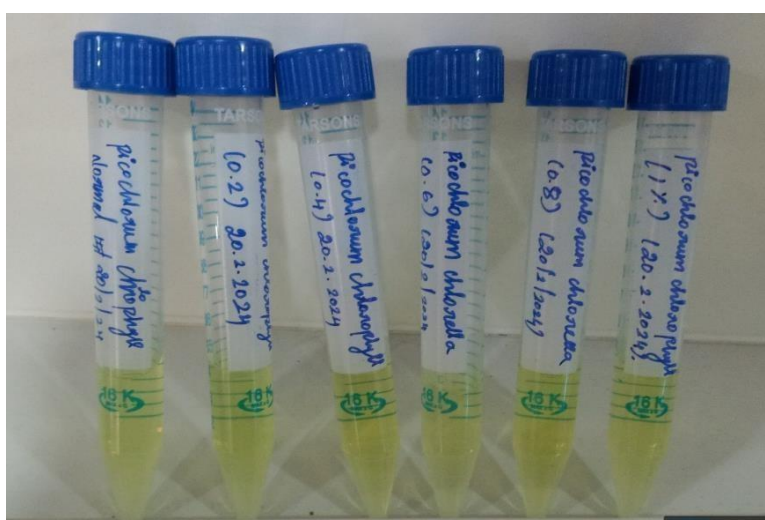


Figure 5.3.1 *Picochlorum* chlorophyll pigment

Table 5.3.1 Absorbance of chlorophyll pigment *Picochlorum*

SL. NO:	Days	Absorption (nm)	Control	0.20% NaHCO ₃	0.40% NaHCO ₃	0.60% NaHCO ₃	0.80% NaHCO ₃	1.00% NaHCO ₃
1	1 st day	470	0.223	0.452	0.244	0.186	0.186	0.214
		644	0.112	0.318	0.121	0.079	0.094	0.091
		661	0.145	0.339	0.149	0.110	0.122	0.132
2	5 th day	470	0.174	0.213	0.218	0.149	0.227	0.199
		644	0.034	0.042	0.014	0.003	0.008	0.01
		661	0.122	0.146	0.069	0.041	0.039	0.046
3	9 th day	470	0.199	0.233	0.234	0.254	0.251	0.199
		644	0.054	0.044	0.019	0.043	0.028	0.011
		661	0.117	0.122	0.073	0.068	0.051	0.054
4	13 th day	470	0.217	0.244	0.253	0.294	0.268	0.2
		644	0.034	0.048	0.032	0.055	0.037	0.024
		661	0.103	0.115	0.096	0.132	0.098	0.083
5	17 th day	470	0.219	0.406	0.419	0.429	0.379	0.444
		644	0.120	0.135	0.130	0.139	0.125	0.147
		661	0.268	0.246	0.234	0.266	0.226	0.286
6	21 st day	470	0.347	0.233	0.192	0.120	0.299	0.35
		644	0.09	0.052	0.056	0.042	0.084	0.098
		661	0.155	0.07	0.083	0.048	0.128	0.185
7	25 th day	470	0.365	0.259	0.212	0.261	0.247	0.173
		644	0.134	0.083	0.083	0.07	0.063	0.059
		661	0.240	0.114	0.102	0.091	0.079	0.075
8	30 th day	470	0.218	0.174	0.204	0.17	0.185	0.104
		644	0.063	0.047	0.063	0.048	0.061	0.044
		661	0.098	0.058	0.1	0.59	0.091	0.055

