PERFORMANCE CHARACTERIZATION OF SELECTED INDIGENOUS MICROALGAE ISOLATED FROM DIFFERENT MARINE AND FRESHWATER SOURCES OF BANGLADESH

Proma Dey

Roll No. 0122/02

Registration No. 1099

Session: 2022-2023

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Aquaculture

Department of Aquaculture

Faculty of Fisheries

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

July 2023

Authorization

I hereby declare that I am the sole author of the thesis. I also authorize the Chattogram Veterinary and Animal Sciences University (CVASU) to lend this thesis to other institutions or individuals for the purpose of scholarly research. I further authorize the CVASU to reproduce the thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

I, the undersigned, and author of this work, declare that the **electronic copy** of this thesis provided to the CVASU Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

The author

July, 2023

PERFORMANCE CHARACTERIZATION OF SELECTED INDIGENOUS MICROALGAE ISOLATED FROM DIFFERENT MARINE AND FRESHWATER SOURCES OF BANGLADESH

Proma Dey

Roll No. 0122/02

Registration No. 1099

Session: 2022-2023

This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made.

……..

Supervisor

Department of Aquaculture

……

(Dr. Helena Khatoon)
Chairman of the Examination Committee

Department of Aquaculture
Faculty of Fisheries
Chattogram Veterinary and Animal Sciences University
Chattogram-4225, Bangladesh

JULY 2023

ACKNOWLEDGEMENTS

First and foremost, all praises to the **Almighty** for giving me the health, ability and strength to accomplish this MS research work as well as the thesis on due time. Without His help, I would not be able to finish this thesis work.

I would like to convey my earnest gratitude to my beloved parents and siblings for their ultimate understanding, inspirations, moral support, kindness and blessings, forbearance and endless love to complete this study.

I sincerely express my deepest sense of gratitude, sincere appreciation, profound regards and immense indebtedness to my honorable teacher and research supervisor **Dr. Helena Khatoon**, Associate Professor, Department of Aquaculture, Chattogram Veterinary and Animal Sciences University for her valuable suggestions, intellectual guidance, constructive and constant inspiration throughout the entire period of the study and in preparations of this manuscript.

My appreciation and gratitude is extended to **University Grants Commission**, Bangladesh and the **Ministry of Science and Technology** for funding required to accomplish my research work.

I wish to express my heartfelt gratitude to our honorable Vice-Chancellor **Prof. Dr. A.S.M Lutful Ahasan** and respective Dean **Prof. Dr. Md. Nurul Absar Khan** for their supportive administrative coordination to fulfill my MS research work.

I would like to express appreciation to the **Diseases and Microbiology Laboratory**, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University for providing laboratory facilities for this study.

At the end, I would like to express cordial thanks to my loving friends, all laboratory attendant and well-wishers for their Co-operation, cheerfulness and inspiration during the course of this study. Last but not least, to everyone who has supported me directly or indirectly in completing this thesis.

The Author

July, 2023

Table of Contents

List of Figures

List of Tables

List of Abbreviations

Words	Abbreviation
sp.	Species
CD	Cell Density
OD	Optical Density
DO	Dissolve Oxygen
BBM	Bold Basal Media
Ppt	Parts Per Thousand
ppm	Parts Per Million
rpm	Rotation Per Minute
rcf/ ×g	Relative Centrifugal Field
pH	Power of Hydrogen
ºC	Degree Celcius
$\mu Em^{-2} s^{-1}$	Microeinsteins Per Second Per Square Meter
VP	Volumetric Productivity
LP	Lipid Productivity
AP	Areal Productivity
DB	Dried Biomass
SGR	Specific Growth Rate
PC	Phycocyanin
APC	Allophycocyanin
PE	Phycoerythrin
Hr	Hour
µ	Micro
mL	Mili Liter
L	Liter
%	Percentage
g	Gram
lbs	Pound
v/v	Volume/Volume
TAN	Total Ammonium Nitrogen
Min	Minute
Mg	Mili Gram
Nm	Nanometer
Cm	Centimeter

Abstract

This study includes information on isolating some indigenous freshwater and marine microalgae and determining their growth rate, pigmennts, and proximate composition in order to take advantage of the great potentiality of local microalgae. For microalgae isolation streak plating method in agar, serial dilution and capillary pipetting was performed and for the evaluation of growth performance. Selected microalgae (*Chlamydomonas* sp., *Navicula* sp., *Gonyostomum* sp., *Nannochloropsis* sp., *Choricystis* sp., *Chromochloris* sp., *Tetraedron* sp., *Chlorobotrys* sp. *Coelastrella* sp., *Kirchneriella* sp. *Oscillatoria* sp1 and *Oscillatoria* sp4) were mass cultured in commercial Bold Basal Medium (BBM) and Conway Medium, harvested at their stationary phases to determine the pigments content (chlorophyll-a, chlorophyll-b, chlorophyll-c, carotenoid and phycobiliproteins) and proximate composition (protein, lipid and carbohydrate). Results showed that, onset of stationary phase (9–14 days) varied among the fourteen species where in the stationary phase, significantly higher ($p<0.05$) cell density was observed in *Gonyoatomum* sp. All microalgae showed chlorophyll-a as the main pigment component, where *Oscillatoria* sp2 showed significantly highest ($p<0.05$) chlorophyll-a (22.72±0.04 µg/mL). On the other hand, highest chlorophyll b (2.75±0.07 µg/mL) and carotenoid content (6.888±0.08 µg/mL) was recorded in *Chlamydomonas* sp. Moreover, total phycobiliproteins was also significantly higher ($p<0.05$) in *Oscillatoria* sp4. (89.80±0.12 mg/g) and significantly lowest ($p<0.05$) in *Nannochloropsis* sp. (0.89±0.04 mg/g). Significantly higher and lower ($p<0.05$) amount of total protein was recorded in *Navicula* sp. (59.5123±0.78% dry weight) and *Krichneriella* sp. (17.276±0.76 % dry weight), respectively. Similarly, highest and lowest ($p<0.05$) amount of lipid content was recorded in *Gonyoatomum* sp. (27.4±0.695 dry weight) and *Oscillatoria* sp3 (9.07±0.07%dry weight), respectively. In addition, carbohydrate content was highest ($p < 0.05$) in *Kirchneriella* sp. The results on amino acids and fatty acids revealed significant differences ($p < 0.05$) across these experimental species in numerous instances.

Keywords: Isolation, Microalgae, Growth, Pigments, Proximate, Fatty acid, Amino acid

Chapter-1: Introduction

Recent years have seen a significant increase in interest in microalgae due to their enormous application possibilities in the biopharmaceutical, nutraceutical, and renewable energy industries. Khan et al., 2018). Because of the high nutritional value, favorable impact on the growth rate of aquatic species due to increased triglyceride and protein deposition in muscle, improved resistance to disease, decreased nitrogen output into the environment, omega-3 fatty acid content, physiological activity, and carcass quality, and potential replacement of common feed stuff, microalgae have the potential to reduce the dependence on conventional raw materials in aquafeed. (Becker, 2004). Additional benefits provided by microalgae include fast growth rates, high productivity, no need for agricultural land, quick harvest cycles, ease of cultivation, high lipid content, and high photosynthetic efficiency (Nascimento et al., 2015). Growth and nutritional property of microalgae varies between species to species. The first and most crucial step in the bioprospecting of microalgae for any commercial use is the selection of the species or strain (Borowitzka, 2013). For the use of microalgae in aquaculture, monitoring of cell growth is considered a fundamental component (Santos-Ballardoa, 2015). According to Safi et al. (2013), one of the key factors determining the nutritional value of microalgae is their protein level. According to Hu et al. (2015), the lipid content of microalgae can range from 20% to 50% of dry weight, and the high oil content of many microalgae species has been used to produce biofuel (Hussain et al., 2017). Although carbohydrates have a lower energy value than other microalgal substances, they constitute the initial raw material used in the biotechnological conversion process to create biofuels (Andreeva et al., 2021). Chlorophylls, carotenoids, and phycobilins are the main pigment groups found in microalgae, and they are regarded as one of the most important products from microalgae (Granado-Lorencio et al., 2009).Chlorophyll and carotenoid have antioxidant properties, are widely used as natural food coloring agents (Hosikian et al., 2010), and play a significant role in the photosynthesis of algae and photosynthetic bacteria (Lamers et al., 2012). They are also used in a variety of pharmaceutical products (Bhagavathy and Sumathi, 2012). According to Ba'saca-Loya et al. (2009), phycobiliproteins are a class of proteins containing covalently bonded linear tetrapyrrole chromophoric groups. According to Wiedenmann (2008),

the most prevalent phycobiliproteins include phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC).

The amount of lipids produced under stress circumstances ranged from 4.5 to 7.5 t ha/year (Choong et al., 2020), or between 20% and 50% of the dry weight of the biomass (Wang et al., 2016), which is anticipated to be between 15 and 25 t ha/year. Additionally, a number of dietary components, including phosphorus, carbon, nitrogen and iron, are acknowledged as some of the most significant influences on the yield of biomass and the accumulation of lipids (White et al., 2013).

For the optimization of mass microalgal production, it is critical to have a thorough understanding of how a particular microalgal strain responds to different culture conditions, such as nutrition delivery (Hyka et al., 2013). There is a shortage of high-quality microalgae available in hatcheries, and a number of species that were once utilized in such hatcheries have since been abandoned due to their poor nutritional value (Ponis et al., 2006). Therefore, choosing an algal species and strain can be regarded as the first and most crucial stage in designing a dependable and financially feasible technique for the mass production of microalgae. To take advantage of the nutritional features, the top performing tropical strains should be chosen because they are better acclimated to the same environment, perform better, and are more resilient. Despite being a crucial component of microalgal technology, measuring and monitoring cell growth has received little attention (Havlik et al., 2013).To utilize microalgae commercially in aquaculture, pharmaceuticals and other sectors, screening of microalgal species should be done through performance characterization. The characterisation of microalgae in Bangladesh has, however, received very little study attention. In order to choose possible strains that will provide competitive advantages for any commercial usage, the current approach compares the growth rate and nutritional characteristics of various freshwater and marine microalgae (*Chlamydomonas* sp., *Navicula* sp., *Gonyostomum* sp., *Nannochloropsis* sp., *Choricystis* sp., *Chromochloris* sp., *Tetraedron* sp., *Chlorobotrys* sp. *Coelastrella* sp., *Kirchneriella* sp. and four strains of *Oscillatoria* sp1, *Oscillatoria* sp2, *Oscillatoria* sp3 and *Oscillatoria* sp4).

The ultimate aim of this research is to select potential strains of microalgae for commercial application with the characteristics of fast growth, suitable pigments and proximate composition. The specific objectives are:

- To isolate and identify microalgae from different marine and freshwater sources of Bangladesh
- To determine the growth phases of selected isolated microalgal species
- To determine pigment, proximate and bio-chemical composition of selected isolated microalgal species

Chapter-2: Review of Literature

2.1 Microalgae

One of the contemporary biotechnologies is microalgae culture. The first unialgal cultures were produced by Beijerinck in 1890 using Chlorella vulgaris, and Warburg pioneered the use of such cultures for research into plant physiology in the early 1900s. Many of these early experiments are summarized in the famous book compiled by Burlew (1953), which was published after 1948 at Stanford (USA), Essen (Germany), and Tokyo. With research on the utilization of algae as microbial protein sources and as photosynthetic gas exchangers for space travel, interest in applied algal culture has remained strong. *Chlorella* was first grown commercially on a big scale in Japan in the early 1960s. In the early 1970s, a *Spirulina* harvesting and cultivation plant was established in Lake Texcoco, Mexico. By 1980, 46 large-scale factories in Asia were producing more than 1000 kg of microalgae (primarily *Chlorella*) per month (Kawaguchi, 1980), and in 1996, about 2000 t of *Chlorella* were traded in Japan alone. Dai Nippon Ink and Chemicals Inc. established a commercial *Spirulina* plant in Thailand. In the USA, other *Spirulina* plants have been developed, including Microbio in California and Cyanotech in Hawaii. When Western Biotechnology Ltd. and Betatene Ltd. (now Cognis Nutrition & Health) built manufacturing facilities in Australia in 1986, *Dunaliella salina*'s use as a source of -carotene rose to become the third-largest microalgae sector. Other commercial facilities in Israel and the USA quickly followed these. Around the same time as these algae, large-scale production of cyanobacteria (blue-green algae) started in India. In the USA and India, numerous plants that produce *Haematococcus pluvialis* as a source of astaxanthin have recently been created. As a result, in a relatively short time of only around 30 years, the microalgal biotechnology industry has greatly expanded and diversified (Perumal et al., 2012).

2.1.1 *Chlamydomonas* sp.

Taxonomic Classification:

Kingdom: Plantae

Phylum: Chlorophyta

Class: Chlorophyceae

Order: Chlamydomonadales

Family: Chlamydomonadaceae

Genus: *Chlamydomonas* (Ehrenberg, 1834)

Green algae belonging to the genus *Chlamydomonas* can be found in freshwater, saltwater, and even snow. Hoham et al., 2002). *Chlamydomonas* are unicellular, motile, spherical or slightly cylindrical creatures with or without papillae. Chloroplasts are often cup-shaped and green in color (Guiry, 2007). The genus is distinguished by having two anterior flagella that are equal in length to one another (Harris, 2009). When one of the flagellar microtubules is injured, the cell may disintegrate both of them to give extra parts for rebuilding the damaged microtubules (Gelfand, 1991)

2.1.2 *Navicula* sp.

Taxonomic Classification:

Kingdom: Chromista

Phylum: Ochrophyta

Class: Bacillariophyceae

Order: Naviculales

Family: Naviculaceae

Genus: *Navicula* (Bory de Saint-Vincent, 1822)

Navicula is a single-living, motile diatom. It has a boat-like shape and a yellowish brown tint. It has the raphe that surrounds the cell wall, which is found in the cytoplasm close to the cell wall. (Bellinger and Sigee, 2010). On each side of the raphe, there are two chloroplasts (Cupp, 1943). Chloroplast is present along with the pyrenoids and a nucleus with two sizable vacuoles are all also present. True *Navicula* species exhibit lanceloate valves with narrow axial areas surrounded by thin striae that are radiating at the center but parallel to the cell apices (Bellinger and Sigee, 2010).

2.1.3 *Gonyostomum* sp.

Taxonomic Classification:

Phylum: Ochrophyta

Class: Raphidophyceae

Order: Raphidomonadales

Family: Vacuolariaceae

Genus: *Gonyostomum* (Diesing, 1866)

The vegetative cells of *G.* semen are drop-shaped. The cells are up to 100 µm long, (Drouet et al., 1935). As they lack a cell wall and are solely protected by a cell membrane, they can vary greatly in size and shape. Numerous oval, bright green chloroplasts are present throughout the cells. Chlorophyll a is the pigment responsible for its bright green color. The pigments chlorophyll c1 and c2, diadinoxanthin, trans-neoxanthin, cis-neoxanthin, α and β carotene, violaxanthin, zeaxanthin and alloxanthin are also present in *G.* semen (Sassenhagen et al., 2014). Trichocysts, which are tiny organelles found beneath the cell membrane, erupt under physical strain and emit slimy threads (Lebret et al., 2012).

2.1.4 *Nannochloropsis* sp.

Taxonomic Classification:

Kingdom: Chromista

Phylum: Ochrophyta

Class: Eustigmatophyceae

Order: Eustigmatales

Family: Monodopsidaceae

Genus: *Nannochloropsis* (Hibberd, 1981)

Unicellular, planktonic *Nannochloropsis* sp. have either 2-4 m diameter subspherical or 3-4 1.5 m cylindrical cells (Al-Hoqani et al., 2016). The *Nannochloropsis* sp. are

small, non-motile spheres that can't be separated by either light or electron microscopy (Andersen et al., 1998). They develop primarily in marine habitats but can also be found in fresh and brackish water. they typically have a yellow-green chloroplast with the primary pigments being chlorophyll a and the xanthophylls violaxanthin and vaucheriaxanthin (Lubián et al., 2000).

2.1.5 *Choricystis* sp.

Taxonomic Classification:

Kingdom: Plantae

Phylum: Chlorophyta

Class: Trebouxiophyceae

Order: Incertae sedis

Family: Coccomyxaceae

Genus: *Choricystis* (Fott, 1976)

The Coccomyxaceae family contains the genus *Choricystis*. The predominant production of 2 (occasionally 4) autospores per sporangium and the absence of pyrenoids are characteristics of *Choricystis.* Many different *Choricystis* species can be found in freshwater lake plankton, but they can also occasionally be found in terrestrial or aerial habitats connected to various substrates. The genus *Choricystis* is known for its small cells, lack of mucilage, absence of pyrenoids, and autosporulation, which is frequently characterized by the production of two autospores (Zidarova et. al., 2009).

2.1.6 *Chromochloris* sp.