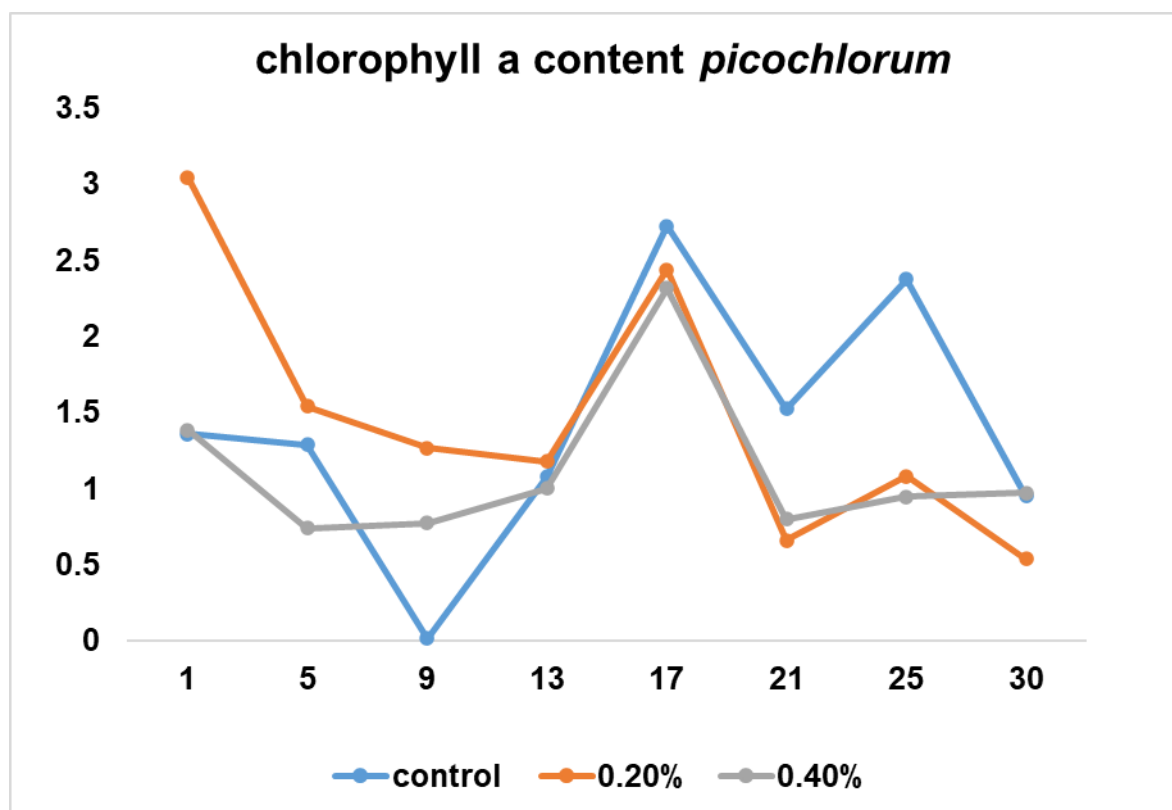


Figure 5.3.2 Chlorophyll a content of *Picochlorum*

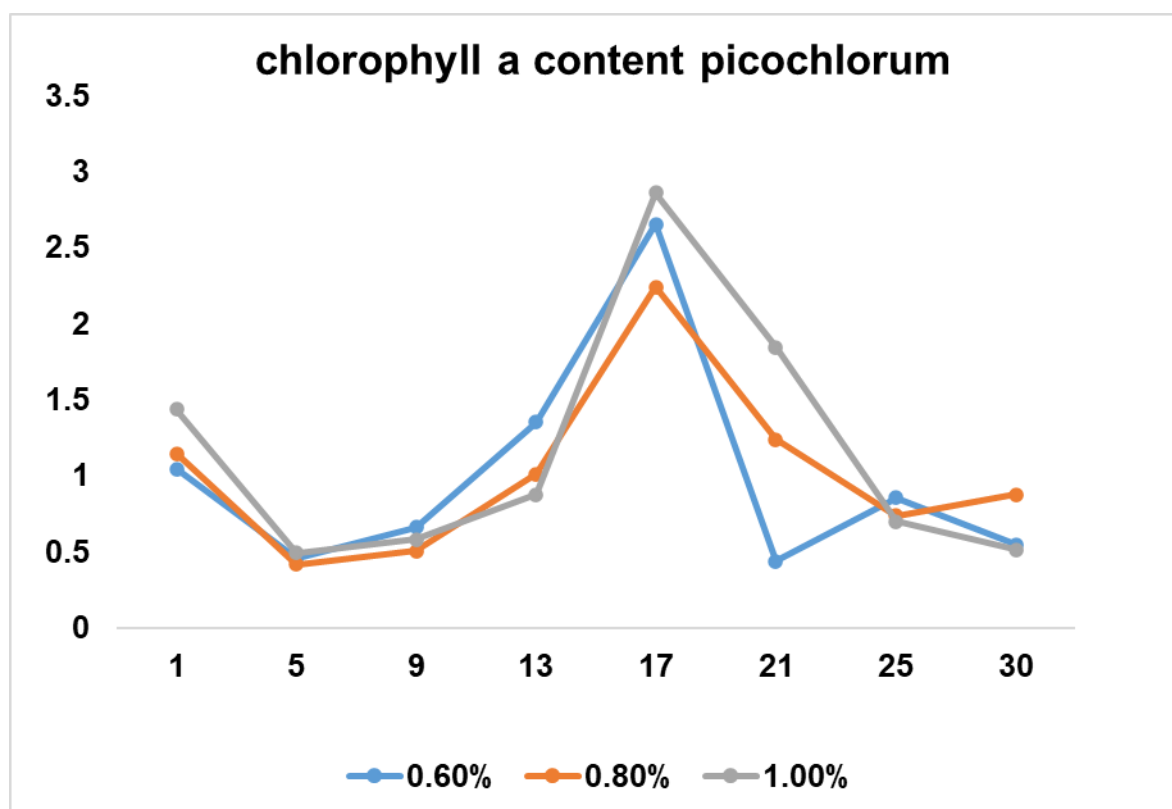


Figure 5.3.3 Chlorophyll a content of *Picochlorum*

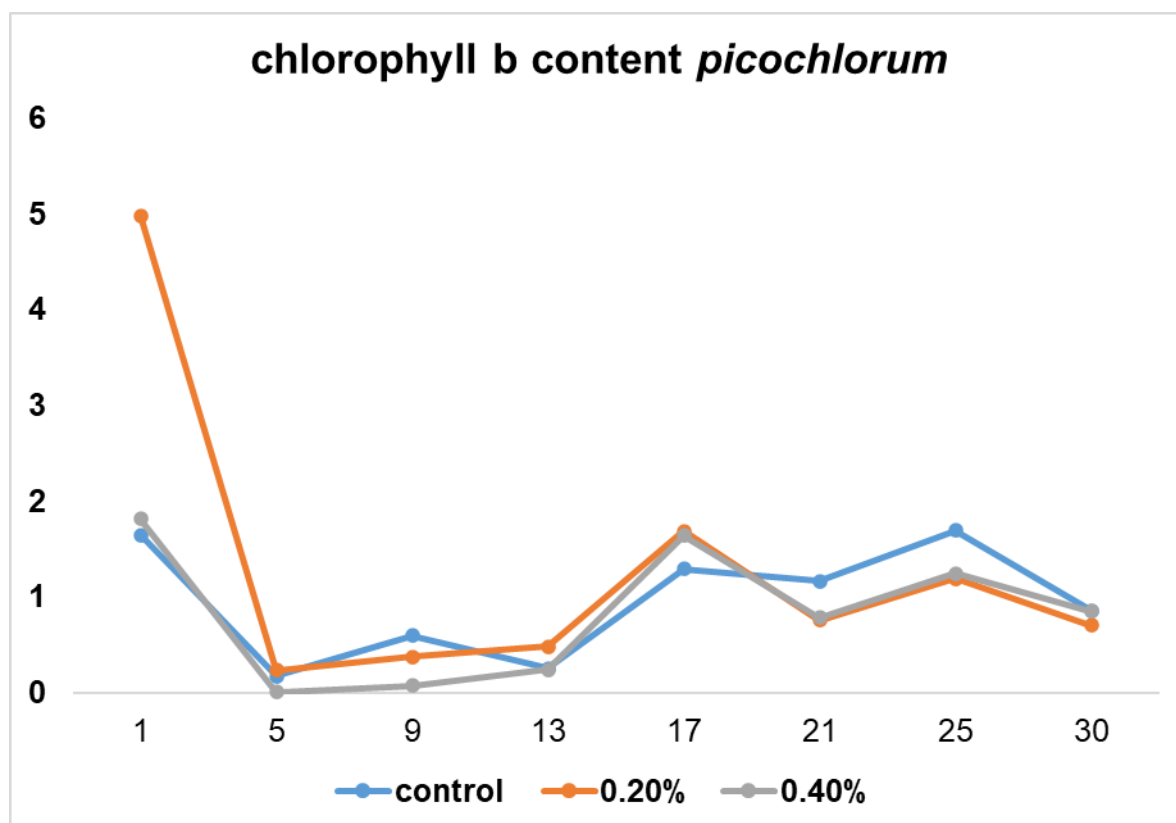


Figure 5.3.4 Chlorophyll b content of *Picochlorum*

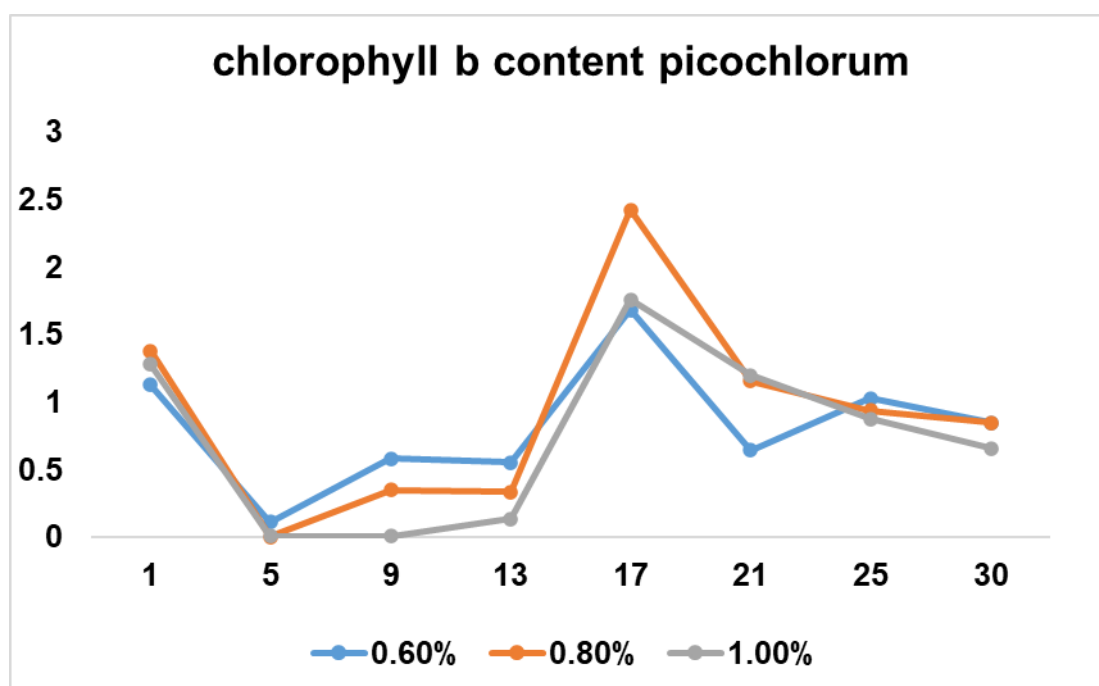


Figure 5.3.5 Chlorophyll b content of *Picochlorum*

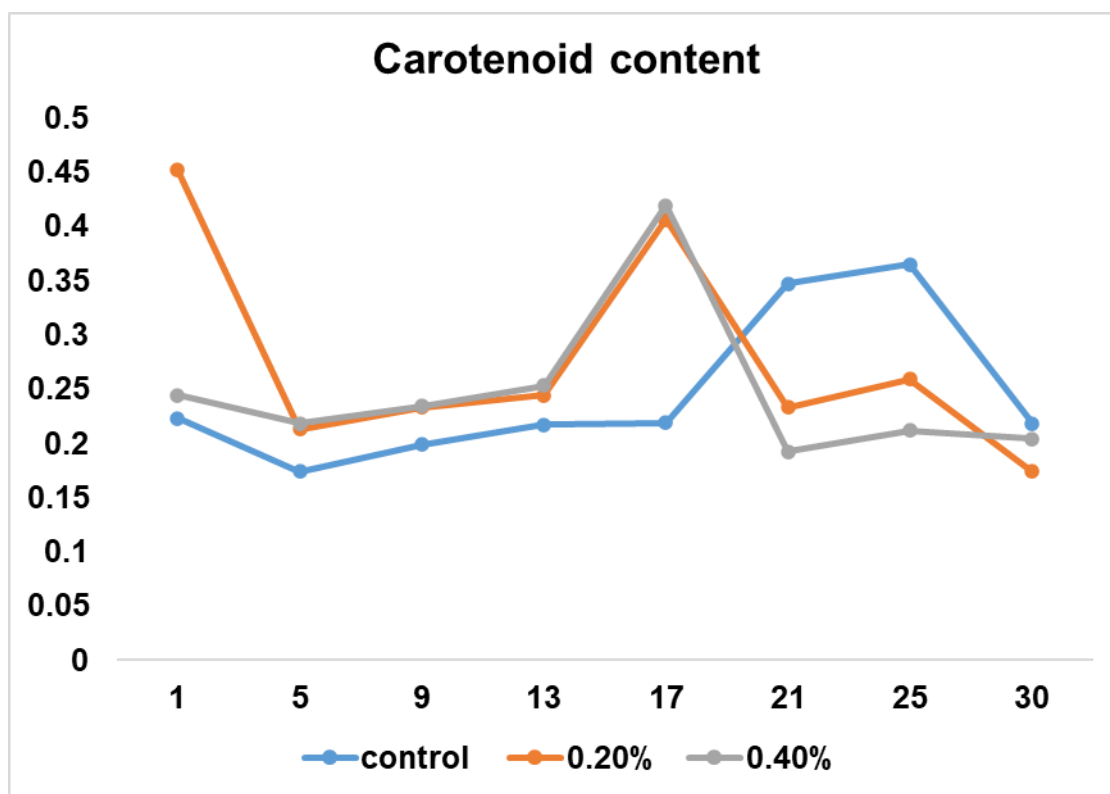


Figure 5.3.6 Carotenoid content *Picochlorum*

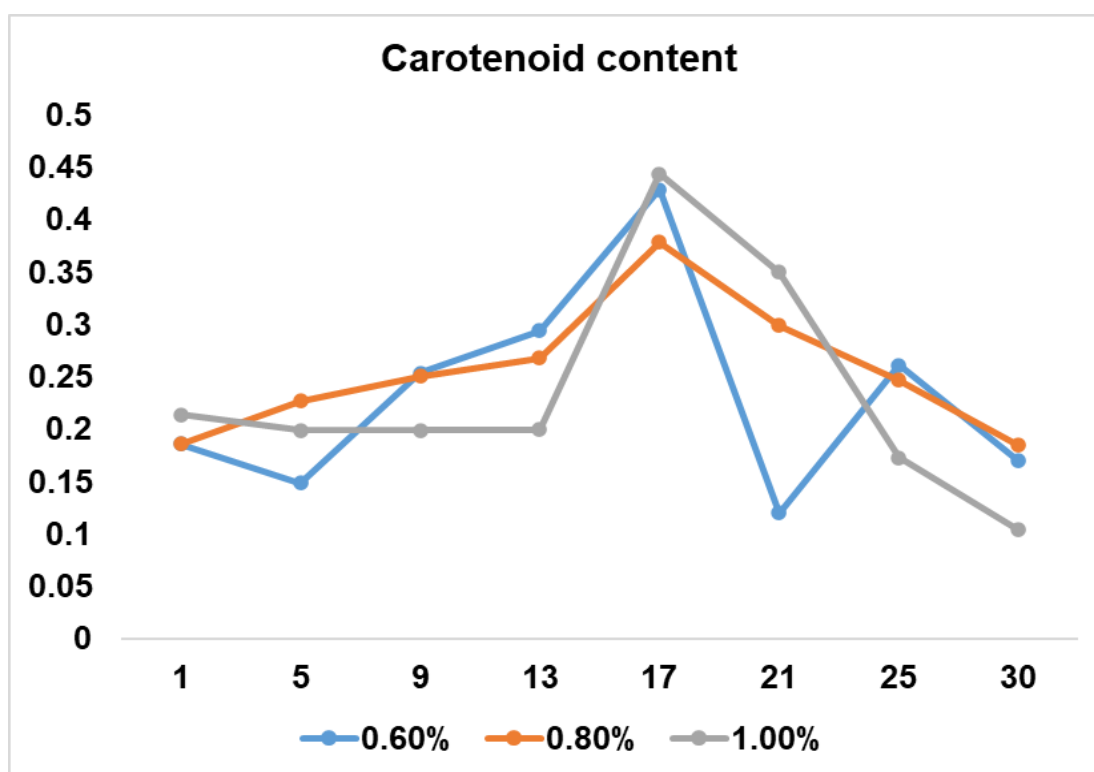


Figure 5.3.7 Carotenoid content *Picochlorum*

5.3.2 Estimation of chlorophyll pigment chlorella

Micro algae growth was analysed using the pigment levels. it was measured at 644.8 λ , 661.6 λ and 470 λ in Milton Roy UV-vis spectrophotometer (Lichtenthaler, 1987). The reading was mention in Table 5.3.2.1 Absorbance of chlorophyll pigment *Chlorella*.

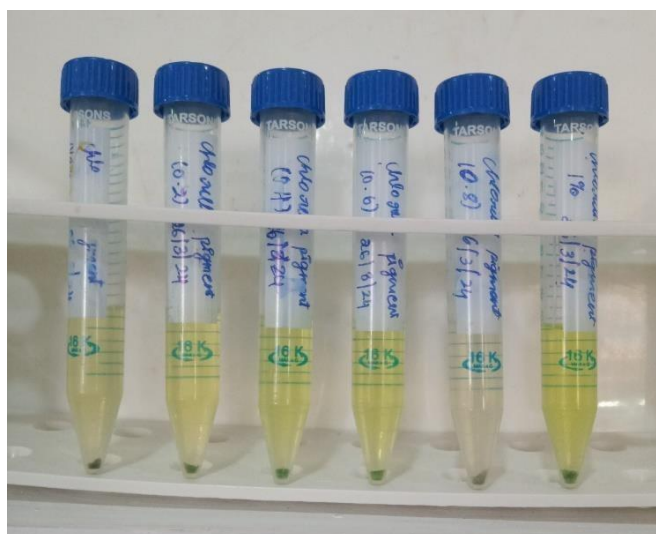


Figure 5.3.2.1 Estimation of chlorophyll pigment *Chlorella*

Table 5.3.2.1 Absorbance of chlorophyll pigment *Chlorella*

SL. NO :	Days	Absorption (nm)	Control	0.20% NaHC O ₃	0.40% NaHC O ₃	0.60% NaHC O ₃	0.80% NaHCO ₃	1.00% NaHCO ₃
1	1 ST day	470	0.32	0.30	0.259	0.278	0.95	0.323
		644	0.096	0.096	0.072	0.076	0.086	0.092
		661	0.162	0.152	0.097	0.112	0.131	0.154
2	5 th day	470	0.289	0.32	0.277	0.274	0.313	0.325
		644	0.103	0.088	0.07	0.075	0.095	0.096
		661	0.177	0.151	0.105	0.114	0.163	0.164

3	9 th day	470	0.189	0.232	0.15	0.239	0.183	0.191
		644	0.054	0.083	0.048	0.08	0.057	0.056
		661	0.06	0.127	0.053	0.118	0.082	0.07
4	13 th day	470	0.2	0.228	0.196	0.214	0.227	0.19
		644	0.058	0.063	0.059	0.057	0.063	0.054
		661	0.081	0.08	0.074	0.067	0.079	0.056
5	17 th day	470	0.194	0.197	0.186	0.205	0.186	0.175
		644	0.046	0.054	0.052	0.05	0.051	0.045
		661	0.56	0.079	0.071	0.068	0.069	0.055
6	21 st day	470	0.322	0.291	0.269	0.325	0.278	0.35
		644	0.065	0.071	0.054	0.068	0.056	0.081
		661	0.087	0.103	0.067	0.09	0.067	0.123
7	25 th day	470	0.312	0.303	0.314	0.34	0.33	0.362
		644	0.064	0.082	0.073	0.077	0.068	0.073
		661	0.092	0.116	0.117	0.116	0.104	0.105
8	30 th day	470	0.248	0.314	0.273	0.221	0.216	0.243
		644	0.058	0.059	0.078	0.054	0.079	0.05
		661	0.073	0.072	0.128	0.076	0.142	0.061