Taxonomic Classification:

Kingdom: Plantae

Phylum: Chlorophyta

Class: Chlorophyceae

Order: Sphaeropleales

Family: Scenedesmaceae

Genus: *Chromochloris* (Kol and Chodat, 1934)

Chromochloris sp. is a straightforward 4-m-long, unicellular, haploid, coccoid alga with several mitochondria, which are commonly seen as a tubular network, and a single interconnected chloroplast that makes up about 40% of the cell volume and includes starch granules. The majority of mitochondria are closely related to either the nucleus or the chloroplast. Neither pyrenoids nor flagella (cilia) were seen, though. Although it can also split into 16, 32, or 64 cells, *Chromochloris* sp. typically produces two or four daughter cells from each division. Although the timing of cell division is unknown to be regulated, it is found that the daughter cells are of the same size (Roth et al. 2017).

2.1.7 *Tetraedron* sp.

Taxonomic Classification:

Kingdom: Plantae

Phylum: Chlorophyta

Class: Chlorophyceae

Order: Sphaeropleales

Family: Hydrodictyaceae

Genus: *Tetraedron* (Kützing, 1845)

The coccal, unicellular green algae genus *Tetraedron* is easily recognized by its distinctive polygonal shape. It belongs to the family of common freshwater planktonic algae that is widely found in tropical and temperate environments (Stoyneva et al., 2012). The *Tetraedron* consists of solitary, unattached cells that can be triangular and flat, pyramidal, polyhedric. The angles can be whole, with or without spines, or they can be differently lobed to create dichotomous or trichotomous processes with spine-tipped tips (Prescott, 1951). Cells are 12 µm in diameter. One pyrenoid and one parietal chloroplast are present in each cell (Jadhavar and Papdiwal, 2016). Like many

of its relatives in the Chlorococcales, Tetrahedron reproduces asexually by enlarging polyhedral cells to generate autospores, which can range in number from 4 to 32 depending on the species. This organism can also reproduce by creating motile zoospores. (Pickett-Heaps, 1972; Starr, 1954).

2.1.8 *Chlorobotrys* sp.

Taxonomic Classification:

Kingdom: Chromista

Phylum: Ochrophyta

Class: Eustigmatophyceae

Order: Eustigmatales

Family: Chlorobothryaceae

Genus: *Chlorobotrys* (Bohlin, 1901)

The Eustigmatophyceae family contains the genus *Chlorobotrys*. In culture, the cells are essentially spherical with an average diameter of 12-5 zm (range: 9.25-16.6 zm). Their adjacent faces are gently flattened against one another and they are typically seen in pairs. The cell wall is smooth, colorless, thick, and somewhat rigid, yet it also exhibits a noticeable degree of flexibility. The refractile appearance of the cell wall under a light microscope adds another distinctive feature. *Chlorobotrys* sp. cells are distinguished by the presence of a single, substantial, colorful, refractile globule in each one, in addition to the mucilage and cell wall (Hibberd, 1974).

2.1.9. *Coelastrella* sp.

Taxonomic Classification:

Kingdom: Plantae

Phylum: Chlorophyta

Class: Chlorophyceae

Order: Sphaeropleales

Family: Scenedesmaceae

Genus: *Coelastrella* (Chodat, 1922)

Coelastrella species are elliptical until citriform and coccoid. They can be found as aggregations of a few cells or as single-celled microalgae. The sculptured cell walls of these species, which have 16–40 meridional ribs and either have or do not have polar thickenings, make them unique (Uzunov et al., 2008; Kaufnerová and Eliá, 2013). The cell wall has two layers. The cells are uninucleate, with numerous noticeable vacuoles, one parietal cup-shaped chloroplast, and one pyrenoid surrounded by two or three starch plates (Tschaikner et al., 2007a). Asexual reproduction occurs when the parental cell wall ruptures, releasing 2–16 autospores (Guiry and Guiry 2020). The distinctive differences in the wall sculptures, together with other morphological characteristics including cell morphology, chloroplast, and pyrenoid structures, are helpful for identifying the species (Hanagata et al., 1996; Tschaikner et al., 2007b).

2.1.10 *Kirchneriella* sp.

Taxonomic Classification:

Kingdom: Plantae

Phylum: Chlorophyta

Class: Chlorophyceae

Order: Sphaeropleales

Family: Selenastraceae

Genus: *Kirchneriella* (Schmidle, 1893)

Colonies of cells often make up *Kirchneriella*, which is covered in a thin layer of mucilage. Typically, a colony contains 4-16 individuals, though occasionally they live alone. A single chloroplast with one pyrenoid is found inside each crescent-shaped cell. They are dispersed erratically throughout the mucilage (Shubert and Gärtner, 2015).The size and form of a cell can be used to identify between different species (Komárek and Fott, 1983). Autospores that are arranged serially within the mother

cell are used by *Kirchneriella* to replicate. They are expelled when the mother cell wall ruptures (Da Silva et al., 2013).

2.1.11 *Oscillatoria* sp.

Taxonomic Classification:

Kingdom: Bacteria

Phylum: Cyanobacteria

Class: Cyanophyceae

Order: Oscillatoriales

Family: Oscillatoriaceae

Genus: *Oscillatoria* (Gomont, 1892)

Oscillatoria is a genus of blue-green algae.This filamentous unbranched algae reproduces by fragmentation. The steady, repetitive oscillation that gives it its name is considered to be caused by mucilage secretion that pulls the filament away from the direction of excretion. Trichomes, which are composed of rows of cells, make up each filament of oscillatoria. The trichome's tip oscillates back and forth like a pendulum. Reproduction only occurs through vegetative methods. The filament typically splits into a number of pieces known as hormogonia. Each hormogonium is made up of one or more cells that divide in one direction to form a filament (Britannica, 2016).

2.2 Role of Microalgae

A microalga is a significant source of nutrition and is frequently employed, either directly or as an additional source of fundamental nutrients, in the aquaculture of other organisms. Microalgae are used as a source of food in aquaculture farms that raise larvae of molluscs, echinoderms, crustaceans, and fish. A vital food supply for shellfish aquaculture is a low bacterial and high microalgal biomass. Microalgae can serve as the initial link in a series of other aquaculture activities. For instance, a microalga plays a significant role in brine shrimp farming as a food supply. In order to provide a convenient source of live feed for the aquaculture of larval fish and crustaceans, brine shrimp develop latent eggs known as cysts that may be maintained

for extended periods of time and then hatched on demand. The visual appeal of finfish raised in captivity is one of the other uses of microalgae in aquaculture. One such instance is the employment of a microalga to make salmon flesh pinker in salmon farming. This is accomplished by supplementing the diet of farmed fish with natural pigments that contain carotenoids, such as Astaxanthin made from the microalgae Haematococcus (Perumal et al., 2012).

2.3 Isolation of Microalgae

The first and most important step in the bioprospecting of microalgae for any commercial use is the selection of the species or strain (Barclay and Apt, 2013). Sample collection, isolation, purification, identification, maintenance, and characterization of prospective products are among the stages involved in the screening of microalgae species (Gong and Jiang, 2011). Depending on the types of microalgae, 18S ribosomal RNA genes for eukaryotic microalgae and 16S ribosomal RNA for prokaryotic microalgae can be used to sequence algal taxonomy. There aren't many studies on the isolation and molecular characterization of the algae in Bangladesh, despite the fact that this method is now widespread in other parts of the world. Tarin et al. are the first to report on microalgae isolation in Bangladesh. In order to assess their potential as feedstock and biofuel production, they isolated and characterized *Chlorella vulgaris* and *Anabaena variabilis* from natural and manmade water bodies at Dhaka University and Khulna in 2016. By examining partial 18S rDNA sequences, *Pithophora polymorpha* and *Spirogyra maxima*, two newly reported microalgae from Bangladesh, were successfully identified (Alfasane et al., 2019). Islam et al. (2021) have described four marine microalgae (*Chlorella* sp., *Nannochloropsis* sp., *Tetraselmis* sp., and *Chaetoceros* sp.) isolated from Bangladesh's Cox's Bazar coast in terms of growth performance, pigments, and nutritional composition. In Bangladesh, local microalgae and their potential, particularly in freshwater habitats, have received very little attention.

2.4 Growth factors of microalgae

Growth of microalgae is affected by some factors such as length of photoperiod, temperature, pH and light intensity (Wahidin et al., 2013).

2.4.1 Light

Intensity of light is an important factor for microalgae cultivation. Generally, for biomass growth, microalgae depend on enough carbon source (about 40-50% carbon) and light to carry out photosynthesis process (Moheimani, 2005). Requirement varies on the basis of the conditions. For an Erlenmeyer flask; 1000 lux is suitable where 5000-10000 lux required for larger volume (FAO, 1996). The use of fluorescence light for indoor culture can promote a better growth and cell division of microalgae (Laing, 1991). However, maximum exposure of light can become limiting factor to microalgae density. Kaewpintong (2004) reported that cell growth rate increase depending on light intensity, but until a definite stage and after that the growth decrease. It is supported by Lavens and Sorgeloos (1996) that higher light intensity may result in photo-inhibition.

2.4.2 Temperature

Temperature is the 2nd most prior factor for culturing microalgae. Besides, above 27 ºC will make the algae die (Laing, 1991). Various microalgae are viable with very low temperature than its optimum (upto 15 ºC lower), but crossing limit above by 1-4 ºC can create a great damage (Teresa et al., 2010). Changing of light intensity will influence temperature which indirectly affects growth of microalgae (Huang et al., 2013). Optimum temperature of microalgae culture ranged from 20 to 24 ºC.

2.4.3 pH

pH plays an important role for culturing microalgae. Many cellular activities disrupt in microalgae cell because of pH level failure (Lavens and Sorgeloos, 1996). pH is directly related with CO_2 accessibility and for that reason it is also essential for photosynthesis. Higher pH concentration may reach at limiting values at pH 9 (FAO, 1996).

2.4.4 Nutrient composition of media

Nitrate, phosphate and silicate are some examples of macronutrients essential for microalgae growth (Lavens and Sorgeloos, 1996). Nitrogen is considered as the most important and common type of nutrient in the culture medium (Thompson et al., 1989). Zinc, cobalt, boron, iron and manganese are the most commonly used trace metals (Probert and Klaas, 1999). Others are thiamine (B1), cyanocobalamin (B12) (FAO, 1996). Lourenço (2006) reports direct interaction of nutrients on growth of

microalgae. Commercial media contain all types of micronutrients and macronutrients to help the growth of microalgae.

2.4.5 Mixing and aeration

Homogenize mixing and aeration is also an important factor for microalgae growth. Kaewpintong (2004) found fine development of cell growth in an aerated culture system (bioreactor) than non-aerated system.

On the contrary, excess liquid pressure, velocity, over turbulence and excess bubbles can create stress to the cell which result cell damage (Eriksen, 2008). It is also important to prevent sedimentation. So, to ensure better contacts with cell and nutrients it is also important to maintain homogenous conditions through balance aeration.

2.5 Microalgal growth

Five phases can be seen in the growth of microalgae (Lavens and Sorgeloos, 1996). These can be categorized into the following phases: lag phase, exponential phase, linear growth phase, stationary growth phase, decline or death phase, and so forth. Microalgae exhibit each phase when the conditions are right.

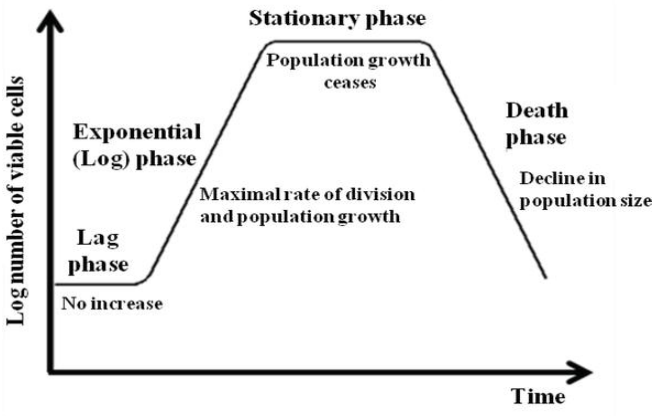


Figure 12: Microalgae growth curve (Teresa et al., 2010)

Here, the cell is in the lag phase, when it is still alive but not yet prepared to divide. Cell density started to rise over the course of the second phase. During the exponential development phase, microalgae frequently quadruple their biomass (Chisti, 2007). They enter a stationary phase where the pace of growth is balanced. Microalgae are most dense in this phase compared to all other phases. Finally, the dying stage, during which the cell density began to decrease. Growth is now starting

to be constrained by physical parameters like pH, CO2, and nutrition levels (FAO, 1996).

2.6 Pigments of Microalgae

2.6.1 Chlorophyll

Chlorophylls, carotenoids, and phycobilins are the main pigment groups found in microalgae, and they are regarded as one of the most important products from microalgae (Granado-Lorencio et al., 2009). The two primary forms of chlorophylls are chlorophyll-a and chlorophyll-b, and chlorophyll is one of the important bioactive substances that can be isolated from microalgae (Aris et al., 2010). Chlorophyll-b participates indirectly in photosynthesis by transforming the light it absorbs into Chlorophyll-a, which is the major pigment responsible for turning light energy into chemical energy (Nayek et al., 2014). Having antioxidant properties, chlorophyll and its derivatives are employed as natural food coloring agents (Hosikian et al., 2010) and are also commonly found in pharmaceutical products (Bhagavathy and Sumathi, 2012).

2.6.2 Carotenoid

Carotenoid is a naturally occurring, lipid-soluble pigment that ranges in color from yellow to red. It predominates in plants and is crucial for photosynthesis in both algae and bacteria (Lamers et al., 2012). However, a number of variables, including salinity, light intensity, nutritional shortage, and temperature, have an impact on the formation of carotene. (2009) Kleinegris et al. Natural foods including egg yolk, chicken, and fish have been found to utilise carotenoids widely as coloring agents. More than 750 carotenoids have been discovered, but only a select few, including astaxanthin, canthaxanthin, lutein, lycopene, and -carotene, have been employed commercially (Vlchez et al., 2011). The main benefit of employing microalgae as a carotenoids carrier is their beneficial effects on human health because of the abundance of other antioxidants they contain.

2.6.3 Phycobiliproteins

Phycobiliproteins are a class of proteins that have linear tetrapyrrole chromophoric groups covalently linked (Ba´saca-Loya et al., 2009). Phycoerythrin (PE), Phycocyanin (PC), and Allophycocyanin (APC) are the three most prevalent

phycobiliproteins (Wiedenmann, 2008). Phycocyanin is a pigment used commercially in the pharmaceutical sector because it shields the photosystems from free radicals and can stop the oxidative damage caused by free radicals. According to Mathivanan et al. (2015), phycocyanin is a water-soluble, intensely fluorescent, blue-green light-harvesting pigment that is unique to some algae species and is a member of the Rhodophyta and Cryptophyta families. It is specifically found in cyanobacteria. Phycobiliproteins are widely employed as nutraceuticals, natural colors, and in various biotechnological applications in the food, cosmetics, pharmaceutical, and diagnostics industries (Becker, 2004).

2.7 Proximate composition of microalgae

The approximate composition of microalgae is typically the percentage composition of several essential elements like water, protein, fat, carbohydrate, and ash(Ganguly et al., 2018). When compared to lipid and carbohydrate, the protein content of microalgae often makes up a larger fraction (Lavens and Sorgeloos, 1996). Microalgae, particularly Chlorella sp., have a total protein concentration of between 43-50%(Phukan et al., 2011). Some algae, especially blue-green and green algae, have very high protein contents that can be employed as functional food ingredients, ranging from 40 to 60% (of dry matter). In terms of protein content, amino acid purity, and nutritional acceptability, algal proteins have a high nutritional value (Reyes et al., 2012). The lipid content of microalgae typically ranges from 20 to 50% of the dry weight of the cell, but it can reach as high as 80% in some circumstances **(**Michael and Borowitzka, 2018). Microalgae can overproduce lipids or carotenoids when they are under stress, such as when there is a lack of nutrients, high salt, or intense light.

The species of microalgae in biomass depends on the type of microalgae and growth conditions. Although carbohydrates have a lower energy value than other microalgal substances, they constitute the initial raw material used in the biotechnological conversion process to create biofuels (Andreeva et al., 2021; Aytenfisu et al., 2018). The species of microalgae and growing conditions can affect the number of lipids, the proximity or location of double bonds in the carbon chain, and the amount of carbohydrates in the biomass (Villarruel-Lopez et al., 2017; Park et al., 2014).

In order to discover the possible species for various aquaculture applications, the goal of this project is to isolate microalgae from Kaptai Lake, Halda River, Sonadia, Maheshkhali and Naf estuary. Additionally, isolated microalgae will be characterized based on growth, pigments, and proximate composition.

Chapter 3: Materials and Method

3.1 Sample collection:

The microalgae samples were collected from March to July from different freshwater bodies and marine water stations of Chattagram, Bangladesh. Freshwater microalgae samples were collected from Halda River($22^{o}51'$ N, $91^{o}84'$ E), and Kaptai Lake ($22^{o}64'$ N, $92^{o}19'$ E), Sonadia (21.28′ N, 91.5′ E), Maheshkhali($21^{o}31'$ N, $91^{o}59'$ E), and the Naf estuary ($20^{o}47'$ N, $92^{o}28'$ E), were the three locations of Bay of Bengal from where marine microalgae samples were collected.