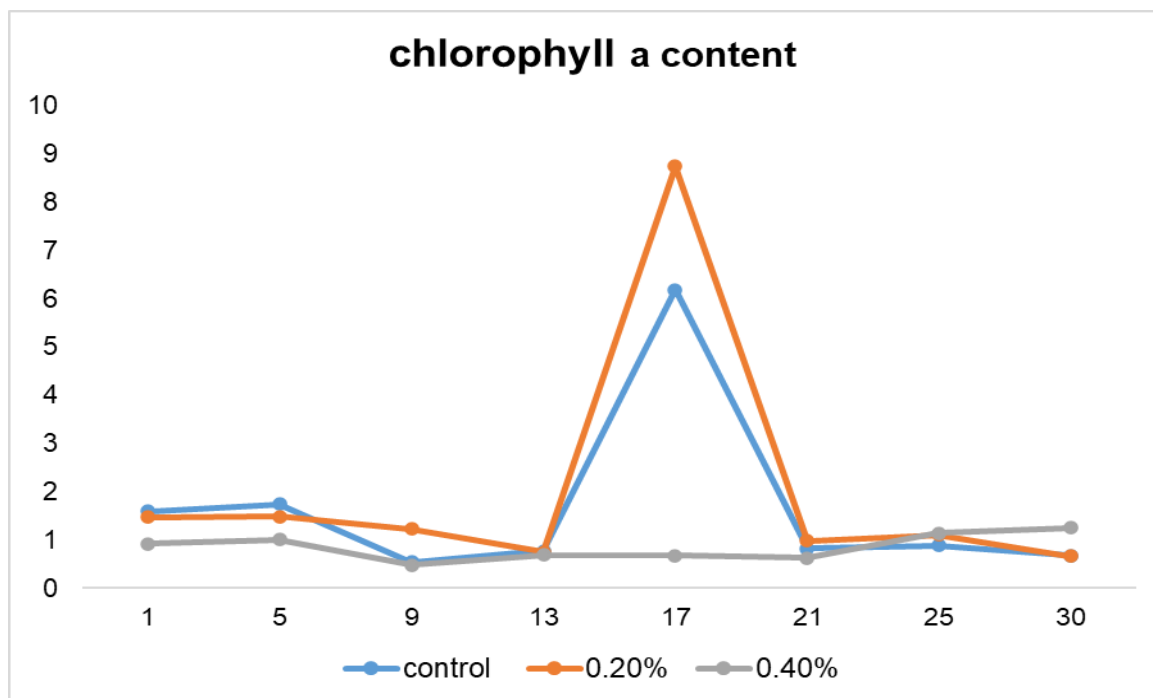


Figure 5.3.2 Chlorophyll a content of Chlorella

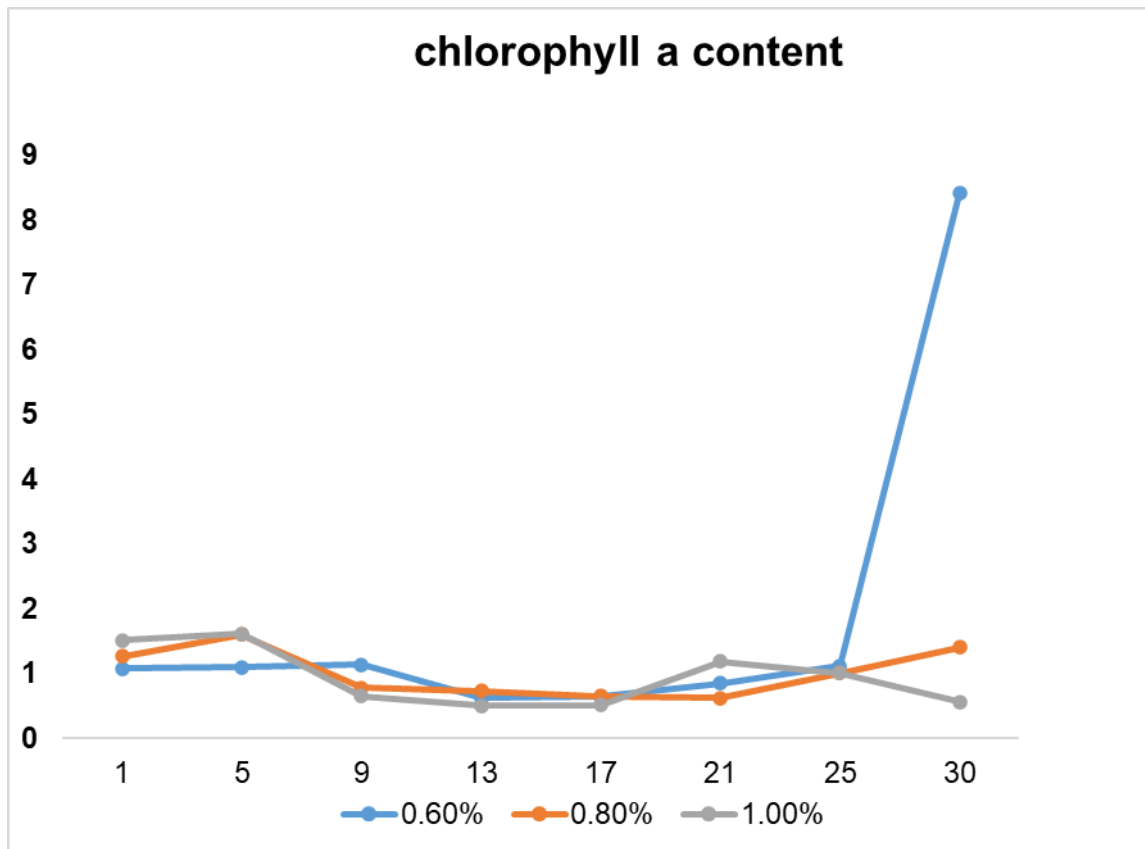


Figure 5.3.2.3 Chlorophyll a content of Chlorella

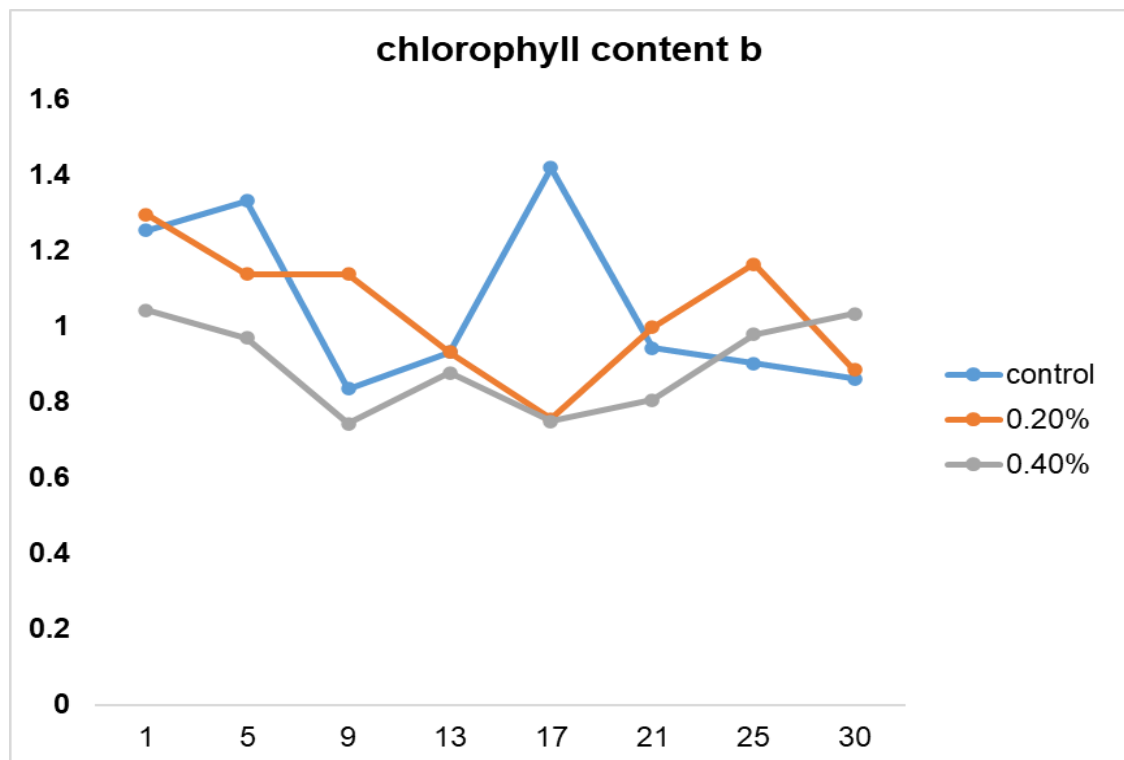


Figure 5.3.2.4 Chlorophyll b content Chlorella

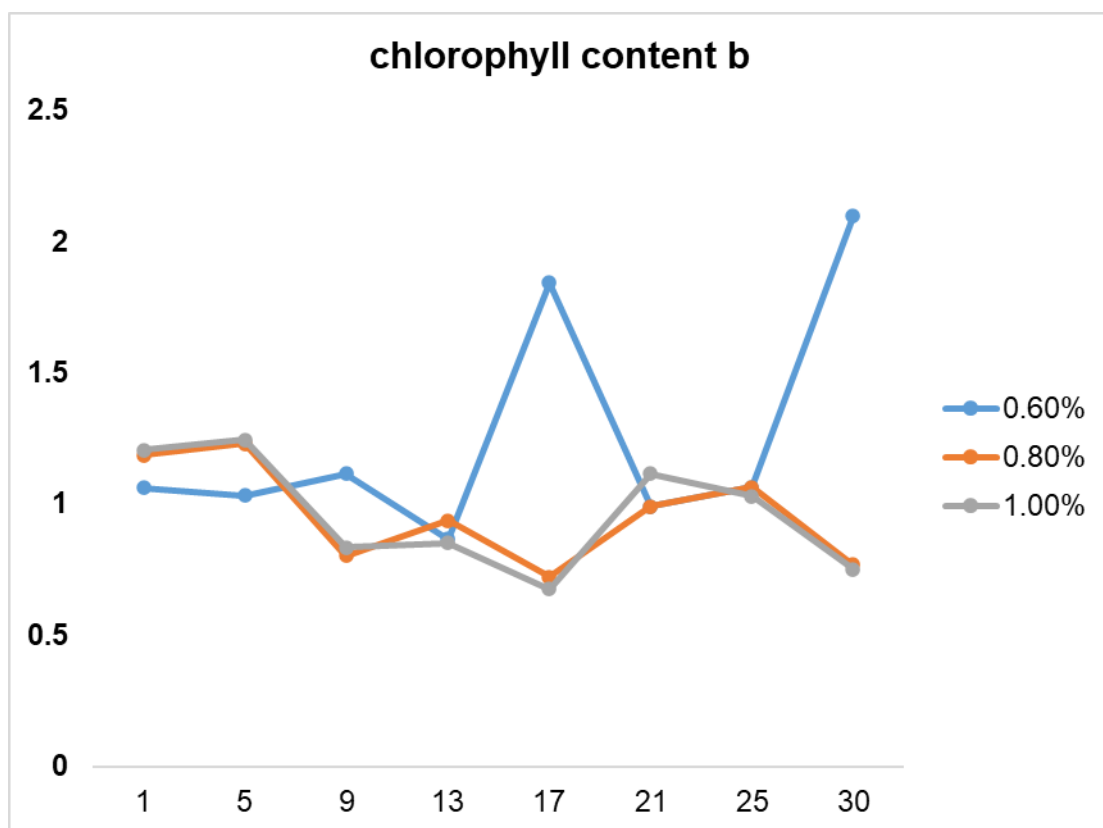


Figure 5.3.2.5 Chlorophyll b content *Chlorella*

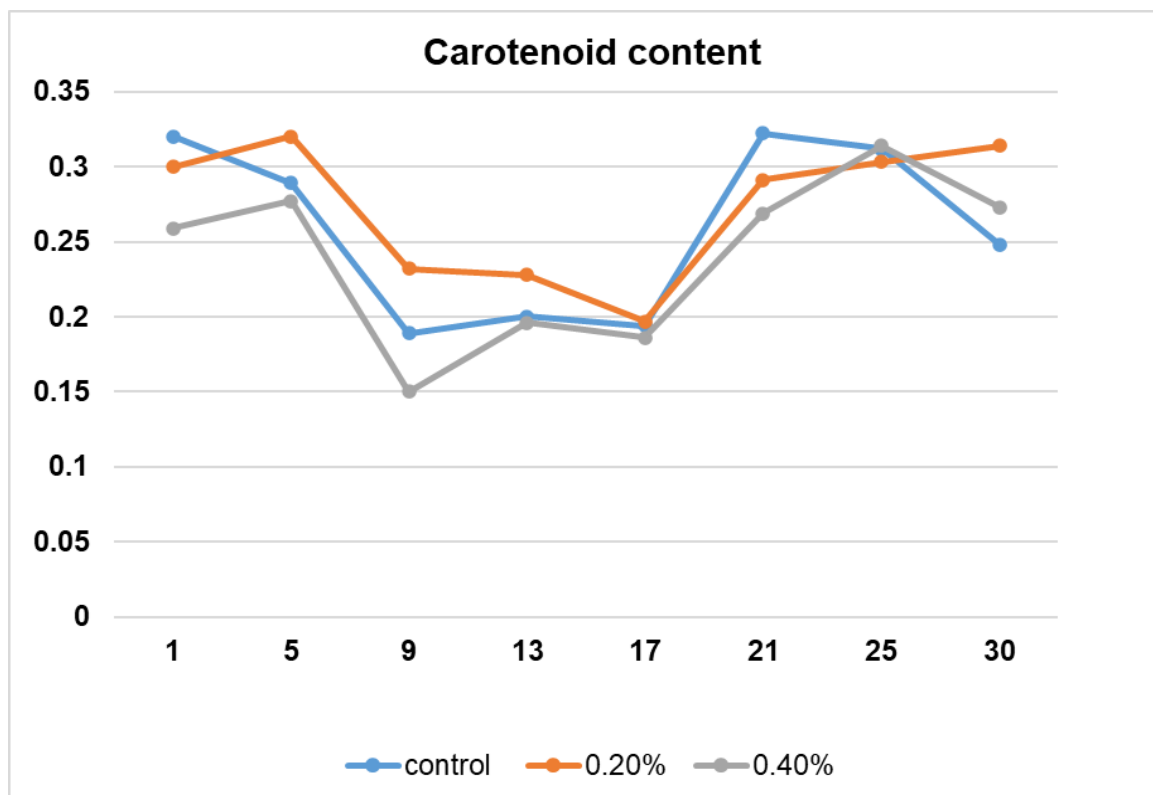


Figure 5.3.2.6 Carotenoid content of *Chlorella*

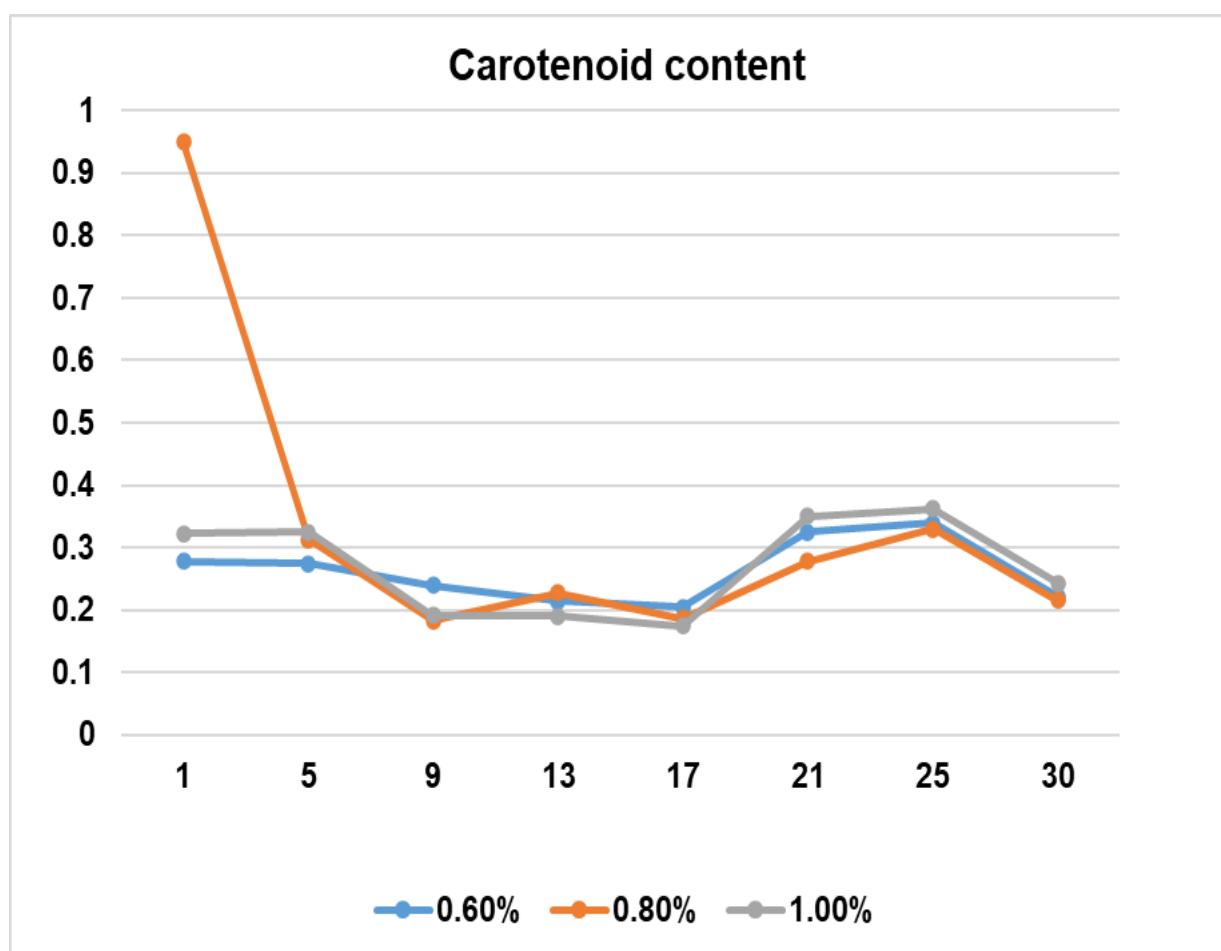


Figure 5.3.2.7 Carotenoid content of Chlorella

5.3.3 ESTIMATION OF CARBOHYDRATE *PICOCHLORUM*

Estimation of carbohydrate in *Picochlorum*, the colour variant was appeared and the reading was taken from the uv-vis spectrophotometer in 490(nm). The absorbance was mentioned in Table 5.3.3.1 Absorbance of Carbohydrate content *Picochlorum*.