3.2 Determination of water quality parameters

Some water quality parameters such as water temperature, pH, dissolved oxygen (DO) and salinity were measured on spot using glass thermometer, a handheld pH meter (pHep-HI98107, HANNA, Romania), dissolve oxygen meter (DO-5509, Lutron) and a handheld ATC refractometer (YEGREN) respectively. Before usage, all the instruments were calibrated. In addition, total ammonium nitrogen (TAN), nitrite–nitrogen (NO2-N) and soluble reactive phosphorous (SRP) were later determined on laboratory by using Parsons et al. (1984) analytical methods.

3.2.1 Determination of total ammonia nitrogen (TAN)

Total ammonia nitrogen was determined using the chemical method of Parson et al. (1984). Total ammonia nitrogen was determined according to Parson et al. (1984). Standard stock solution was prepared by weighing 9.343 g of anhydrous grade $(NH_4)_2SO_4$ (dried at 110°C for 1 hour, cooled in desiccator before weighing) and dissolving in 1000 ml deionized water. From the stock solution (1000 mg L^{-1} of total ammonia-nitrogen), a series of standard solutions (0.01, 0.03, 0.05, 0.07, 0.1, 0.3, 0.5 and 1.0 mg L^{-1}) were prepared by mixing with appropriate ratio of deionized water.

Samples and standard solutions (10 ml) were placed in test tube. Then 0.4 ml of phenol solution (20 g of analytical grade phenol was dissolved in 200 ml of 95% v/v ethyl alcohol) and 0.4 ml of sodium nitroprusside (1 g of $Na_2[Fe(CN)_5\ NO]_2H_2O$ was dissolved in 200 ml of DDH_2O water) were added in each testube. Finally, 1 ml of oxidizing solution was added and cooled at. The test tubes were covered with parafilm (the color is stable for 24 hours after the reaction period) and kept at room

temperature (20-27°C) for 1 hour. After 1 hour, the extinction was measured at 640 nm using spectrophotometer (Nano Drop Spectrophotometer, Model-Nanoplus, Germany). Oxidizing solution was prepared by mixing 100 ml of alkaline reagent (100 g of sodium citrate and 5 g of sodium hydroxide were dissolved in 500 ml of DDH2O) and 25 ml of sodium hypochlorite solution [commercial hypochlorite (e.g. clorox) which should be about 1.5 N].

3.2.2 Determination of nitrite-nitrogen (NO_2-N)

Nitrite was determined using the chemical method developed by Parsons et al. (1984). Standard stock solution was prepared by weighing 4.93 g anhydrous grade $NaNO_2$ (dried at 110°C for 1 hour, cooled in desiccator before weighing) and dissolving it in 1000 mL deionized water. From the stock solution (1000 mg L^{-1} of NO_2-N), a series of standard solutions (0.01, 0.03, 0.05, 0.07, 0.1, 0.3, 0.5 and 1.0 mg L^{-1}) were, prepared by mixing with deionized water.

Samples and standard solutions (10 ml) were taken in test tube. Then 0.2 ml of sulfanilamide solution was added. Sulfanilamide solution was prepared by dissolving 5 g of sulfanilamide in a mixture of 50 mL of concentrated hydrochloric acid and diluted to 500 ml with DDH_2O. After more than 2 minutes but less than 10 minutes, 1 ml of NED reagent (0.5 g of the N- (1-napthyl)-ethylenediamine dihydrochloride was dissolved in 500 ml of distilled water) was added and mixed immediately using vortex mixture. Between 10 minutes and 2 hours afterwards, the extinction was measured at a wavelength of 543 nm by using the Nano Drop Spectrophotometer (Model-Nanoplus, Germany).

3.2.3 Determination of soluble reactive phosphorous (SRP)

Soluble reactive phosphorous (SRP) was determined according to Parsons et al. (1984). Standard stock solution was prepared by weighing 4.39 g of anhydrous grade potassium dihydrogen phosphate, KH_2PO_4 (dried at 110°C for 1 hour, cooled in dessicator before weighing) and dissolving in 1000 ml deionized water. From the stock solution (1000 mg $L^{-1}$of PO_4-P) a series of standard solutions (0.01, 0.03, 0.05, 0.07, 0.1, 0.3, 0.5 and 1.0 mg L^{-1}) was prepared by mixing with deionized water.

Samples and standard solutions (10 ml) were placed in test tubes and 1 ml of mixed reagent was added. After 5 minutes and preferably within the first 2-3 hours, the

extinction was measured at 885 nm by using Nano Drop Spectrophotometer (Model-Nanoplus, Germany). Mixed reagent was prepared by mixing 100 ml of ammonium molybdate (15 g of analytical reagent grade ammonium paramolybdate $(NH_4)6Mo_7O_{24}$ was dissolved in 500 ml of distilled water), 250 ml sulfuric acid, 100 ml ascorbic acid (27 g of ascorbic acid was dissolved in 500 ml of distilled water) and 50 ml of potassium antimonyl-tartrate solution (0.34 g of potassium antimonyl-tartrate (tartar emetic) was dissolved in 250 ml of water).

3.3 Collection of microalgae

Sample was collected by using 60 µm mesh size plankton net and the collected samples were taken in 500 ml sample bottle containing nutrient solution (Bold basal medium for freshwater microalgae, and Conway medium for marine and coastal water microalgae). After that, until they were brought to the lab, the sample bottles with sample were maintained at refrigerated condition. It is crucial to remember that the collected microalgae must be kept in nutrient solution to keep them alive.

3.4 Centrifugation

The collected microalgae samples were concentrated by centrifuging at 4000 rpm for 5 minutes. The separation efficiency of centrifugation depends on speed and time and it should be remembered that excessive centrifugal force can sometimes damage fragile cells (Singh et al., 2016). After centrifugation, the supernatant fraction containing low density cells such as bacteria was discarded, while the concentrate containing microalgae and higher-density cells was kept. After several centrifuging cycles, a higher concentration of desired concentrate containing microalgal strain was achieved and it was used for isolation. Several studies have shown that centrifugation aids in concentrating algal cells and reducing the bacterial population. Though this technique does not generate an axenic algal culture by itself, it will ensure the purity of cultures by facilitating posterior techniques (Fernandez-Valenzuela et al., 2021).

3.5 Determination of microalgal diversity

Filtered raw freshwater samples from each of the sampling site were preserved by adding a few drop of lugol's iodine to determine the microalgal diversity. Then the samples were observed under microscope and microalgal diversity was determined by

using phytoplankton identification books such as Belcher and Swale (1976); John et al. (2002) and Bellinger and Sigee (2010).

3.6 Isolation of Microalgae

3.6.1 Streak Plating Technique (Phang and Chu 1999)

3.6.1.1 Agar plate preparation

For preparing the agar medium, 1.5 % agar was added to 1 L of BBM or Conway media and solution was sterilized in an autoclaved at 121˚C temperature for 15 min under 150 lbs pressure. Then the agar medium was cooled to about 50 °C and vitamin solution was added into it while mixing by gently rotating the flask to ensure mixing of the nutrients and avoid bubble formation. After that, the warm medium was aseptically poured into the sterile petri dish where the agar was at least ½ or ¾ the depth of the petri dish, and agar medium was left to cool and solidify and stored in plastic bags in a refrigerator until further use.

3.6.1.2 Streaking in agar plate

After preparation of the agar plate 1-2 drops of the concentrated natural collection was placed near the periphery of the agar. Concentarte will be allowed to streak through loop in plates in axenic condition and to keep for at least seven days to grow microalgae. Repeated streak-platings will be carried out to peak up single colony from earlier streaked plates and to make free from bacteria. From last streaked plates, the single colonies will be picked up by loop and allowed to grow in tubes and vials. Before putting in the tubes and vials, the single cell growth and purity of single species will be confirmed after observing under microscope. Then, the pure culture of isolated microalgae will be maintained in BBM or conway media in tube, vial and volumetric flask in the laboratory for further use and study. Following the above procedure, the important microalgae will be isolated, allowed to grow in culture media (BBM and Conway media) and maintained in the laboratory.

3.6.2 Serial dilution

For serial dilution, test tubes were filled with 9 ml of culture medium prepared in the last lab. Tubes were labeled as 10^{-1} –10^{-10} to indicate dilution factor. After that, test tubes were put in the biological safety cabinet where test tube cap was removed and

its neck was flamed and 1 ml of enrichment sample was added to the test tube (10^{-1}) and mixed gently. Then, 1 ml of this dilution was taken and added to the next tube (10^{-2}) and then mixed gently and this procedure was repeated for the remaining tubes (10^{-3} –10^{-10}). After completing the dilution procedure, test tubes were incubated under controlled temperature and light conditions. After 2–4 weeks, the cultures were examined microscopically by withdrawing a small sample aseptically from each dilution tube where unialgal culture found in one of the higher-dilution tubes, e.g., 10^{-6} –10^{-10}. When the tubes contain two or three different species, capillary pipetting method was applied for isolation of single algal species.

3.6.3 Picking up method (Capillary method)

First, a micropipette was made from a glass pasteur pipette for the capillary picking up method. The tip of the pipette was held in the hotter part of the flame while being supported on the left by a hand and on the right by forceps. Once the pipette reached a soft, pliable condition, it was quickly removed from the flame with a gentle pull to create a thin tube. The forceps was then moved to the proper spot of the thin tube where it was gently bent until it broke and formed a micropipette. The micropipettes were then sterilized at 126°C for 15 minutes and attached to a piece of soft rubber tubing when ready for use. These were used to pick up single cells from the collected sample under a microscope.

After sample collection, the microalgal samples are immediately placed in sterile Bold basal medium (BBM) for freshwater algae or Conway medium for marine algae. Individual cells will be extracted using a micropipette in a series of tries. 4-6 drops of sterile Bold basal medium or Conway medium was placed in the groove of a glass slide. The microalgal sample will then be introduced, and a drop will be examined under an inverted microscope. One drop of the sample will be added to the following one after the target organism has been confirmed. To isolate a single cell in its purest form and to ensure its purity, the method will be performed numerous times (Guillard, 1995). In order to preserve the isolated microalgal species, either BBM or CM was used for growth.

3.7 Morphological identification of microalgae

Morphological identification of isolated microalgae was done microscopically at 40X magnification and based on the morphological characteristics using phytoplankton

identification books (Belcher and Swale, 1976; John et al., 2002 and Bellinger and Sigee, 2010).

3.8 Preparation of culture media for microalgal growth

3.8.1 Preparation of BBM (Bold basal medium)

For the preparation of BBM, distill water was used as a source of freshwater. Standard BBM was used for the culture of microalgae. To prepare 1L of BBM, first 10mL of each stock solution (1-6), 1 mL of stock solution (7, 8 and 10) and 0.7ml of boric acid solution (9) was added into 940 mL of dH_2O. Finally, 0.5ml of filter sterilized vitamin solution (11) was poured into it. Table- 3.1 showed the different constituents of BBM.

Table 3.1 Constituents of bold basal medium

Name of chemicals			Quantity
1. Potassium dihydro phosphate (KH_2PO_4)			8.75 g/500ml
2. Calcium chloride dehydrate ($CaCl_2$. $2H_2O$)			1.25g/500ml
3. Magnesium sulfate hydrate ($MgSO_4$. $7H_2O$)			3.75g/500ml
4. Sodium nitrate ($NaNO_3$)			12.5g/500ml
5. Dipotassium phosphate (K_2PO_4)			3.75g/500ml
6. Sodium chloride (NaCl)			1.25g/500ml
7. $Na_2EDTA.2H_2O$			10g/L
Potassium hydroxide (KOH)			6.2g/L
8. Iron (2) sulfate 7-hydrate ($FeSO_4.7H_2O$)			4.98g/L
Sulphuric acid (H_2SO_4)			1ml/L
9. Boric acid (H_3BO_3)			5.75g/500ml
10. Trace metal solution			g/L
Boric acid (H_3BO_3)			2.86g
Manganese (2) chloride tetrahydrate ($MnCl_2$. $7H_2O$)			1.81g
Zinc sulfate hydrate ($ZnSO_4$. $7H_2O$)			0.222g
Sodium molybdate dihydrate ($Na_2MoO_4.2H_2O$)			0.390g
Copper (2) sulfate pentahydrate ($CuSO_4$. $5H_2O$)			0.079g
Cobalt nitrate hexahydrate ($Co(NO_3)_2$. $6H_2O$)			0.0494g
11. Vitamins			0.5ml
Thiamine HCl	200mg	To 950 ml of dH_2O	
Biotin	1g/L	1ml from each of the two	

Cyanocobalamin	1g/L	primary stocks was added then and made to 1L

3.8.2 Preparation of conway Medium (Tompkins *et al.*, 1995)

Conway medium was used for the marine microalgae culture. Conway medium consists of various macronutrients, trace metals, and vitamins. 1 ml of solution A, 0.5 ml of solution B, and 0.1 ml of solution C are added to 1litre of the filtered and sterilized sea water to prepare the medium.

Table 3.2 Constituents of Conway medium

Solution A- Macronutrients

Chemical name	Molecular formula	Proportions
Sodium/Potassium nitrate	$NaNO_3$ /KNO_3	100.0 0 g/116.0 0 g
EDTA Disodium salt	$C_{10}H_{16}N_2O_8$	45.00 g
Boric acid	H_3BO_3	33.60 g
Sodium di-hydrogen orthophosphate	$NaH_2PO_4.4H_2O$	20.00 g
Ferric chloride hexahydrate	$FeCL_3.6H_2O$	1.30 g
Manganese (II) chloride tetrahydrate	$MnCL_2.4H_2O$	0.36 g
Deionized/distilled water	H_2O	1 L

Solution B- Trace Metal solution

Chemical name	Molecular formula	Proportions
Zinc chloride	$ZnCl_2$	2.10 g
Cobalt (II) chloride hexahydrate	$CoCl_3.6H_2O$	2.00 g
Ammonium molybdate tetrahydrate	$(NH_4)6MO_7O_{24}.4H_2O$	0.90 g
Copper (II) sulfate pentahydrate	$CuSO_4.5H_2O$	2.00 g
Zinc chloride	$ZnCl_2$	2.10 g

Deionized/distilled water	H_2O	1 L

Solution C- Vitamin's Solution

Chemical name	Molecular formula	Proportions
Thiamine	Vitamin B1	200 mg
Cyanocobalamin	Vitamin B12	10 mg
Deionized/distilled water	H_2O	100 mL

3.9 Determination of growth curve

The isolated microalgae were cultivated using the BBM and conway media (Stein, 1980). Cultures were grown at a temperature of 24 ± 1°C in a 350 mL culture volume of a sterile 500 mL borosilicate Erlenmeyer flask for each species with three replicates where 2% pure culture stocks were added in each flask. Microalgae cultures were maintained at 24 hr light condition at 150 μEm-2s^{-1} intensity with continuous gentle aeration at a rate of 4.53 ± 0.53 mg/L. The experiment was continued until the death phase and finally completed the growth curve depending on cell density (cells.ml^{-1}) and optical density (absorbance). In case of cyanobacteria, growth curve was determind depending on chlorophyll-a and optical density (absorbance).

3.9.1 Determination of cell density

Microalgae cell count was carried out every day by using a Neubauer hemacytometer (0.0025 mm^2, 0.1 mm deep chambers, Assistent, Germany). Before use, hemacytometer was cleaned with distilled water to make sure it is free from dust, lint and grease. To facilitate counting, Lugol's iodine was added to microalgae sample for fixation and staining. Then small drop of properly mixed sample was placed into the counting chamber and the cells were allowed to settle 3-5 minutes for better counting. Under the magnification of 40X, microalgae cells were counted for both chambers of the hemacytometer. The cells were counted by using the following formulae (Lavens and Sorgeloos, 1996):

$$\text{Cell count (cell/ml) for 5 squares} = \frac{\text{Total number of cells counted}}{10\times4} \times 10^6$$

$$\text{Cell count (cell/ml) for 25 squares} = \frac{\text{Total number of cells counted}}{50\times4} \times 10^6$$

Where 10 and 50 = the squares of the 2 hemacytometer chambers and 4 x 10^{-6} = the volume of samples over the small square areas, that were equivalent to 0.004 mm^3 (0.2 mm x 0.2 mm x 0.1 mm), expressed in cm^3 (ml).

3.9.2 Determination of maximum absorbance (optical density)

Optical density (OD) was measured every day for the growth curve analysis. BBM without any microalgae cells were used as blank. Maximum absorbance value for each microalga was used to perform the growth curve by OD. Maximum absorbance were measured at the wavelength of302 nm for *Chlamydomonas* sp., 301 nm for *Navicula* sp., 300 nm for *Gonyostomum* sp., 450nm for *Nannochloropsis* sp., 342 nm for *Choricystis* sp., 318 nm for *Chromochloris* sp., 342 nm for *Tetraedron* sp., 301 nm for *Chlorobotrys* sp. 301 nm for *Coelastrella* sp., 437 nm for *Kirchneriella* sp., 600 nm for *Oscillatoria* sp1, 443 nm for *Oscillatoria* sp2, 530 nm for *Oscillatoria* sp3 and 475 nm for *Oscillatoria* sp4. as those wavelengths gave maximum absorbance when the culture samples were scanned between 300 to 700 nm, using a spectrophotometer (Nano Drop Spectrophotometer, Model-Nanoplus, Germany).

3.10 Experimental design for pigment and proximate composition determination

In large sterile 2L borosilicate Erlenmeyer flasks, having 1.7L pure BBM and Conway media were used for this experiment. Each of the microalgae species was cultured to maintain similar environmental condition (Temperature: 24 ± 1°C; Light: 150 μEm-2s-1 intensity) until stationary phase. From the fresh cultured sample, carotenoid and chlorophyll were analyzed at the end of their exponential phase. As phycobiliprotein and proximate composition analysis required dry biomass, all the cultures were harvested at the end of their exponential phase by centrifugation (Hitachi* High-speed Refrigerated Centrifuge, himac CR 21g-II, China) depending on the growth curve experiment, and dried at 40°C temperature by using hot air oven and finally preserved at a refrigerator (4°C) for further use.

3.11 Determination of biomass

Biomass determination is prerequisite for productivity analysis. Biomass were determined by filtering of 1ml microalgae sample from each replication of individual microalgae through a pre-weighted (after marking of filter paper rinsed with 10ml distill water and dried at 100°C for 4hr in hot air oven) glass microfiber filter paper, which was further rinsed with 10ml distill water for three times. Then the filter paper

with biomass was oven dried at 100°C for 4hours. After that, final weight of filter paper was taken followed by 15min of desiccation and dry biomass was calculated according to Ratha et al. (2016).

3.12 Determination of productivity

Biomass productivity, areal productivity and lipid productivity were calculated according to Mercado et al. (2020) and Sukkrom et al. (2016). Productivity data was calculated at the end of the exponential phase. Specific growth rates (SGR) and Cell doublings per day were calculated according to Daniel and Srivastava (2016) and cell duplication time was calculated according to Chiu et al. (2008).

3.12.1 Volumetric productivity

Following equation was used to calculate volumetric productivity:

$$\text{Volumetric productivity/VP (mg L}^{-1}\text{ day}^{-1}\text{)} = \frac{(X_2 - X_1)}{(t_2 - t_1)}$$

Where, X_1 and X_2 were the biomass concentrations (mg L^{-1}) on days t_1 (start of study) and t_2 (end of the study).

3.12.2 Areal productivity

Following equation was used to calculate areal productivity:

$$\text{AP (mg cm}^{-2}\text{ day}^{-1}\text{)} = \frac{VP \times V}{A}$$

Where, VP = volumetric productivity, V = total volume of the culture, A = surface area occupied ground.

3.12.3 Lipid productivity analysis

Lipid productivity (LP) was calculated by using the following equation:

$$\text{LP (mg L}^{-1}\text{ day}^{-1}\text{)} = \frac{\text{Biomass Productivity (mg } L^{-1}\ day^{-1}\text{) x (\% lipid)}}{100}$$

3.12.4 Specific growth rates (SGR)

SGR was calculated using the formula as follows:

$$\text{SGR (r)} = \frac{\ln Nt - \ln No}{\Delta t}$$

Where, Nt is the final cell count and No is the initial cell count; t is the number of days.

3.12.5 Cell duplication time

Cell duplication time was calculated using the following formula:

Cell duplication time td= 0.693/μ

3.12.6 Cell doublings per day

Cell doublings per day was calculated using the following formula:

Cell doublings per day (K) = $\frac{\ln Nn - \ln Ni}{\ln 2(tn - ti)}$

Where, Nn is the final cell count and Ni is the initial cell count; tn is the final time in days and ti is the initial time in days.

3.13 Determination of pigments

3.13.1 Extraction of microalgae for chlorophyll determination

For the extraction of microalgae, first 1ml of $MgCO_3$ solution (after proper shaking) was filtered through the filter paper (47 mm Ø Whatman® GF/C glass microfiber filter papers) by using filtering apparatus, so that most of the area of filter was covered. Then, 10ml of each algae sample was filtered and the edges which were not coated with residue were trimmed away. After that, the filter paper was fold and place into 15ml centrifuge tube where the middle of the filter is facing downward. Into the centrifuge tube, 2ml of 90% acetone was added and grind for 1 minute, again 8ml of 90% acetone was added and grind for 30 sec. Then, the sample was refrigerated in the dark for 1 hour. After 1 hour, sample was centrifuged at 3000 rpm for 10 minutes and transferred the acetone extract into another centrifuge tube and centrifuged at low speed (500 rpm) for 5 minutes. Lastly, the absorbance of acetone extract was measured against 90% acetone as blank.

3.13.2 Determination of chlorophyll

Chlorophyll concentration was determined according to Jenkins (1982). The clean extract was transferred to a 1cm cuvette and OD was measured at 750, 664, 647 and 630nm wavelength. OD at 664, 647, and 630 nm were used for chlorophyll determination where, OD at 750 nm was used as turbidity correction factor and subtracted from each of the pigments OD values before using them in the equations. Concentrations of chlorophyll a, b, and c in the extract were calculated by inserting the corrected optical densities in following equations (Jeffrey and Humphrey, 1975):

a) C_a = 11.85(OD664) - 1.54(OD647) - 0.08(OD630)

b) C_b = 21.03(OD647) - 5.43(OD664) - 2.66(OD630)

c) C_c = 24.52(OD630) - 7.60(OD647) - 1.67(OD664)

Where: C_a, C_b, and C_c = concentrations of chlorophyll a, b, and c, respectively, mg/L, and OD664, OD647, and OD630 = corrected optical densities (with a 1 cm light path) at the respective wavelengths. After the determination of the concentrations of pigments in the extract, the amount of pigments per unit volume was calculated as follows:

$$\text{Chlorophyll a}, \text{mg/m}^3 = \frac{\text{Ca} \times \text{extract volume, L}}{\text{volume of sample, m}^3}$$

3.13.3 Determination of carotenoids

1 mL aliquot of the algal suspension from each culture was taken at their stationary phase and centrifuged at 1000g for 5 min. Then, the obtained pellet was extracted with 3 mL of ethanol: hexane 2:1 (v/v). After that, 2mL of water and 4 mL hexane (Sigma, USA) were added to the mixture, shaken vigorously and centrifuged again at 1000 ×g for 5 min. Finally, absorbance of the separated hexane layer was determined using spectrophotometer at a wavelength of 450 nm. The amount of extracted carotene from the samples in micrograms was determined by multiplying the absorbance ($A_{450)}$ with 25.2 (Shaish, 1992).

3.13.4 Determination of phycobiliproteins

As phycobiliproteins estimation required dried biomass, the cultures were harvested by centrifugation at 6000 rpm for 5 min and the harvested pellets were dried at 40°C for overnight. 40 mg of dried powder was then soaked in 10 mL phosphate buffer (pH 7.0; 0.1 M), mixed properly using vortex mixture, and then stored for 24 hr at 4°C. After that, the samples were centrifuged at 6000 rpm for 10 min. Finally the supernatant was collected and spectrophotometric (Nano Drop Spectrophotometer, Model-Nanoplus, Germany) absorbance was measured against the phosphate buffer solution as blank at the wavelength of 562, 615, 652 and 720 nm where 720 nm measured the absorbance of the cellular debris.

The amount of phycocyanin (PC), and allophycocyanin (APC) in the sample was calculated from the absorbance according to Bennett and Bogorad (1973) and phycoerythrin (PE) was calculated according to Siegelman and Kycia (1978).

Phycocyanin (PC) mg/mL= $\{A_{615} - A_{720}) - 0.474 \times (A_{652} - A_{720})\} /5.34$

Allophycocyanin (APC) mg/mL= $\{A_{652} - A_{720}) - 0.208 \times (A_{615} - A_{720})\} / 5.09$

Phycoerythrin (PE) mg/mL = $\{A_{562} - (2.41 \times PC) - (0.849 \times APC)\} /9.62$

Total phycocyanin, phycoerythrin, and allophycocyanin (mg/g) were calculated according to Silveira et al. (2007) as follows:

$$P = (\text{Pigment concentration} \times V) / DB$$

Where, V= solvent volume, DB= dried biomass

Total phycobiliproteins (mg/g) were further calculated from the sum of the phycocyanin, phycoerythrin, and allophycocyanin contents in dried microalgae biomass.

3.14 Determination of proximate compositions

3.14.1 Protein determination

Protein was determined according to Lowry et al. (1951). For that, 5 mg of each freeze dried biomass was taken and prepared 25 mL well mixed (tissue homogenizer) microalgae solution by using distilled water. From that 25 mL, 0.5 mL solution was taken from each type of samples, and 0.5 mL of 1N NaOH was added and kept in hot water bath (100°C) for 5 min. After that, the samples were put into cold water bath. 10 mins after cooling, 2.5 mL of mixed reagent (50 mL of Reactive 2 (2g of Na_2CO3 in 100 mL of 0.1 NaOH) and 1 mL of Reactive 1 (1% NP tartrate), mixed) was added in each types of sample. After proper mixing by using vortex mixture, 0.5 mL of folin reagent was added into every sample. Then, the samples were kept in dark place for 30 min and spectrophotometric absorptions were measured at 750 nm wavelength. 2000 µg/L of standard (albumin) stock solution was prepared to produce a calibration graph, from which series of standards at various dilutions (20 µg/L, 40 µg/L, 80 µg/L, 100 µg/L and 200 µg/L) were also prepared and protein analysis procedure was applied as described above. From the obtained absorbance a standard graph was plotted according to the standard results and protein content was determined for each type of samples accordingly.

3.14.2 Carbohydrate determination

Carbohydrate was determined according to Dubois et al. (1956). 5mg of each freeze dried biomass was taken and prepared 25 mL well mixed (tissue homogenizer) microalgae solution by using distilled water. From that 25mL, 1mL solution was taken from each type of samples, and 1 mL 5% phenol and 5 mL of sulfuric acid were poured into it maintaining every 30 sec reaction period. After that, the samples were put in the cold water bath. After cooling, spectrophotometric absorptions were taken for each types of solution at 488 nm wavelength to estimate carbohydrate. 1000 µg/L of standard (glucose) stock solution was prepared to produce a calibration graph, from which, a series of standards at various dilutions (20 µg /L, 40 µg /L, 60 µg /L, 100 µg /L and 140 µg /L) were also prepared and carbohydrate analysis procedure was applied as described above. From the obtained absorbance a standard graph was plotted according to the standard results and carbohydrate content was determined for each type of samples accordingly.

3.14.3 Lipid determination

Lipid was determined according to Bligh and Dyer (1959), and Folch et al. (1957). Aluminum dishes were labeled and weighted as initial weight for each types of sample. In a centrifuge tube, 50 mg of each sample was taken and diluted into 5x volume using distilled water. Then, 3 mL methanol: chloroform (2:1, v/v) was added and mixed (tissue homogenizer) properly. After that, all the tubes were centrifuged at 1000 rpm for 4 min at 4°C. After centrifugation, the supernatants were transferred into clean tubes by Pasteur pipette, and placed them in ice. In the sample tubes, again 3 mL of methanol: chloroform (2:1, v/v) was mixed (tissue homogenizer) properly and centrifuged again at the same conditions, and the supernatants were transferred to the previous supernatants tubes. In the combined supernatants, 1.5 mL of 0.9% NaCl was poured and mixed well by using vortex mixture and kept in the refrigerator for 1 hr at 4°C temperature. Then the tubes were centrifuged at 1000 rpm for 10 min at 4°C temperature that results in two separate layer from which the upper layer of methanol and chloroform was discarded, while, the lower layer was transferred in previously prepared aluminum dish. After that, the solvent was evaporated at 60°C using hot air oven. Afterwards, the aluminum dishes were weighed to get the final weight and lipid weight in the samples was determined by subtracting initial weight from the final weight.

3.15 Determination of fatty Acid

Two steps transesterification also known as 2TE method with a little modification of Griffiths *et al.*, 2010 was used to determine the fatty acid composition. In a lipid extraction beaker, 500mg microalgae powder dissolved in 70ml diethyl ether. Digital Soxhlet Apparatus (FOOD ALYTRD40) was used for lipid extraction. Diethyl ether was removed by placing the test tubes in the Hot Air Oven at 60°c. Then, 1.5 ml of methanolic NaOH was added into the lipid extract and mixed properly through Sonication at 80°C for 5 minutes. Upon cooling at room temperature (25°C), 2ml of BF_3 methanol was poured into the mixture and again sonicated for 30 minutes at 80°C. After cooling at 25°C, 1ml of isooctane and 5ml of saturated NaCl was poured and well mixed through shaking. Then two layers were observed. Fatty acid methyl-esters (FAMEs), an organic substance in the upper layer was transferred to a new test tube. 1ml sample from the test tube was taken into 1.5 ml Eppendorf vial for further fatty acid methyl-esters analysis through GCMS-Gas Chromatography Mass Spectrophotometry (GC-2020plus, SHIMADZU, Japan). Separation of FAMEs was done with a capillary column (30m length, 0.25mm internal diameter, 0.15 μm film thickness, and phase ratio is 250). Helium gas was used as a carrier gas with 1.42 ml/min flow rate. The column temperature program was : 180° to 280°c at 5 °c /min and then at 280°c. Detection of FAMEs were done by comparing the retention time with standard (FAME mix C8-C24; Sigma- Aldrich; Germany).

3.16 Determination of amino acid

The Moore and Stein technique (1951) was slightly modified in order to identify amino acids. 1 g dried biomass of microalgae was first hydrolyzed for 24 hours at 110 ±2 °C in 25 mL of previously prepared acidic hydrolysis solution (6 M HCl + 0.1% phenol). The samples were stabilized using a little quantity of SDB/Na (Sample Dilution Buffer) after cooling. The samples' pH was then adjusted using a basic neutralizing agent to range between 2.1 and 2.3. The hydrolysates were then filtered and diluted with SDB/Na before being put into the injection vials. SYKAM S 433 amino acid analyzer with UV detector was used for the analysis. With a constant flow rate of 0. 5 mL/min of nitrogen gas at a temperature of 60^0C and a reproducibility of 3%, nitrogen gas was employed as the carrier gas. Sigma-Aldrich, Germany's AA-S-18 standard wease is used to measure the concentration of amino acids. The amount

of amino acids was measured in mg/g, which was then converted to % of all amino acids

3.17 Statistical analysis

Mean and standard error (SE) of mean were calculated using MS excel. When assumptions were met, all statistical analyses regarding the growth parameters, proximate composition, pigments content and fatty acid content was performed using the IBM SPSS (v. 26.0). Descriptive statistics were performed for different treatments; thereafter, a test for homogeneity of variance was done. The collected data were analyzed using a one-way analysis of variance (ANOVA). Significant differences amongst treatments were analyzed using Tukey HSD multiple comparison tests at 95% confidence interval level.

Chapter-4: Result

4.1 Water quality parameters of the sampling sites:

Table 4.1 shows the physicochemical parameters of water collected from different marine and freshwater sampling sites, where variations in the physical and chemical parameters were observed.

Table 4.1: Water quality parameters of the sample water collected from different freshwater and marine water sampling sites

Parameters	**Halda river**	**Kaptai lake**	**Naf Estuary**	**Sonadia**	**Maheshkhali**
Temperature (˚C)	30℃	31℃	33℃	29.5℃	31℃
DO (mg/L)	7.5	7.3	7.5	7.3	7.4
Ph	8.1	8.3	8.5	8.1	7.5
Salinity (ppt)	0	0	15	25	30
Total Ammonia nitrogen (TAN) (mg/L)	0.004	0.007	0.003	0.004	0.002
Soluble reactive phosphate (mg/L)	0.046	0.037	0.3365	0.005	0.022
Nitrite-Nitrogen (mg/L)	0.023	0.052	0.085	0.037	0.086

4.2 Chorophyll content of water collected from the sampling sites:

Table 4.2 shows Chlorophyll content of water collected from different marine and freshwater sampling sites, where variations in the physical and chemical parameters were observed.

Table 4.2: Chlorophyll content of different freshwater and marine water sampling sites:

Sampling Station	Chl-a (µg/ml)	Chl-b (µg/ml)	Chl-c (µg/ml)
Halda	1.9805	1.2887	0.9886
Kaptai	0.1605	0.3647	0.1218
Teknaf	0.2552	0.1706	0.3248

Sonadia	0.9343	0.1864	0.1796
Maheshkhali	1.2330	1.00662	1.4046

4.3 Isolated Microalgae from different sampling sites

Water sample collected from different marine and freshwater sampling sites contained a variety of species.Species listed in table 4.3 were isolated from water collected from different sampling locations.