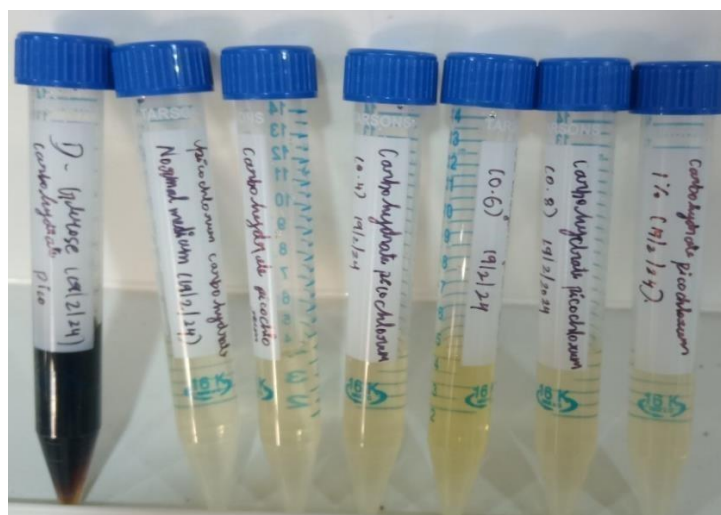


Figure 5.3.3.1 Colour variation of carbohydrate Picochlorum Table
5.3.3.1 Absorbance of Carbohydrate content Picochlorum

S No;	Days and Absorption 490 (nm)	Control	0.20% NaHCO ₃	0.40% NaHCO ₃	0.60% NaHCO ₃	0.80% NaHCO ₃	1.00% NaHCO ₃
1	1 ST day	0.367	0.240	0.248	0.250	0.250	0.308
2	5 th day	0.236	0.258	0.261	0.3777	0.618	0.240
3	9 th day	0.409	0.384	0.400	0.525	0.412	0.470
4	13 th day	0.321	0.421	0.283	0.430	0.455	0.397
5	17 th day	0.228	0.659	0.748	1.182	1.218	1.161
6	21 st day	0.352	0.260	0.185	0.577	0.280	0.260
7	25 th day	0.256	0.345	0.378	0.251	0.280	0.271
8	30 th day	0.191	0.411	0.294	0.211	0.382	0.355

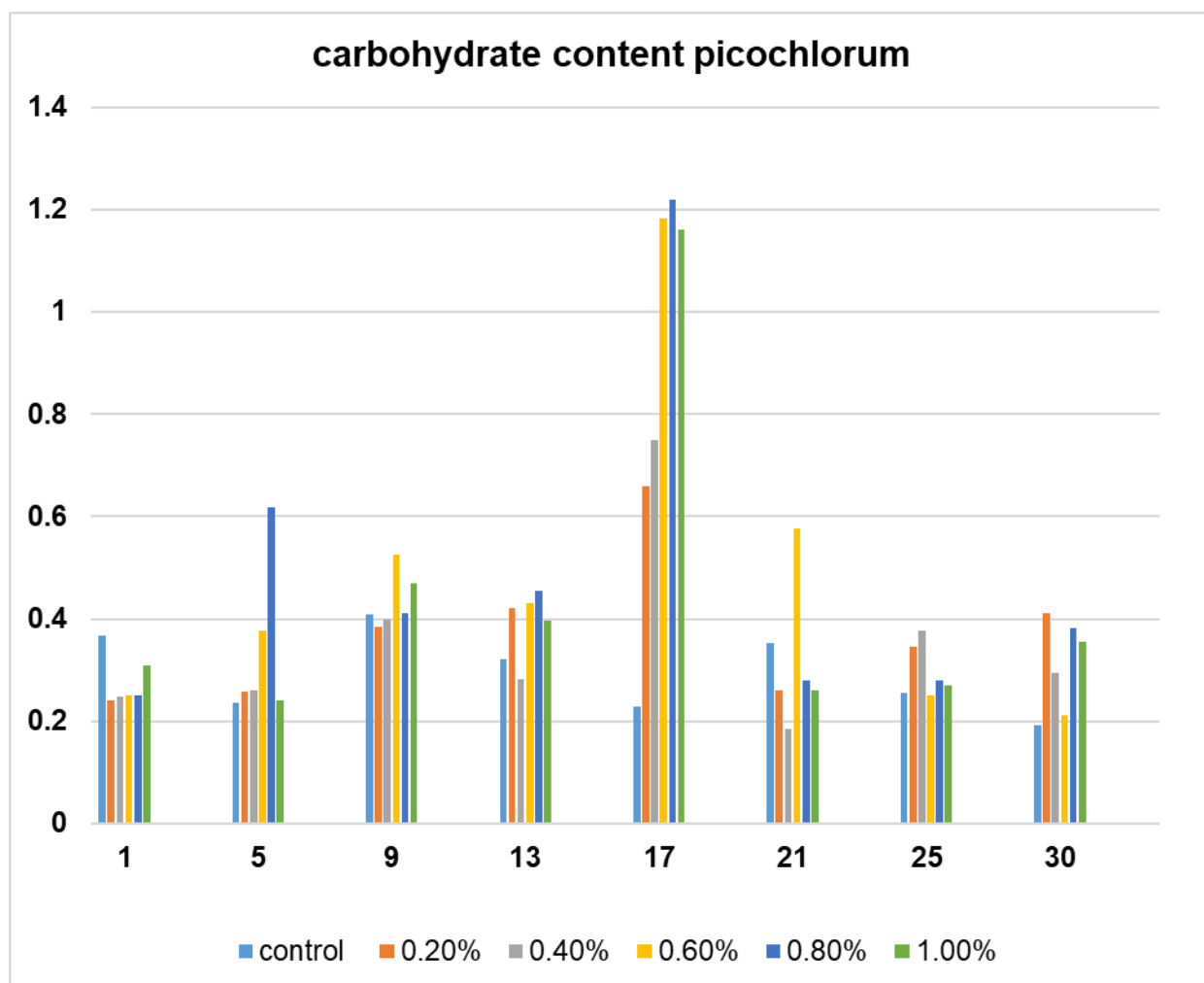


Figure 5.3.3.2 Carbohydrate content Picochlorum

5.3.4 Estimation of carbohydrate *Chlorella*

Estimation of carbohydrate in *Chlorella*, the colour variant was appeared and the reading was taken from the UV- vis spec photometer in 490(nm). The absorbance was mentioned Table 5.3.4.1 Absorbance of Carbohydrate content *Chlorella*.

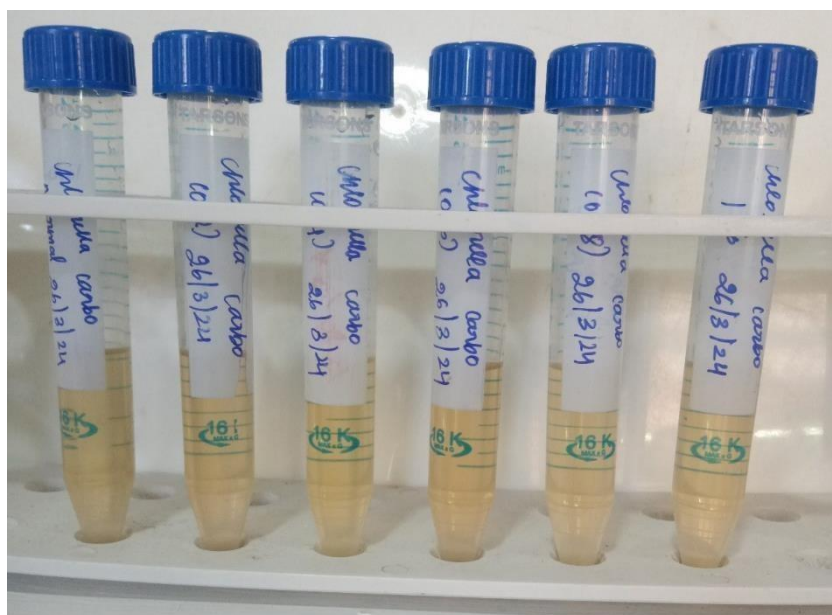


Figure 5.3.4.1 Colour variation of carbohydrate Chlorella

Table 5.3.4.1 Absorbance of Carbohydrate content Chlorella

SL No;	Days and Absorption 490 (nm)	Control	0.20% NaHCO ₃	0.40% NaHCO ₃	0.60% NaHCO ₃	0.80% NaHCO ₃	1.00% NaHCO ₃
1	1 ST day	0.397	0.367	0.431	0.330	0.326	0.291
2	5 th day	0.309	0.422	0.498	0.331	0.46	0.373
3	9 th day	0.103	0.220	0.091	0.200	0.142	0.217
4	13 th day	0.230	0.250	0.252	0.205	0.203	0.253
5	17 th day	0.261	0.347	0.339	0.194	0.496	0.240
6	21 st day	0.195	0.239	0.192	0.224	0.182	0.148
7	25 th day	0.320	0.229	0.254	0.284	0.251	0.314
8	30 th day	0.253	0.265	0.296	0.256	0.232	0.214

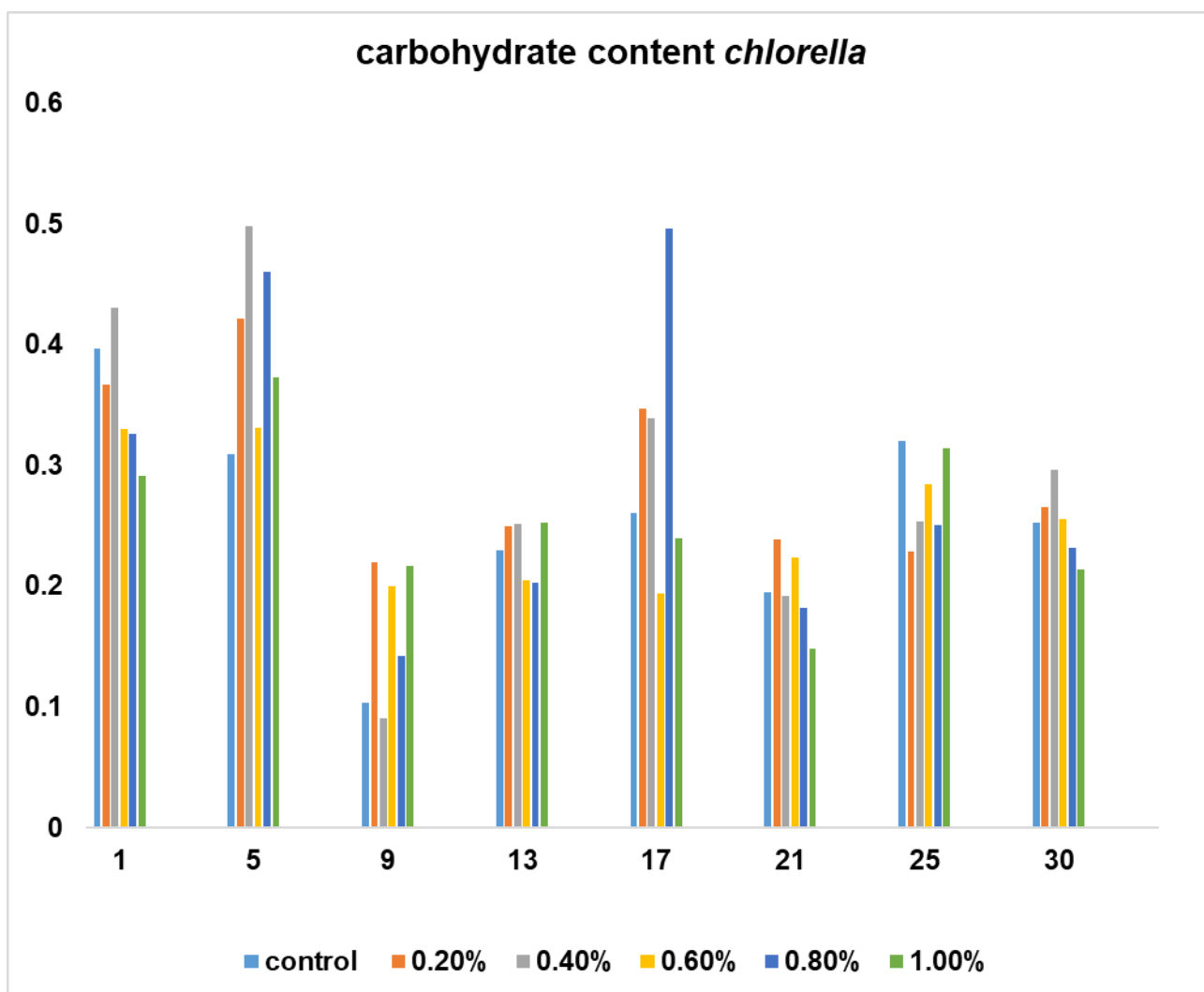


Figure 5.3.4.2 Carbohydrate content *Chlorella*

5.3.5 ESTIMATION OF PROTEIN *PICOCHLORUM*

Estimation of protein *Picochlorum*. In presence of protein the blue colour was appeared. The standard used for bovine serum albumin and the absorbance read at 595 (nm) against blank. The absorbance mentioned in Table 5.3.5.1 Absorbance of protein content *Picochlorum*.

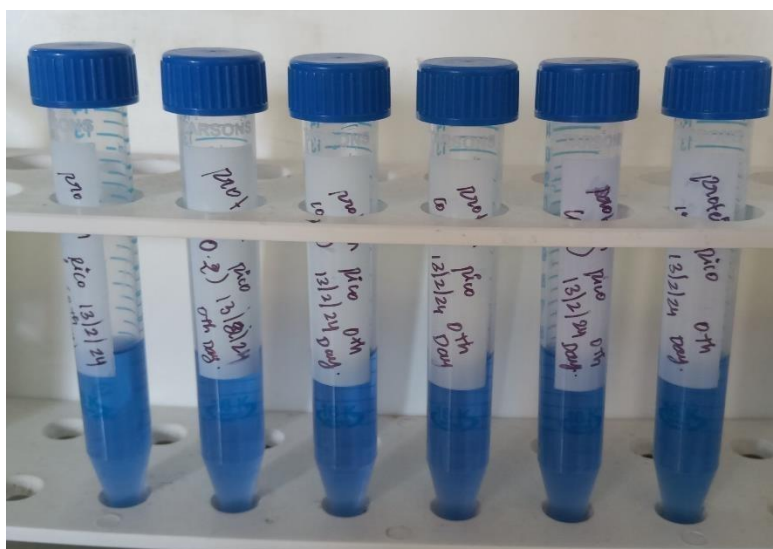


Figure 5.3.5.1 Protein estimation *Picochlorum*

Table 5.3.5.1 Absorbance of protein content *Picochlorum*

SL No;	Days and Absorption 595 (nm)	Control	0.20% NaHCO ₃	0.40% NaHCO ₃	0.60% NaHCO ₃	0.80% NaHCO ₃	1.00% NaHCO ₃
1	1 ST day	0.838	0.815	0.828	0.833	0.836	0.82
2	15 th day	0.813	0.831	0.834	0.825	0.821	0.838
3	30 th day	0.838	0.818	0.840	0.838	0.837	0.817

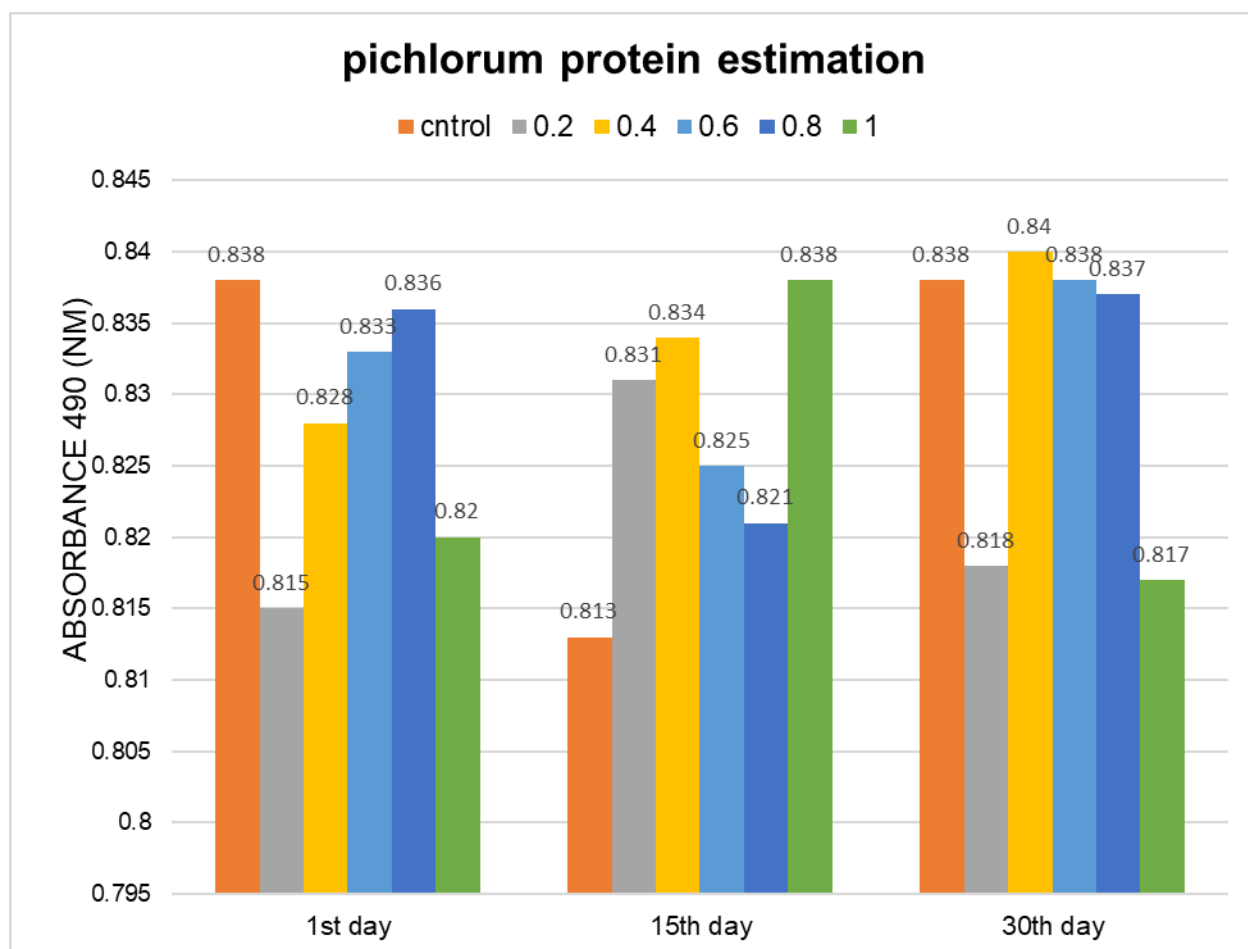


Figure 5.3.5.2 Protein content *Picochlorum*

5.3.6.1 ESTIMATION OF PROTEIN *CHLORELLA*

Estimation of protein *Chlorella*. In presence of protein the blue colour was appeared. The standard used for bovine serum albumin and the absorbance read at 595 (nm) against blank. The absorbance mentioned in Table 5.3.6.1 Absorbance of protein content *Chlorella*.



Figure 5.3.6.1 Protein estimation Chlorella

Table 5.3.6.1 Absorbance of protein content Chlorella

SL No	Days and Absorption 595 (nm)	Control	0.20% NaHCO ₃	0.40% NaHCO ₃	0.60% NaHCO ₃	0.80% NaHCO ₃	1.00% NaHCO ₃
1	1 st day	0.737	0.749	0.73	0.726	0.716	0.714
2	15 th day	0.746	0.717	0.715	0.736	0.731	0.697
3	30 th day	0.678	0.683	0.712	0.639	0.668	0.676

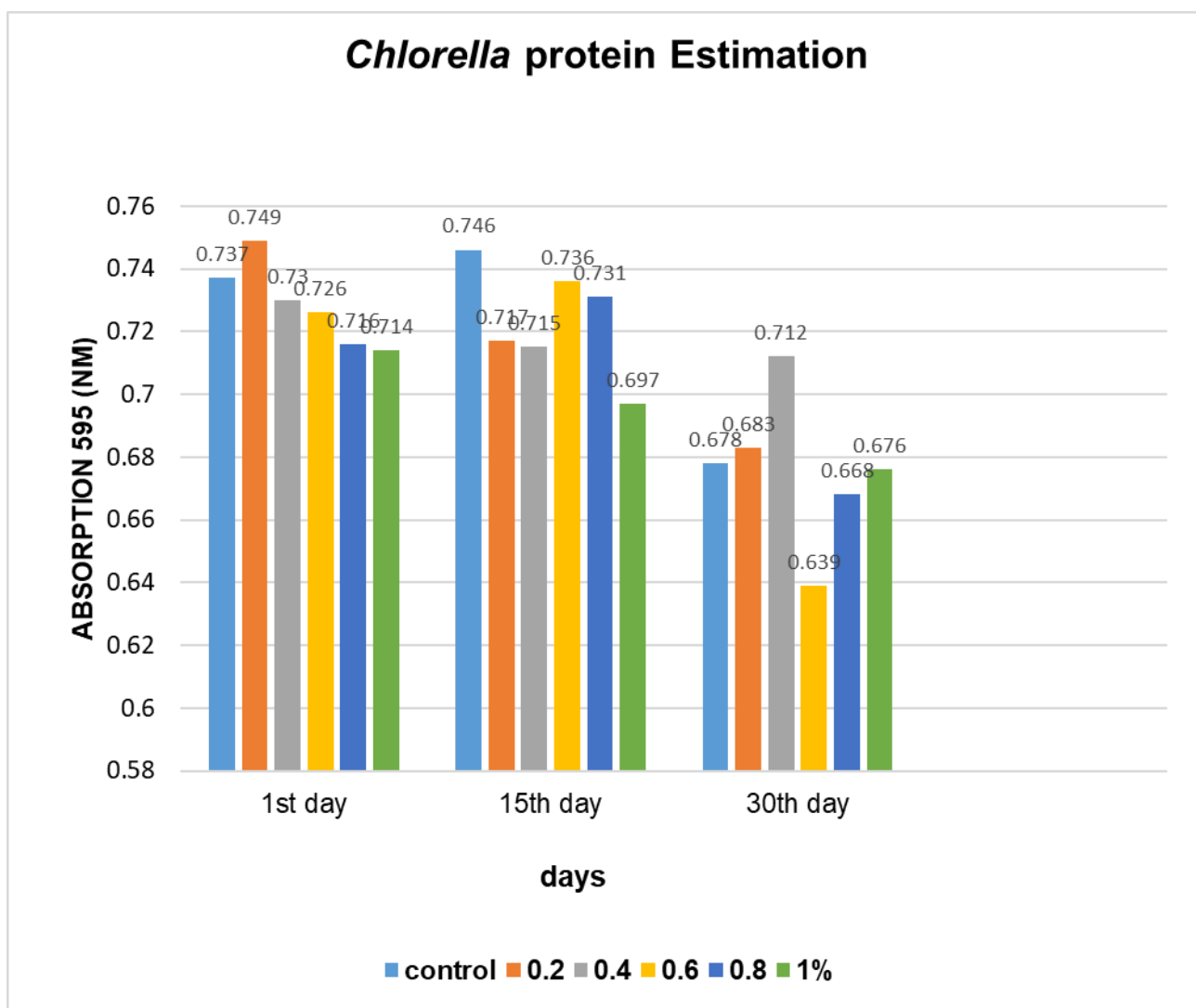


Figure 5.3.6.2 Protein content *Chlorella*

5.3.7 ESTIMATION LIPID *PICOCHLORUM*

Estimation of Lipid *Picochlorum*. In presence of lipid the pink colour was appeared. The standard used for vanillin and the absorbance read at 520 (nm). The absorbance mentioned Table 5.3.7.1 Absorbance of lipid *Picochlorum*.