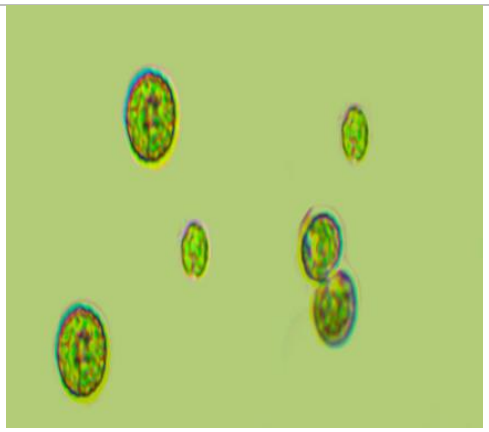
Table 4.3: Isolated microalgae from different freshwater and marine water sampling sites:

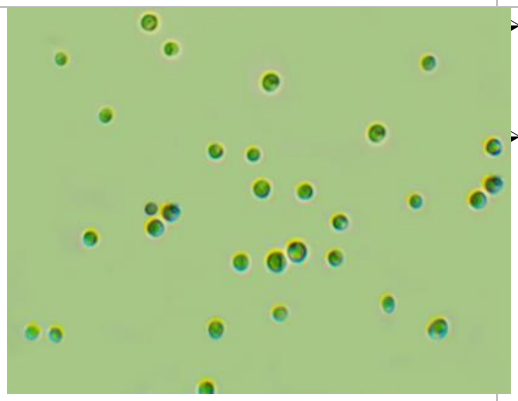
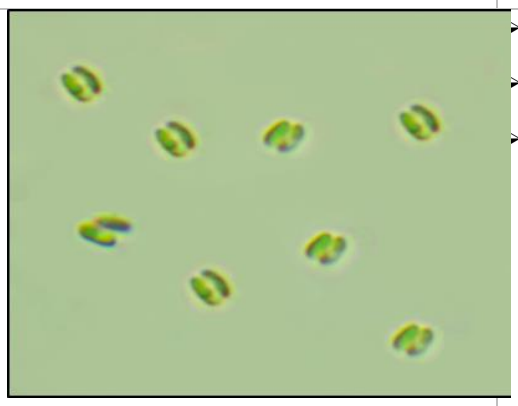
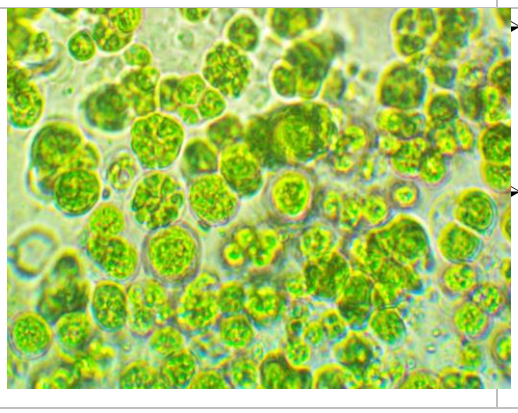
Halda river	Kaptai lake	Teknaf	Sonadia	Maheshkhali
Auxenochlorella sp.	*Coenochloris* sp.	*Gonyostomum* sp.	*Navicula* sp.	*Nitzschia* sp.
Chlorobotrys sp.	*Desmodesmus* sp.	*Pandorina* sp.	*Chlorella* sp.	*Oscillatoria* sp.
Tetraspora sp.	*Coelestrella* sp.	*Euglena* sp.	*Aphanocece* sp.	
Choricystis sp.	*Coccomyxa* sp.	*Chlamydomonas* sp.	*Nannochloropsis* sp.	
Scenedesmus sp.	*Choricystis* sp.	*Chlorella* sp.		
Coccomyxa sp.	*Chromochloris* sp.	*Nannochloropsis* sp.		
Carteria sp.	*Klebsormidium* sp.	*Trachelomonas* sp.		
Chlorella sp.	*Scenedesmus* sp.			
Kirchneriella sp.	*Chlorella* sp.			
Trachelomonas sp.	*Kirchneriella* sp.			
Tetraedron sp.	*Oscillatoria* sp.			
Eudorina sp.				
Chlamydomonas sp.				
Westella sp.				
Oscillatoria sp.				

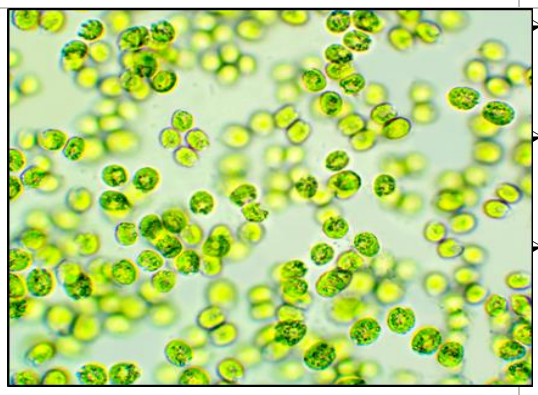
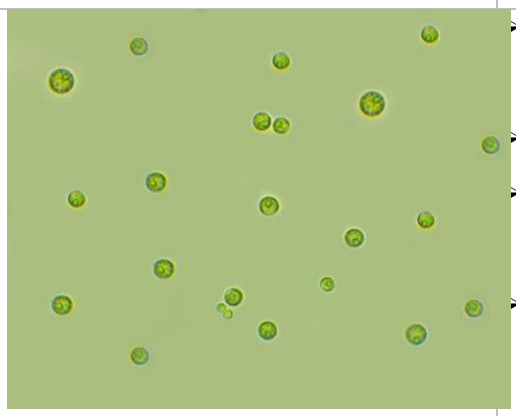
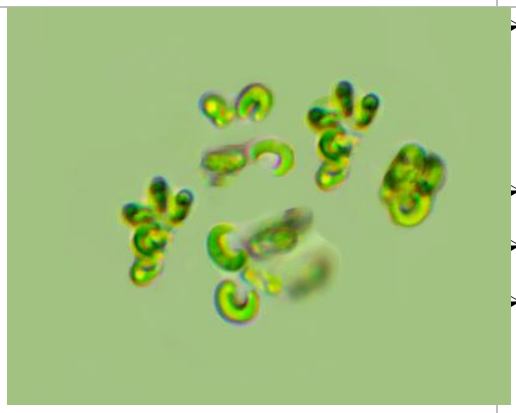
4.4 Characterization of isolated microalgae

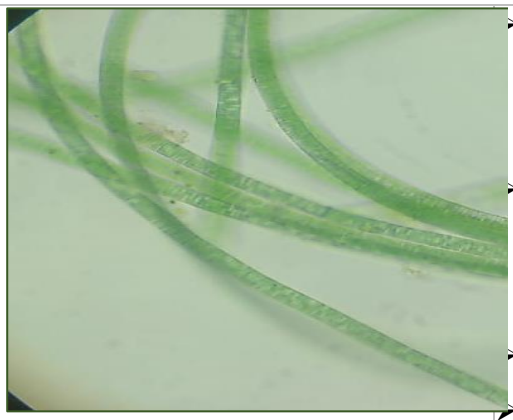
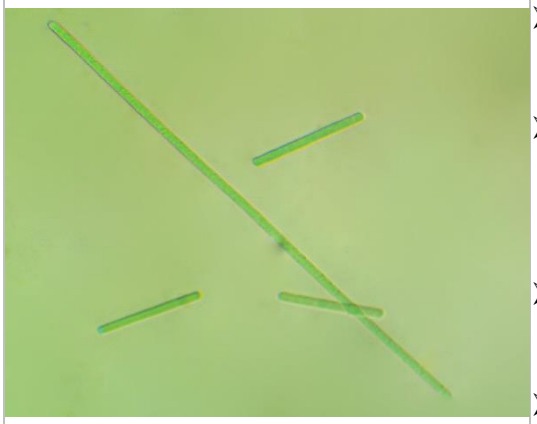
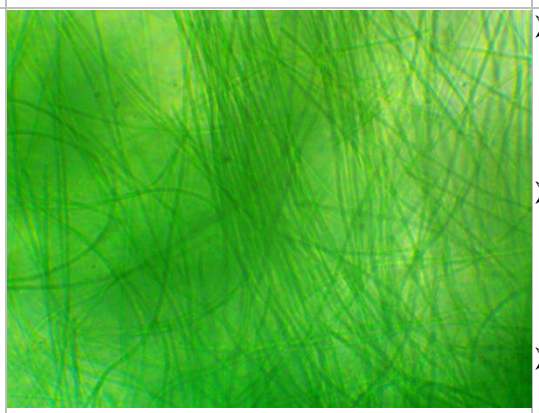
Fourteen species of microalgae were isolated in this study (Table 4.3). According to John and Whitton (2002), isolated microalgae were *Chlamydomonas* sp., *Navicula* sp., *Gonyostomum* sp., *Nannochloropsis* sp., *Choricystis* sp., *Chromochloris* sp., *Tetraedron* sp., *Chlorobotrys* sp. *Coelastrella* sp., *Kirchneriella* sp. of which the largest one is *Chlorobotrys* sp. (12µm) and the smallest one is *Gonyostomum* sp. (2µm). Among the fourteen microalgae, *Chromochloris* sp., *Coelastrella* sp., *Kirchneriella* and *Oscillatoria* sp3 were isolated from the Kaptai lake, where *Choricystis* sp., *Tetraedron* sp., and *Oscillatoria* sp4 from Halda river. Marine microalgae *Chlamydomonas* sp., *Gonyostomum* sp., *Nannochloropsis* sp., and *Oscillatoria* sp1 were isolated from Naf estuary where *Navicula* sp. and *Oscillatoria* sp2 were isolated from Sonadia and Maheshkhali, respectively.

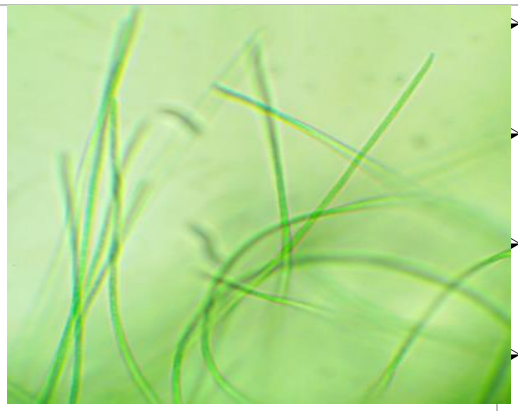
Table 4.4: Characteristics of isolated microalgae

Microalgae species	**Figure**	**Characteristics**
Chlamydomonas sp.		➢ Generally oval and/or circular in shape ➢ Cells are 10 µm in diameter ➢ *Chlamydomonas* are unicellular and motile. ➢ Species have two anterior flagella that are equal in length to one another
Navicula sp.		➢ *Navicula* sp. are single-celled ➢ Cells are 8 to 10 µm in length ➢ Boat-like shape and a yellowish brown tint was observed

Gonyostomum sp.		➢ Cells of *Gonyostomum* sp. are drop-shaped. ➢ The cells are very small measuring 2 µm. ➢ Cell of *Gonyostomum* sp. possess two differently shaped flagella
Nannochloropsis sp.		➢ *Nannochloropsis* are small, round or ovoid and nonmotile ➢ Have a diameter of about 2.5 to 3.3 micrometers
Choricystis sp.		➢ Species are relatively small ➢ Cells are found in pairs ➢ The cell size is about 3-6 µm
Chromochloris sp.		➢ *Chromochloris* sp. is a simple 4-µm, unicellular, haploid, coccoid alga ➢ Neither flagella (cilia) nor pyrenoids were visually observed.

Tetraedron sp.		➢ *Tetraedron* is unicellular algae with flattened, triangular, square or polygonal cells. ➢ Cells are about 5 µm in length and 10 µm in diameter ➢ Spines are also observed in this species
Chlorobotys sp.		➢ Cells are oval, elliptical, or irregularly shaped coccoid unicells. ➢ The coccoid cells are encountered singly, in pairs, or as colonies ➢ The cells are essentially spherical with an diameter of 12 µm
Coelastrella sp.		➢ *Coelastrella* is a green unicellular coccoid microalga. ➢ Cells 4.5 µm wide and 7 µm long ➢ The cells are solitary or form groups ➢ Solitary cells can be lemons shaped to broadly ovoid
Kirchneriell a sp.		➢ Colonies of cells often make up *Kirchneriella* sp. are mostly found in colonies. ➢ A colony contains 4-16 individuals. ➢ Cell size is 4 µm. ➢ Cells are cylindrical, lunate, sickle-shaped, twisted fusiform or spirally twisted.

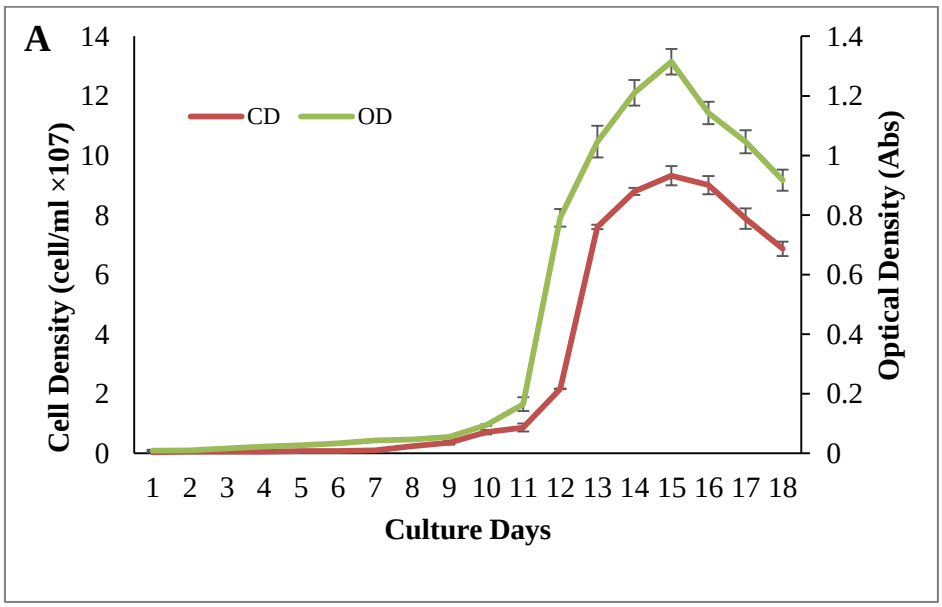
Oscillatoria sp1		➢ Planktonic, cylindrical, thread like structures which appeared in blue-green color. ➢ Species consists of a series of cells forming unbranched filaments or trichomes. ➢ Constricted at cross walls ➢ Cells are 5-7 µm long and 52 µm wide
Oscillatoria sp2		➢ Filamentous, barrel-shaped straight structures with slight bent edges. ➢ Thallus are long and comprises dark blue-green color to blackish blue-green color. ➢ Cells are solitary or in clusters and constricted at cross walls. ➢ Cells are 6-8 µm long and 50 µm wide
Oscillatoria sp3		➢ It contains Clusters like filaments and cells are cylindrical with tapering at the outer edges. ➢ Thallus are short and coiled and trichomes appeared as bright blue-green color. ➢ Cells are 3-4 µm in length and 47 µm in width

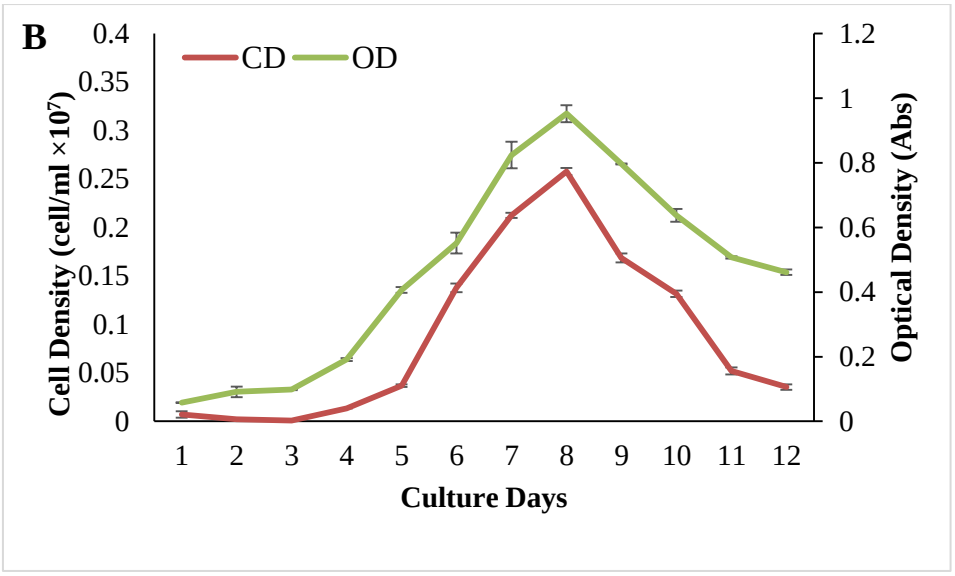
Oscillatoria sp4		➢ Filamentous, thallus are long and visible in dark blue-green color. ➢ Trichomes are straight to slightly curved with bent outer edges ➢ Trichromes are rarely solitary and motile in nature ➢ Cells are 6-8 μm in length and 51 μm width