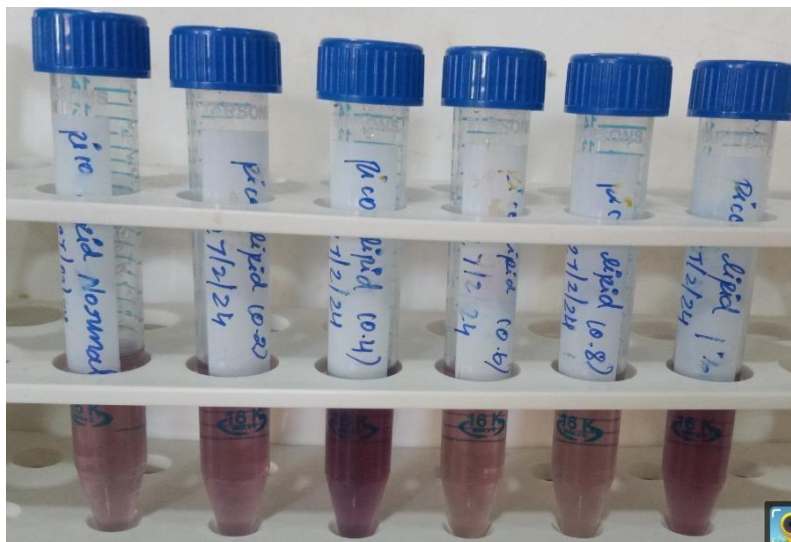


Figure 5.3.7.1 Lipid estimation Picochlorum

Table 5.3.7.1 Absorbance of lipid Picochlorum

SL No;	Days and Absorption 595 (nm)	Control	0.20% NaHCO ₃	0.40% NaHCO ₃	0.60% NaHCO ₃	0.80% NaHCO ₃	1.00% NaHCO ₃
1	1 st day	1.633	1.618	2.006	2.783	0.184	2.918
2	15 th day	0.498	0.64	1.261	0.453	0.439	0.985
3	30 th day	1.512	1.284	0.86	0.61	0.588	1.297

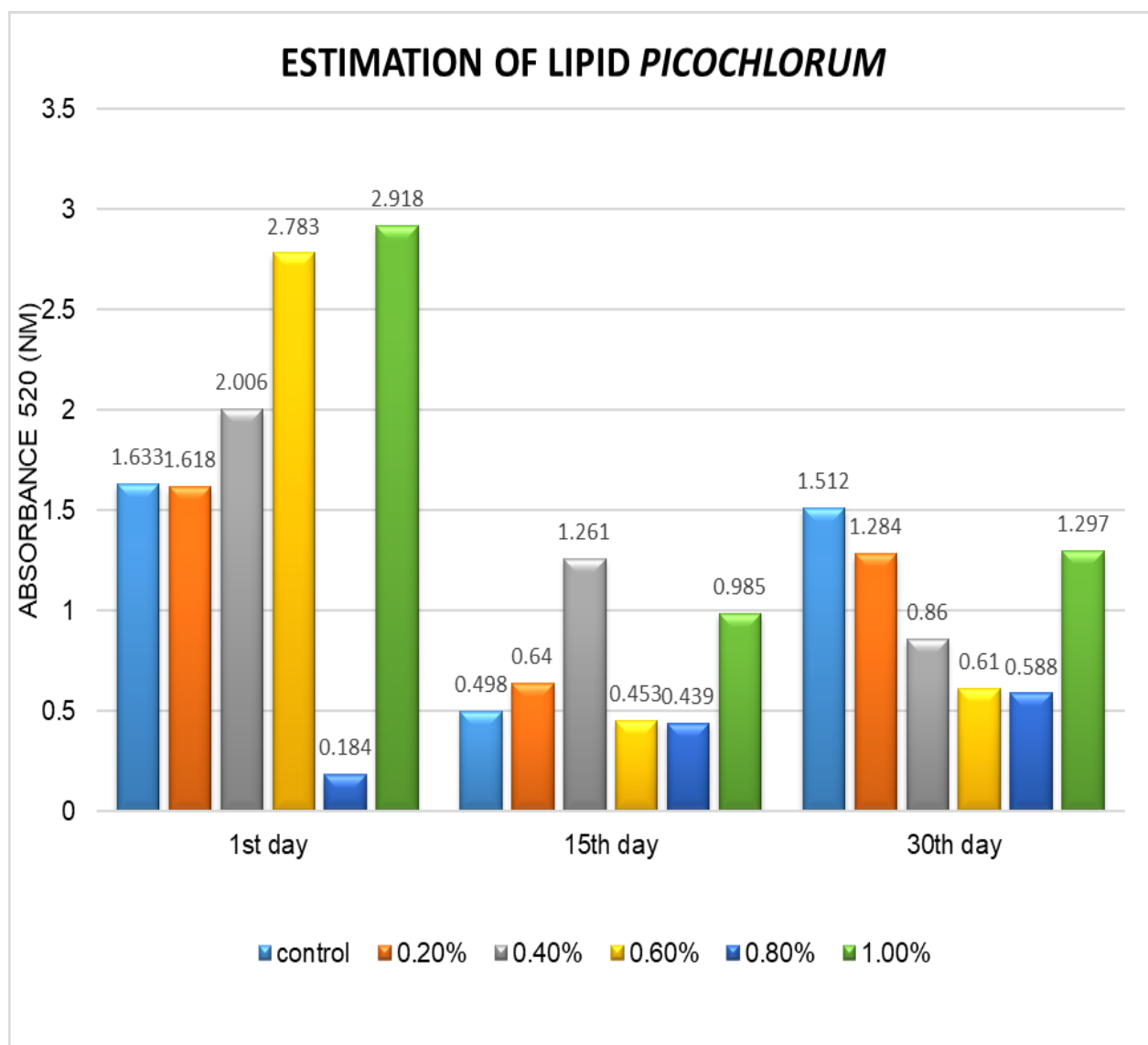


Figure 5.3.7.2 Lipid content *Picochlorum*

5.3.8.1 ESTIMATION LIPID *CHLORELLA*

Estimation of Lipid *Chlorella*. In presence of lipid the pink colour was appeared. The standard used for vanillin and the absorbance read at 520 (nm).

The absorbance mentioned Table 5.3.8.1 Absorbance of lipid *Chlorella* .

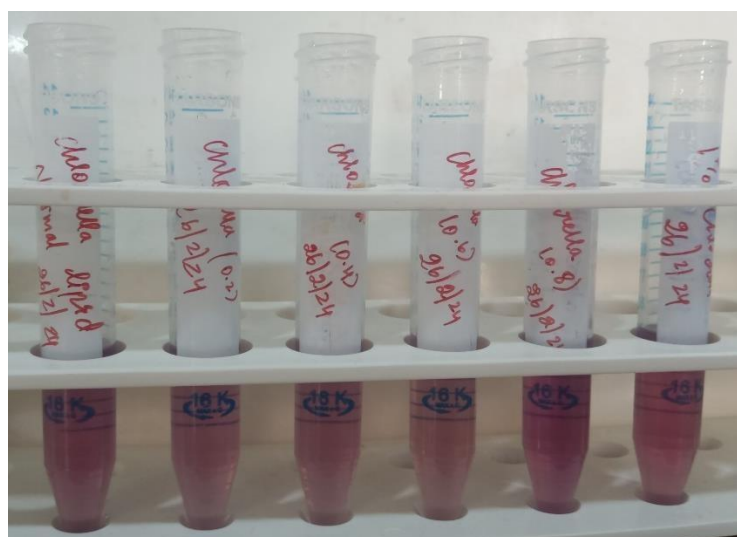


Figure 5.3.8.1 Lipid estimation Chlorella

Table 5.3.8.1 Absorbance of lipid Chlorella

SL No	Days and Absorption 595 (nm)	Control	0.20% NaHCO ₃	0.40% NaHCO ₃	0.60% NaHCO ₃	0.80% NaHCO ₃	1.00% NaHCO ₃
1	1 st day	0.684	0.505	0.504	0.449	0.879	0.855
2	15 th day	0.558	0.487	1.005	0.744	0.969	0.812
3	30 th day	1.868	0.983	1.713	2.204	0.839	0.728