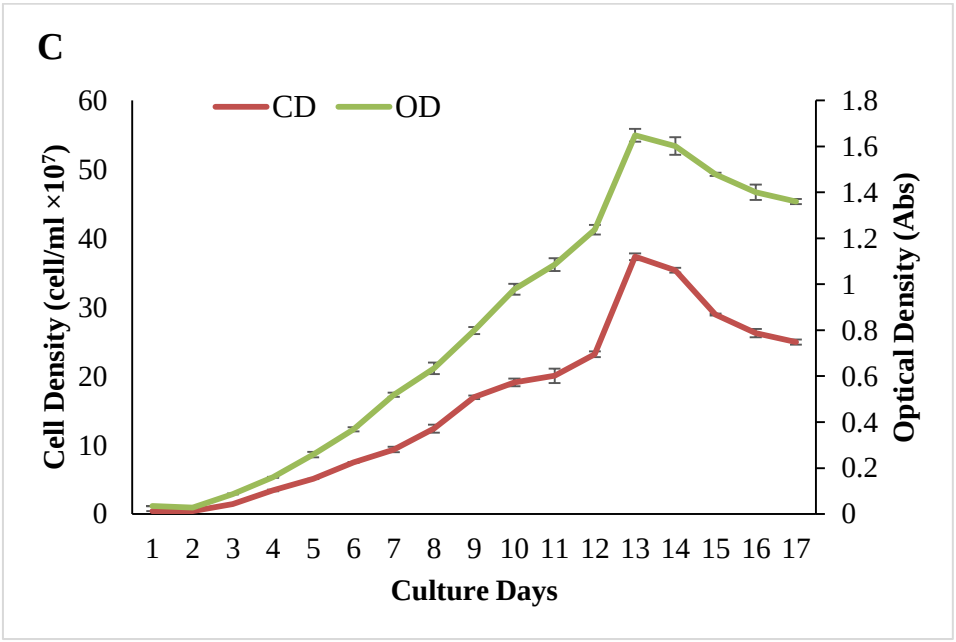
4.5 Growth phases of isolated microalgae

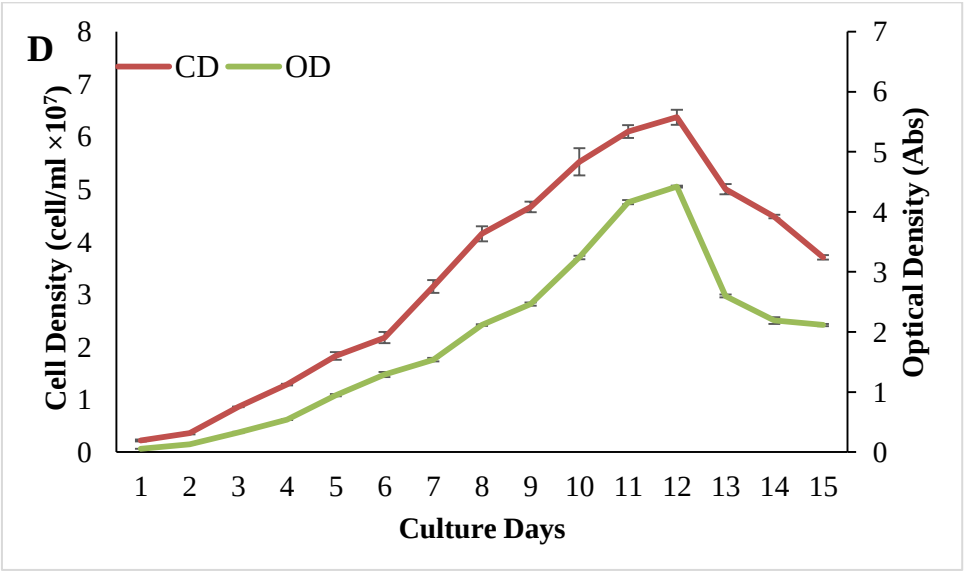
In the present study, different microalgae showed different cell concentration, amount of pigment and proximate composition as those can vary from species to species. Figure 4.3 illustrate the cell density and optical density versus the cultivation time of each of the ten species (*Chlamydomonas* sp., *Navicula* sp., *Gonyostomum* sp., *Nannochloropsis* sp., *Choricystis* sp., *Chromochloris* sp., *Tetraedron* sp., *Chlorobotrys* sp. *Coelastrella* sp., *Kirchneriella* sp. *Oscillatoria* sp1 and *Oscillatoria* sp4) during the cultivation in BBM and Conway media and figure 4.4 showed the growth curve of of *Oscillatoria* sp1 (K), *Oscillatoria* sp2 (L), *Oscillatoria* sp3 (M) and *Oscillatoria* sp4 (N) in terms of chlorophyll-a and optical density(Absorbance). Observation results of growth showed that onset of stationary phase (9-14 days) varied among the fourteen species. Based on the growth curve it is possible to determine the growth phases of those fourteen microalgae. Based on Figure 4.3 and 4.4 the growth phases are almost same for *Nannochloropsis* sp. and *Chromochloris* sp. that showed the lag phase on days 1 to 2, the exponential phase on days 2 to 12, the stationary phase on days 11 to 12, and finally the phase of death from 12 days. *Oscillatoria* sp2 also showed the same growth phases except stationary phase on 10-12 days. Similarly, *Choricystis* sp. and *Kirchneriella* sp. showed the lag phase on 1 to 2 days, the exponential phase on 2 to 9 days, the stationary phase on 8 to 9 days and the death phase from 9 days. *Gonyostomum* sp. and *Oscillatoria* sp3 showed almost same lag phase and exponential phase on days 1-2 and 2 to 13, respectively. *Gonyostomum* sp. showed the stationary phase on days 12 to 13, and the death phase from 13 days, where *Oscillatoria* sp3 showed the stationary phase on days 11-13 and the death phase from 13 days. Though *Coelastrella* sp. and *Oscillatoria* sp4 showed same lag phase on 1-2 days, their other growth phases varies. *Coelastrella* sp showed

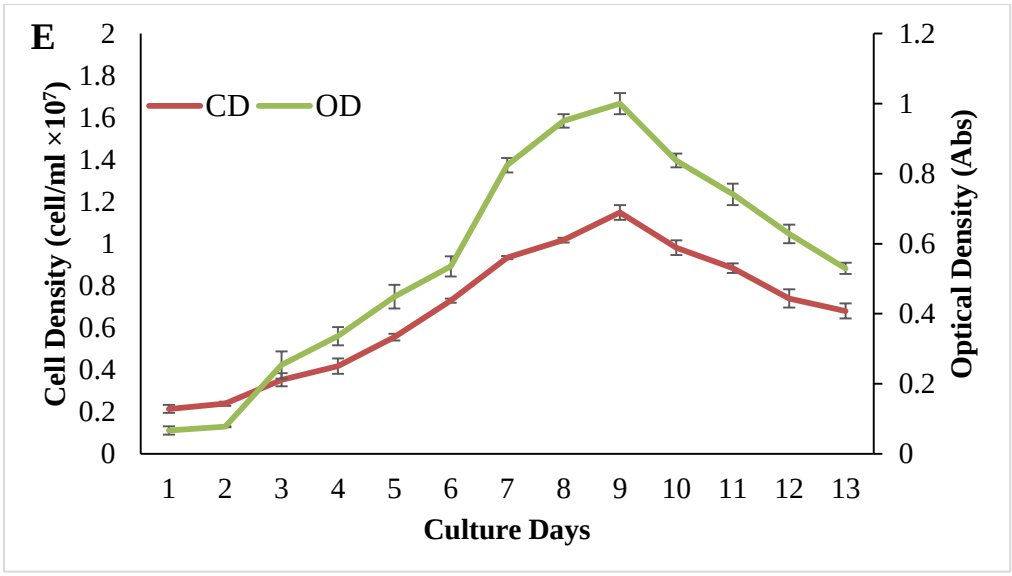
exponential phase on days 2 to 10, the stationary phase on days 9 to 10, and the death phase from 10 days where *Oscillatoria* sp4 showed exponential phase on days 2 to 11, the stationary phase on days 10 to 11, and the death phase from 11 days. Furthermore, in *Tetraedron* sp. from days 1 to 4, days 4 to 20 and days 18 to 20 was recorded as lag phase, exponential phase and stationary phase, respectively. Moreover, *Navicula* sp.and *Chlorobotrys* sp. showed the same lag phase on days 1 to 3. *Navicula* sp. resulted exponential phase on days 3 to 8, stationary phase on days 7 to 8 and death phase from 8 days, where in *Chlorobotrys* sp. from days 3 to 12 and days 11 to 12 was recorded as exponential phase and stationary phase, respectively. Along with this, *Chlamydomonas* sp. and *Oscillatoria* sp1 resulted the lag phase, exponential phase, stationary phase and death phase on days 1 to 7, 7 to 15, 14 to 15 and from 15 days, respectively. In the stationary phase, cell density was significantly higher ($p < 0.05$) in *Gonyostomum* sp. (23.173±0.41 cells/ml) and lower ($p < 0.05$) in *Navicula* sp. (0.213±0.002 cells/ml) compared to the other microalgae. Moreover, in terms of cell density, no significant difference ($p < 0.05$) was found between *Navicula* sp, *Choricystis* sp., *Chromochloris* sp. and *Chlorobotrys* sp. Similarly, *Chlamydomonas* sp. and *Coelastrella* sp. showed almost similar cell density.

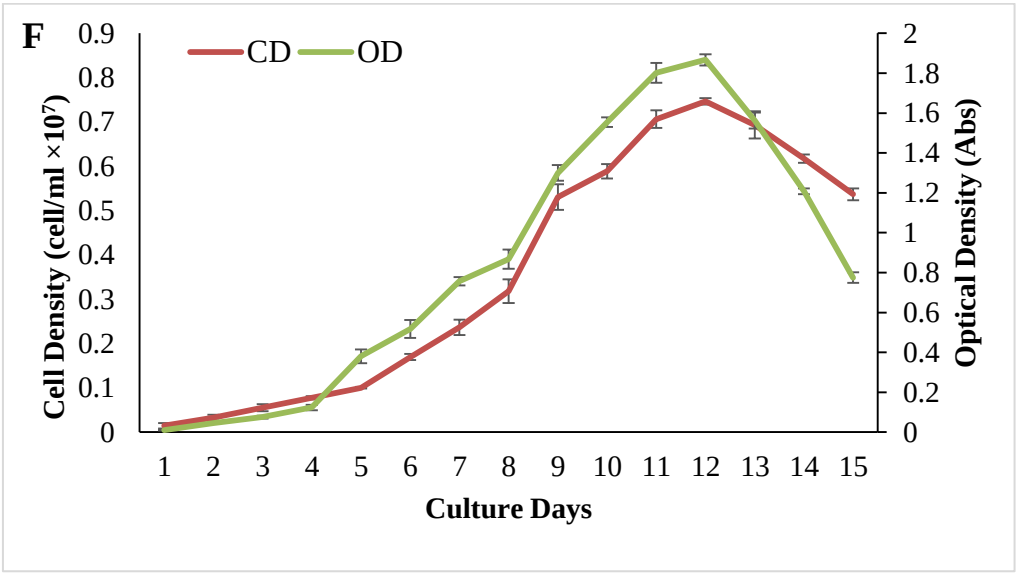


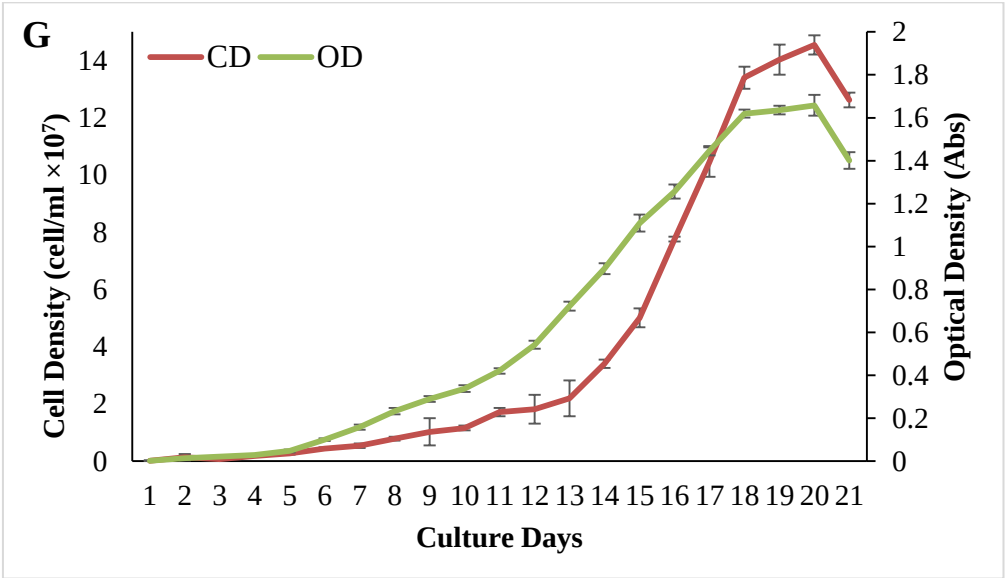

B
CD
OD
Cell Density (cell/ml ×10⁷)
Optical Density (Abs)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1.2
1
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9 10 11 12
Culture Days

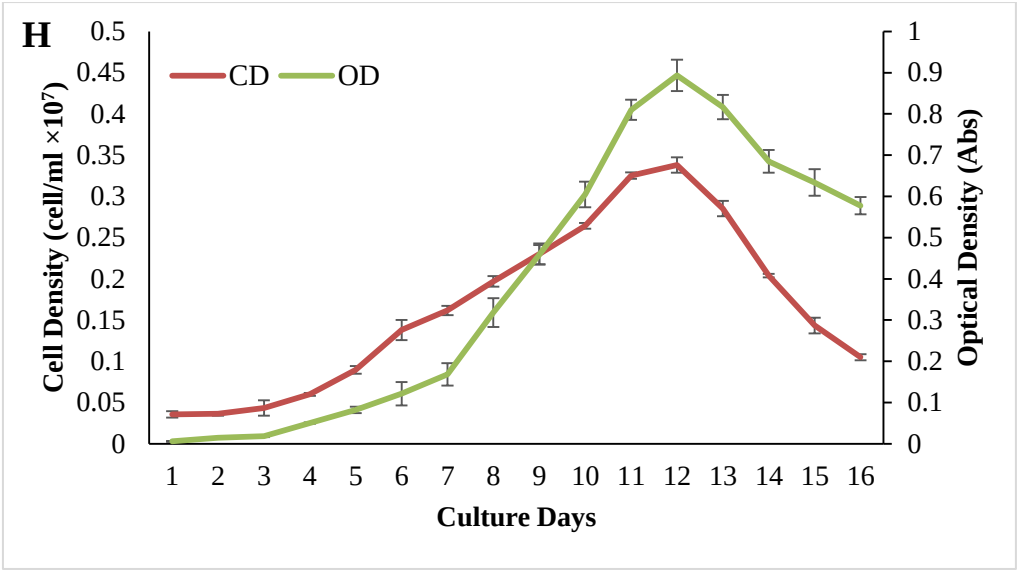

C
CD
OD
Cell Density (cell/ml ×10⁷)
Optical Density (Abs)
60
50
40
30
20
10
0
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Culture Days

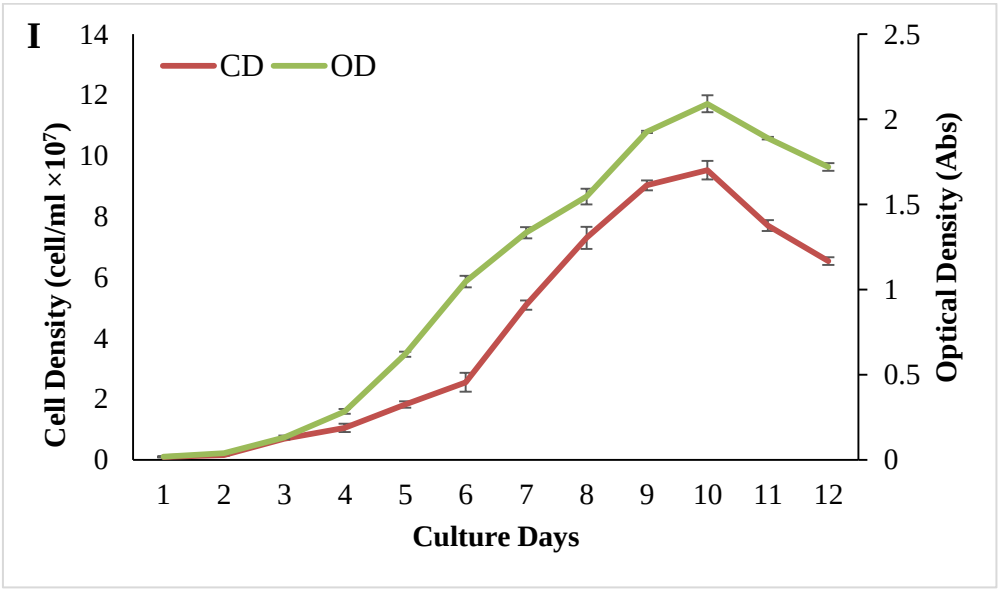

D
CD
OD
Cell Density (cell/ml ×10⁷)
Optical Density (Abs)
8
7
6
5
4
3
2
1
0
7
6
5
4
3
2
1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Culture Days


E
CD
OD
Cell Density (cell/ml ×10⁷)
Optical Density (Abs)
Culture Days
0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2
0 0.2 0.4 0.6 0.8 1 1.2
1 2 3 4 5 6 7 8 9 10 11 12 13


F
CD
OD
Cell Density (cell/ml ×10⁷)
Optical Density (Abs)
Culture Days
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9
0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15


G
CD
OD
Cell Density (cell/ml ×10⁷)
Optical Density (Abs)
Culture Days
0 2 4 6 8 10 12 14
0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21