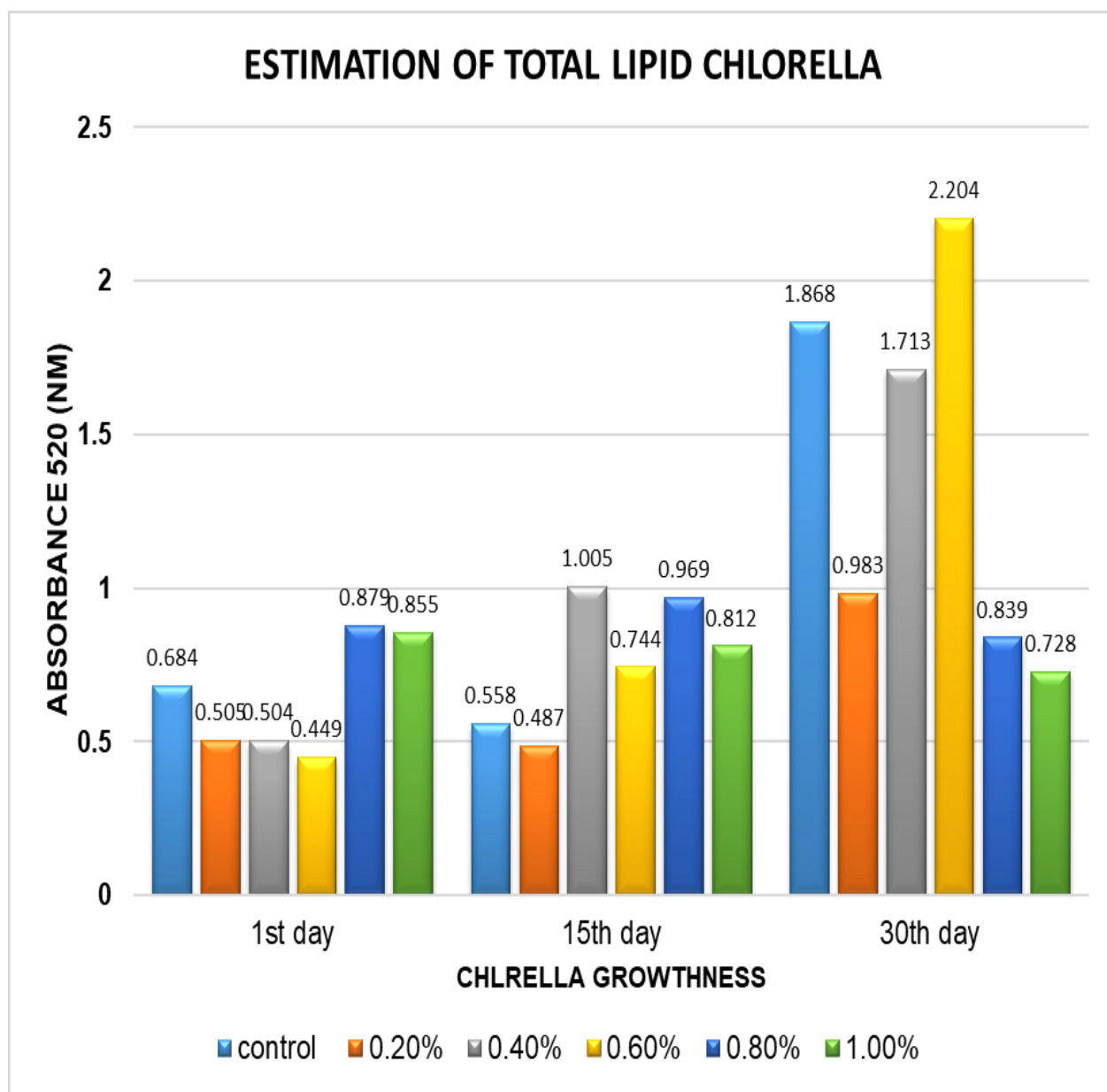
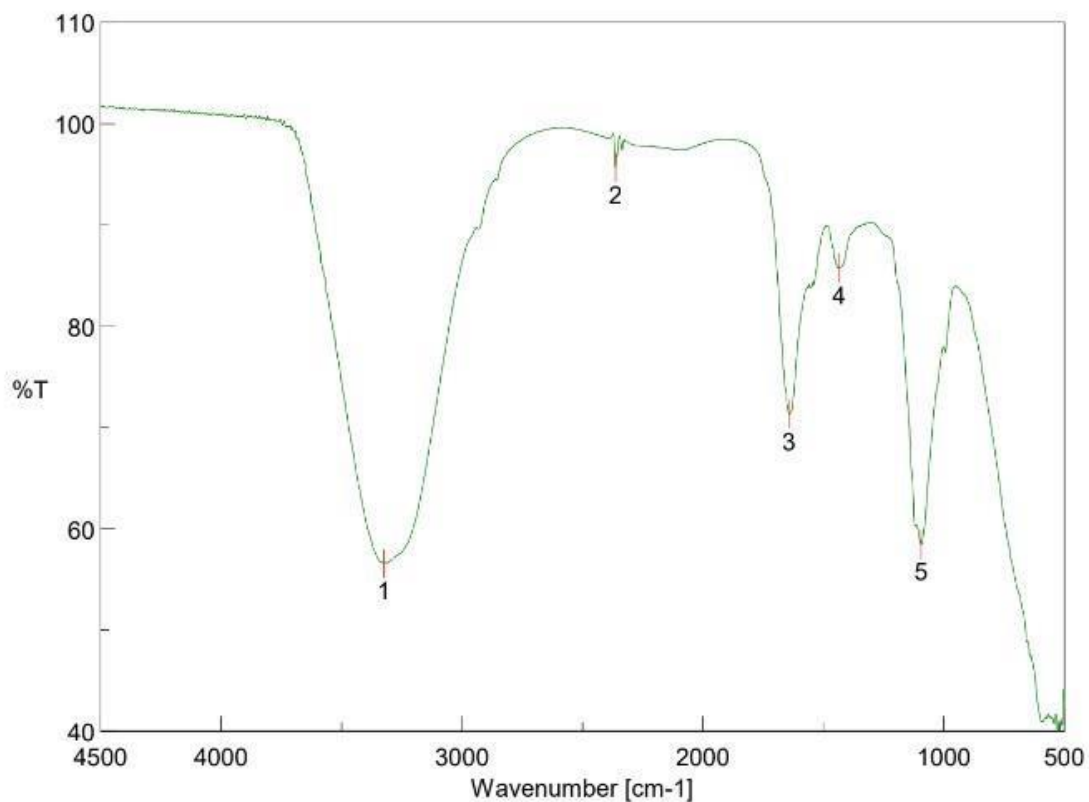


Figure 5.3.8.2 Lipid content Chlorella

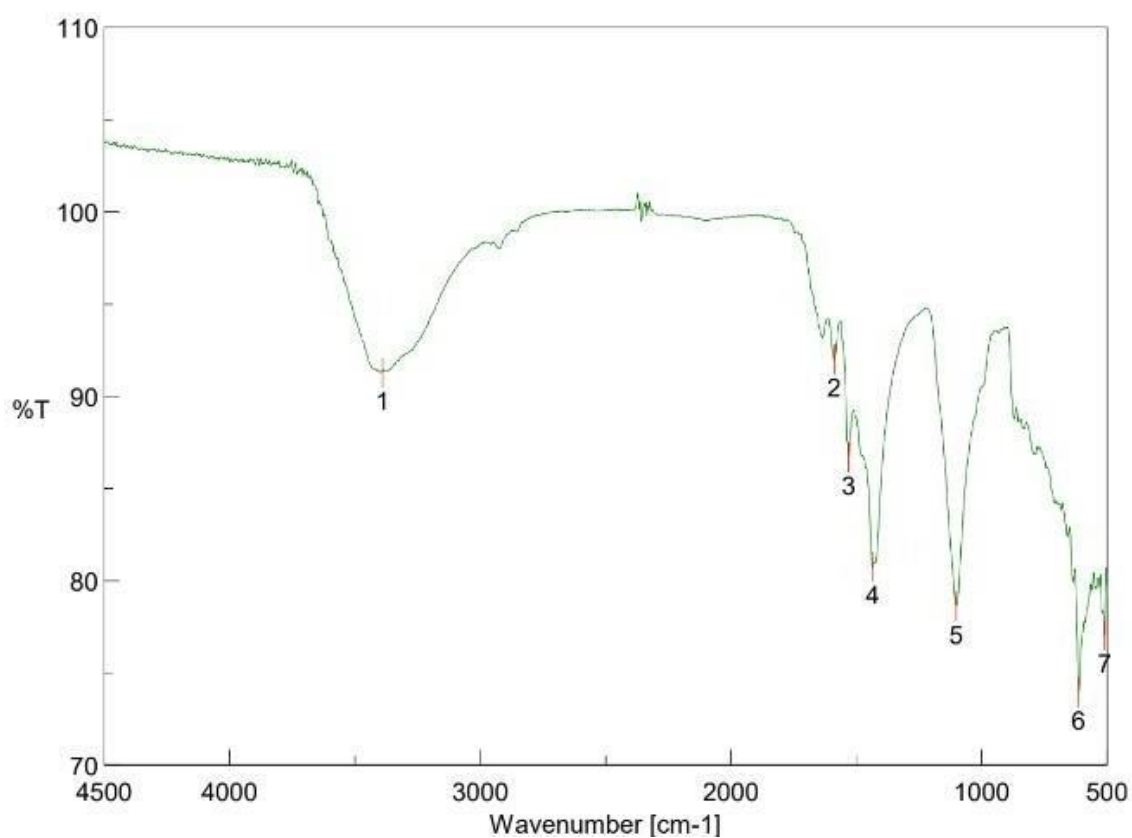
5.4 FTIR RESULT



Figure, 5.4 Normal picochlorum FTIR

Table 5.4 Normal picochlorum peak

No.	Position	Intensity
1	3323.71	56.5422
2	2362.37	95.6676
3	1643.05	71.2819
4	1434.78	85.7179
5	1094.4	58.4375
6	527.436	40.0011



Figure, 5.4.1 0.80% picochlorum FTIR

Table 5.4.1 0.8% Sodium bicarbonate picochlorum peak

No.	Position	Intensity
1	3390.24	91.2907
2	1588.09	91.9819
3	1531.2	86.6936
4	1434.78	80.7552
5	1101.15	78.6213
6	613.252	73.9354
7	510.08	77.0219

5.5 STRUCTURE OF THE MICRO ALGAE

Micro algae was identify under the microscope.

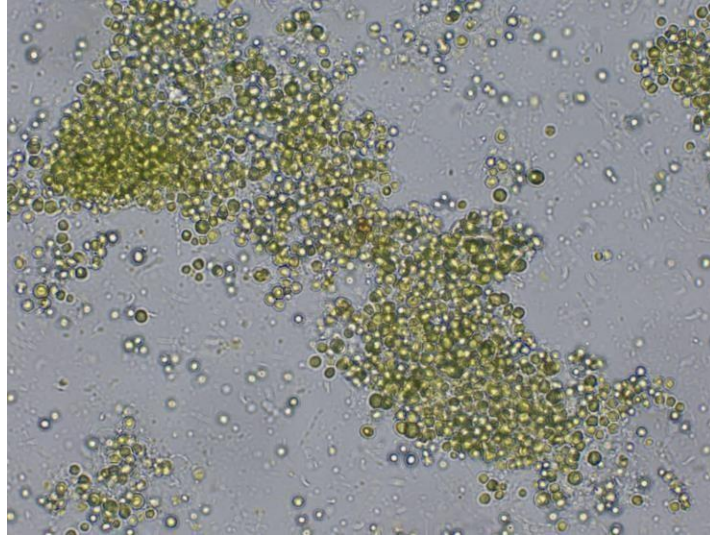


Fig 5.5.1 The image of Picochlorum sp of under microscope

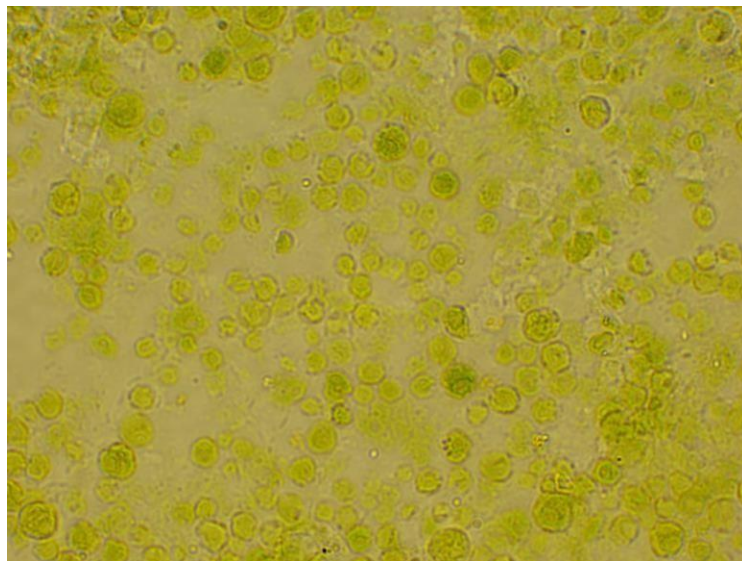


Figure 5.5.2: The image of chlorella sp of under microscope

The comprehensive analysis clearly demonstrated that both Picochlorum and Chlorella species outperformed the 0.8% concentration of sodium bicarbonate medium when compared to standard medium. The microalgae parameters exhibited significantly higher levels under the influence of Picochlorum and Chlorella specie.

CHAPTER-6

6. SUMMARY AND CONCLUSION

This study explores micro algal strains having high growth and CO₂ fixation rate can be cultured on large scale and used throughout the year for the production of saturated as well as long chain unsaturated fatty acids. The potential use of unconventional feed ingredients such as algae, as feed inputs as a replacement of high cost feed stuffs such as fishmeal. The present study two different micro algal culture were used from that *Picochlorum* and *Chlorella* possess better production of PUFA content under using Sodium bicarbonate as a sole inorganic carbon source. As the result of the study compare to *Chlorella* *Picochlorum* shows observe more carbon.

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