H
CD
OD
Cell Density (cell/ml ×10⁷)
Optical Density (Abs)
Culture Days
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16


I
CD
OD
Cell Density (cell/ml ×10⁷)
Optical Density (Abs)
Culture Days
0 2 4 6 8 10 12 14
0 0.5 1 1.5 2 2.5
1 2 3 4 5 6 7 8 9 10 11 12

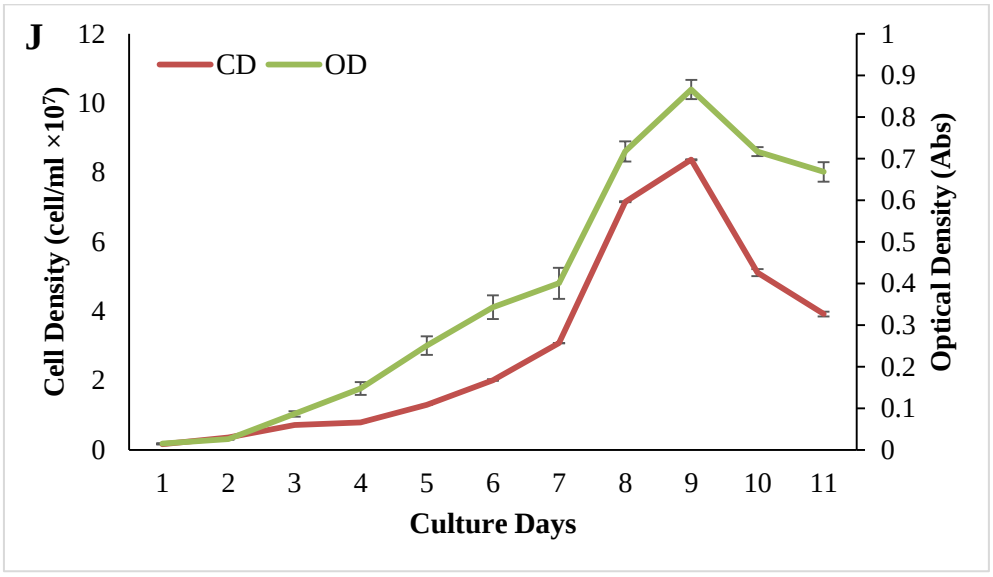
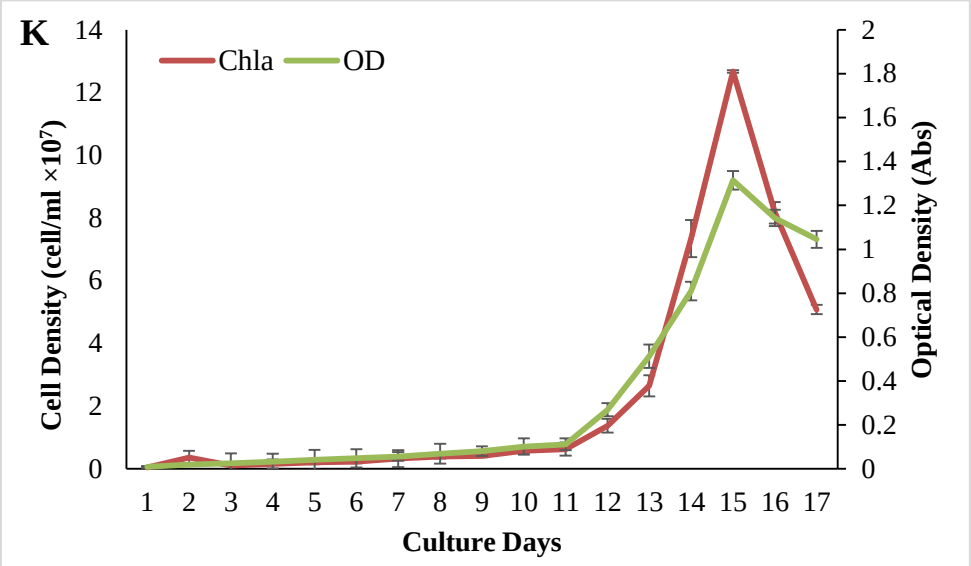
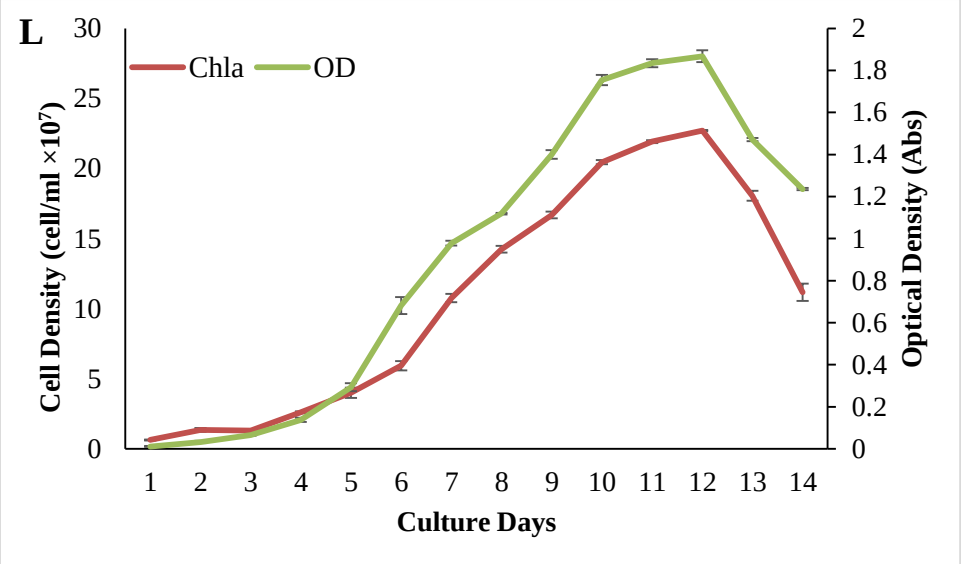

J
CD
OD
Cell Density (cell/ml ×10⁷)
Optical Density (Abs)
Culture Days
0 2 4 6 8 10 12
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
1 2 3 4 5 6 7 8 9 10 11

Figure 4.1: Growth curve in terms of cell density (cells/ml×10^7) and optical density (Absorbance) of marine and freshwater microalgae *Chlamydomonas* sp. (A), *Navicula* sp. (B), *Gonyostomum* sp. (C), *Nannochloropsis* sp. (D), *Choricystis* sp. (E), *Chromochloris* sp. (F), *Tetraedron* sp. (G), *Chlorobotrys* sp. (H), *Coelastrella* sp. (I), *Kirchneriella* sp. (J). Values are means ± standard error. CD and OD represent cell density, and optical density, respectively.





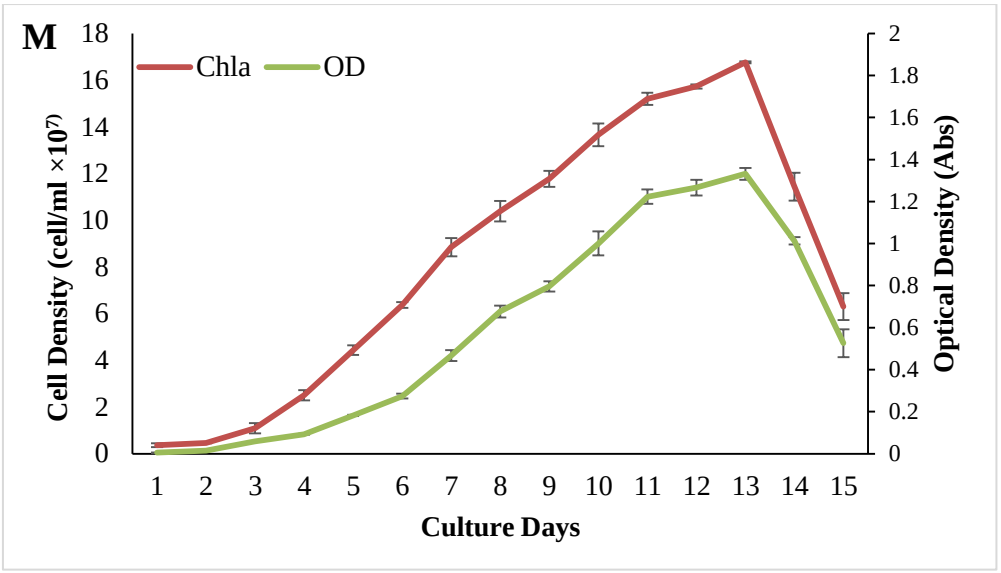


Figure 4.2: Growth curve in terms of chlorophyll-a and optical density (Absorbance) of marine and freshwater microalgae *Oscillatoria* sp1 (K), *Oscillatoria* sp2 (L), *Oscillatoria* sp3 (M) and *Oscillatoria* sp4 (N). Values are means ± standard error. Chla and OD represent chlorophyll a, and optical density, respectively.

4.6 Specific growth rate (SGR), cell duplication time, cell doublings per day and cell density on harvest of isolated freshwater and marine microalgae

Cell duplication time, cell doublings per day (K) and cell density on harvest varied among those different microalgae (Table 4.4) where significantly highest ($p \leq 0.05$) SGR was detected for *Choricystis* sp. and lowest for *Tetraedron* sp. Moreover, *Tetraedron* sp. and *Choricystis* sp. showed significantly ($p \leq 0.05$) highest and lowest cell duplication time, respectively. Along with this, *Navicula* sp. and *Oscillatoria* sp2

showed almost similar SGR and cell duplication time. On the other hand, significantly highest ($p \leq 0.05$) amount of cell doublings was detected for *Navicula* sp. while *Choricystis* sp. resulted the lowest. At the end of the exponential phase, significant highest and lowest ($p \leq 0.05$) amount of cell density was detected from *Gonyostomum* sp. and *Navicula* sp. In terms of cell density, no significant difference was detected from *Navicula* sp., *Choricystis* sp, *Chromochloris* sp. and *Chlorobotrys* sp. Moreover, no significant difference was also found the cell density of *Chlamydomonas* sp. and *Coelastrella* sp.

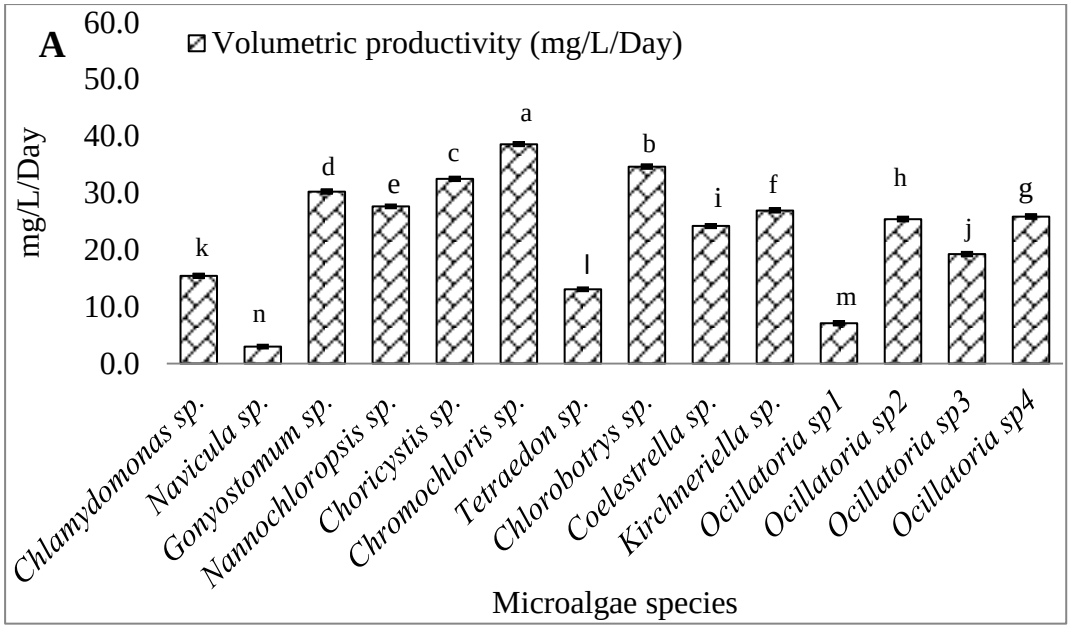
Table 4.5 Cell duplication time (Day), cell doublings per day (K) and cell density on harvest (cells/ml) (mean ± SE) of isolated freshwater and marine microalgae. Values with the different letters within each series indicate significant differences ($p < 0.05$) among the species.

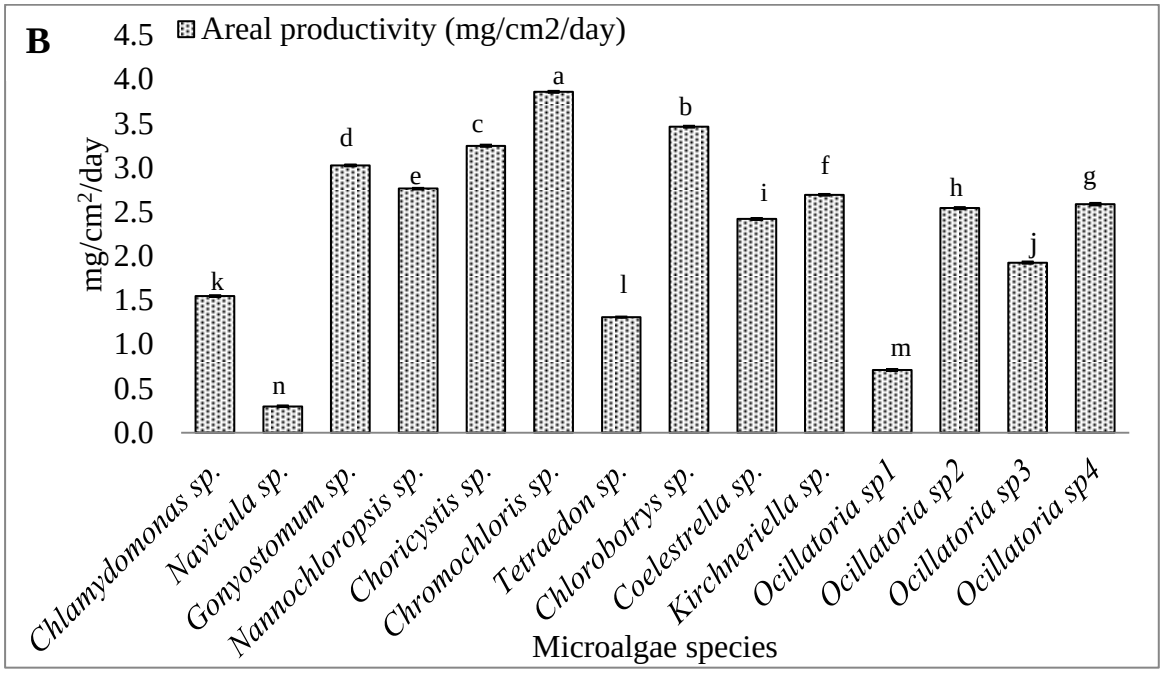
Microalgal Species	Cell duplication time (Day)	SGR(mg/day)	Cell doublings per day (K)	Cell Density (cells/ml×10^7)
***Chlamydomonas* sp.**	1.4 ± 0.0002^{c}	0.49 ± 0.00008^{k}	0.5787 ± 0.003^{abc}	8.7933 ± 0.12^{c}
***Navicula* sp.**	1.06 ± 0.002^{f}	0.65 ± 0.001^{h}	0.829 ± 0.21^{a}	0.2125 ± 0.002^{f}
***Gonyostomum* sp.**	1.12 ± 0.0001^{e}	0.62 ± 0.00008^{i}	0.4806 ± 0.004^{bc}	23.1733 ± 0.41^{a}
***Nannochloropsis* sp.**	1.05 ± 0.00003^{g}	0.66 ± 0.00002^{g}	0.4263 ± 0.01^{bc}	5.601 ± 0.12^{e}
***Choricystis* sp.**	0.78 ± 0.0001^{m}	0.89 ± 0.0001^{a}	0.2829 ± 0.02^{c}	1.0173 ± 0.01^{f}
***Chromochloris* sp.**	1 ± 0.00008^{i}	0.69 ± 0.00005^{e}	0.5269 ± 0.06^{abc}	0.6563 ± 0.02^{f}
Tetraedron sp.	1.86 ± 0.0003^{a}	0.37 ± 0.00005^{m}	0.5183 ± 0.01^{abc}	13.5333 ± 0.52^{b}
***Chlorobotrys* sp.**	1.02 ± 0.0001^{h}	0.68 ± 0.00007^{f}	0.2921 ± 0.02^{c}	0.3252 ± 0.004^{f}
***Coelastrella* sp.**	0.9 ± 0.0001^{k}	0.77 ± 0.0001^{c}	0.7399 ± 0.008^{ab}	9.0317 ± 0.16^{c}
***Kirchneriella* sp.**	0.8 ± 0.0001^{l}	0.87 ± 0.0001^{b}	0.6865 ± 0.01^{ab}	7.1558 ± 0.02^{d}
***Oscillatoria* sp1**	1.58 ± 0.002^{b}	0.44 ± 0.0005^{l}	-	-
***Oscillatoria* sp2**	1.06 ± 0.0004^{f}	0.65 ± 0.0003^{h}	-	-

***Oscillatoria* sp3**	1.19±0.0005[d]	0.58±0.0003[j]	-	-
***Oscillatoria* sp4**	0.98±0.0004[j]	0.71±0.0003[d]	-	-

4.7 Volumetric, areal and lipid productivity of isolated freshwater microalgae

In this study, different freshwater and marine tropical microalgae species were cultured in BBM and Conway media respectively providing uniform parameters to determine the difference in their productivity. Figure 4.4A showed the variation in volumetric productivity for the fourteen microalgae species where significantly highest and lowest ($p \leq 0.05$) amount of volumetric productivity was detected for *Chromochloris* sp. (38.59±0.02 mg/L/Day) and *Navicula* sp. (2.97±0.02 mg/L/Day), respectively. The results also showed that areal productivity varied among those species (Figure 4.4B). The areal productivity varied as 1.54±0.001, 0.29±0.02, 3.02±0.003, 2.76±0.006, 3.24±0.004, 3.86±0.004, 1.31±0.001, 3.46±0.003, 2.41±0.002, 2.69±0.002, 0.71±0.005, 2.54±0.007, 1.92±0.006, and 2.59±0.007 $mg/cm^2/day$ in *Chlamydomonas* sp., *Navicula* sp., *Gonyostomum* sp., *Nannochloropsis* sp., *Choricystis* sp., *Chromochloris* sp., *Tetraedron* sp., *Chlorobotrys* sp. *Coelastrella* sp., *Kirchneriella* sp. *Oscillatoria* sp1, *Oscillatoria* sp2, *Oscillatoria* sp3 and *Oscillatoria* sp4, respectively. Areal productivity was significantly ($p < 0.05$) highest in *Chromochloris* sp. and lowest in *Navicula* sp. Lipid productivity in various microalgae also differs from species to species which is represented in Figure 4.4C. *Chlamydomonas* sp., *Navicula* sp., *Gonyostomum* sp., *Nannochloropsis* sp., *Choricystis* sp., *Chromochloris* sp., *Tetraedron* sp., *Chlorobotrys* sp. *Coelastrella* sp., *Kirchneriella* sp. *Oscillatoria* sp1, *Oscillatoria* sp2, *Oscillatoria* sp3 and *Oscillatoria* sp4 resulted in about3.25±0.11, 0.42±0.002, 8.29±0.22, 5.62±0.23, 4.48±0.28, 8.57±0.19, 3.34±0.12, 7.57±0.18, 5.35±0.11, 6.23±0.01, 1.15±0.004, 3.3±0.03, 1.74±0.01and 4.43±0.03 mg/L/Day of lipid productivity, respectively. Significantly ($p < 0.05$) maximum and minimum amount of lipid productivity were detected from *Chromochloris* sp. and *Navicula* sp. Together with this, similar kind of lipid productivity was detected from *Chlamydomonas* sp., *Tetraedron* sp. and *Oscillatoria* sp2. Moreover, lipid productivity of *Choricystis* sp. and *Oscillatoria* sp4 showed no significant differences.


A
Volumetric productivity (mg/L/Day)
mg/L/Day
60.0
50.0
40.0
30.0
20.0
10.0
0.0
k
n
d
e
c
a
l
b
i
f
m
h
j
g
Chlamydomonas sp.
Navicula sp.
Gonyostomum sp.
Nannochloropsis sp.
Choricystis sp.
Chromochloris sp.
Tetraedon sp.
Chlorobotrys sp.
Coelestrella sp.
Kirchneriella sp.
Ocillatoria sp1
Ocillatoria sp2
Ocillatoria sp3
Ocillatoria sp4
Microalgae species


B
Areal productivity (mg/cm2/day)
mg/cm2/day
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
k
n
d
e
c
a
l
b
i
f
m
h
j
g
Chlamydomonas sp.
Navicula sp.
Gonyostomum sp.
Nannochloropsis sp.
Choricystis sp.
Chromochloris sp.
Tetraedon sp.
Chlorobotrys sp.
Coelestrella sp.
Kirchneriella sp.
Ocillatoria sp1
Ocillatoria sp2
Ocillatoria sp3
Ocillatoria sp4
Microalgae species

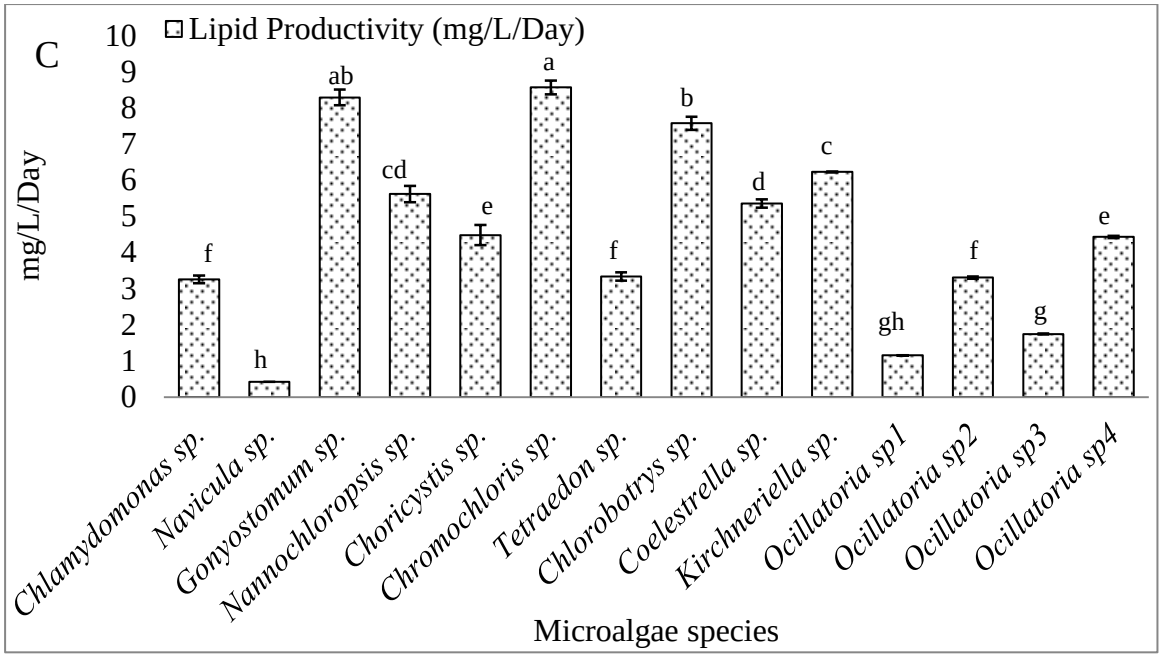


Figure 4.3: Volumetric productivity (A), areal productivity (B) and lipid productivity (C) (mean ± SE) of isolated microalgae. Values with the different letters within each series indicate significant differences ($p < 0.05$) among the species.

4.8 Pigment content of isolated microalgae:

4.8.1 Chlorophyll

Chlorophyll, carotenoid and different phycoboliproteins content were determined under this study where chlorophyll-a, chlorophyll-b chlorophyll-c and carotenoid were recorded as µg/mL and phycobiliprotein content in mg/g.

Different microalgae showed a considerable variation in chlorophyll (Table 4.5), carotenoid (Figure 4.6) and phycobiliproteins (Table 4.5) content. Under this study, chlorophyll-a content of fourteen microalgae species were determined where nine species were green microalgae, one was brown microalgae (Diatom) and four species were cyanobacteria. Significantly highest ($p < 0.05$) amount of chlorophyll-a (22.72±0.04 µg/mL) was recorded in *Oscillatoria* sp2 on day-10. On the other hand, *Navicula* sp. showed significantly minimum ($p < 0.05$) amount of chlorophyll-a (1.048±0.02 µg/mL) on day-6. There was no significant difference was found between chlorophyll-a content of *Gonyostomum* sp. and *Kirchneriella* sp. Chlorophyll-b content of nine green microalgae were recorded where Significantly highest ($p < 0.05$) amount of chlorophyll-b (2.75±0.07 µg/mL) was recorded in *Chlamydomonas* sp. On the other hand, *Choricystis* sp. showed significantly

minimum ($p < 0.05$) amount of chlorophyll-b (0.58±0.04 µg/mL). Chlorophyll-c content of brown microalgae *Navicula* sp. was determined and the value was 0.85±0.005 µg/mL.

Table 4.6: Chlorophyll-a, b and c (means ± SE) of isolated freshwater and marine microalgae, cultured in BBM and Conway media, respectively. Values with the different letters within each series indicate significant differences ($p < 0.05$) among the species.

Microalgal Species	Chlorophyll-a	Chlorophyll-b	Chlorophyll-c
***Chlamydomonas* sp.**	10.27 ± 0.06^{f}	2.75 ± 0.07^{a}	-
***Gonyostomum* sp.**	3.19 ± 0.04^{k}	0.88 ± 0.02^{e}	-
***Nannochloropsis* sp.**	11.47 ± 0.13^{e}	2.007 ± 0.02^{bc}	-
***Choricystis* sp.**	2.54 ± 0.04^{l}	0.58 ± 0.04^{f}	-
***Chromochloris* sp.**	7.8 ± 0.09^{g}	1.86 ± 0.09^{c}	-
Tetraedron sp.	5.51 ± 0.001^{i}	2.27 ± 0.04^{b}	-
***Chlorobotrys* sp.**	6.65 ± 0.04^{h}	1.81 ± 0.1^{c}	-
***Coelastrella* sp.**	4.42 ± 0.1^{j}	1.19 ± 0.03^{d}	-
***Kirchneriella* sp.**	3.15 ± 0.04^{k}	1.17 ± 0.02^{d}	-
***Navicula* sp.**	1.048 ± 0.02^{m}	-	0.85±0.005
***Oscillatoria* sp1**	12.67 ± 0.04^{d}	-	-
***Oscillatoria* sp2**	22.72 ± 0.04^{a}	-	-
***Oscillatoria* sp3**	16.76 ± 0.04^{c}	-	-
***Oscillatoria* sp4**	21.06 ± 0.07^{b}	-	-

4.8.2 Carotenoid

Total carotenoid content varied among those different microalgae (Figure 4.6). Significantly ($p < 0.05$) highest and lowest quantity of carotenoid accumulations was detected at day 13 from *Chlamydomonas* sp. (6.888±0.08 µg/mL) and *Oscillatoria*

sp1 (0.99±0.008 µg/mL), respectively. Moreover, identical amount of total carotenoid was detected from four strains of *Oscillatoria*.

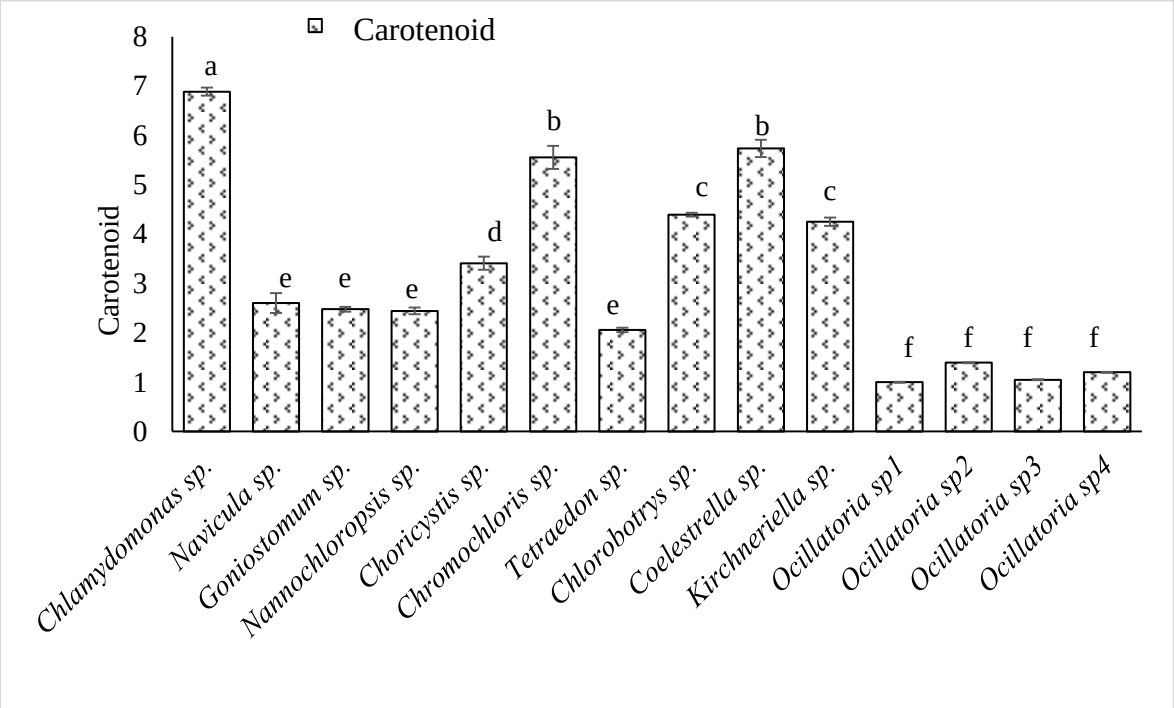


Figure 4.4: Carotenoid content (µg/ml) (mean ± SE) of isolated freshwater and marine microalgae, cultured in BBM and Conway Medium. Values with the different letters within each series indicate significant differences ($p < 0.05$) among the species.

4.8.3 Phycobiliproteins

Phycobiliproteins analysis found that, phycocyanine, allophycocyanin and phycoerythrin content were significantly ($p < 0.05$) highest in *Oscillatoria* sp4. at day 9 of about 69.3±0.012 mg/g, 18.3±0.099 mg/g and 2.2±0.033 mg/g respectively (Table 4.5). Significantly ($p < 0.05$) minimum and maximum ($p < 0.05$) quantity of total phycobiliprotein content was achieved in *Nannochloropsis* sp. (0.89±0.04 mg/g) and *Oscillatoria* sp4. (89.80±0.12 mg/g) at day 10 and day 9, respectively (Table 4.5).

Table 4.7 Different phycobiliproteins (Phycocyanine, Allophycocyanin and Phycoerythrin) along with total phycobiliproteins of isolated marine and freshwater microalgae. Values are means ± SE. Values with the different letters within each series indicate significant differences ($p < 0.05$) among the species.

Algal Species	Phycocyanine Content (mg/g)	Allophycocyanin Content (mg/g)	Phycoerythrin content (mg/g)	Total phycobiliproteins content (mg/g)
***Chlamydomonas* sp.**	1.96 ± 0.11^{e}	0.99 ± 0.01^{g}	0.56 ± 0.009^{fg}	3.50 ± 0.1^{e}
***Navicula* sp.**	1.12 ± 0.07^{f}	1.41 ± 0.03^{f}	0.99 ± 0.03^{d}	3.52 ± 0.11^{e}
***Gonyostomum* sp.**	0.33 ± 0.04^{gh}	1.05 ± 0.05^{g}	0.41 ± 0.03^{fgh}	1.79 ± 0.04^{fg}
***Nannochloropsis* sp.**	0.22 ± 0.008^{gh}	0.51 ± 0.01^{h}	0.16 ± 0.02^{i}	0.89 ± 0.04^{h}
***Choricystis* sp.**	0.52 ± 0.008^{fg}	1.3 ± 0.03^{fg}	0.48 ± 0.02^{fg}	2.301 ± 0.009^{f}
***Chromochloris* sp.**	1.07 ± 0.02^{f}	1.92 ± 0.01^{e}	1.04 ± 0.03^{d}	4.04 ± 0.04^{e}
***Tetraedron* sp.**	0.67 ± 0.008^{fg}	1.03 ± 0.04^{g}	0.62 ± 0.005^{ef}	2.32 ± 0.05^{f}
***Chlorobotrys* sp.**	0.26 ± 0.05^{gh}	0.58 ± 0.04^{h}	0.23 ± 0.02^{hi}	1.07 ± 0.07^{h}
***Coelastrella* sp.**	0.3 ± 0.02^{h}	1.93 ± 0.02^{e}	0.31 ± 0.004^{ghi}	1.98 ± 0.009^{fg}
***Kirchneriella* sp.**	0.1 ± 0.04^{gh}	1 ± 0.03^{g}	0.41 ± 0.05^{fghi}	1.50 ± 0.04^{gh}
***Oscillatoria* sp1**	61.7 ± 0.03^{b}	21.7 ± 0.14^{a}	2.6 ± 0.14^{b}	85.93 ± 0.3^{b}
***Oscillatoria* sp2**	42.6 ± 0.47^{c}	6.01 ± 0.16^{d}	4.67 ± 0.08^{a}	53.28 ± 0.26^{c}
***Oscillatoria* sp3**	35.6 ± 0.09^{d}	7.14 ± 0.06^{c}	0.85 ± 0.02^{de}	43.64 ± 0.06^{d}
***Oscillatoria* sp4**	69.3 ± 0.01^{a}	18.3 ± 0.1^{b}	2.2 ± 0.03^{c}	89.80 ± 0.12^{a}

4.9 Proximate composition of microalgal species:

In order to compare the differences in the biochemical composition, various marine and freshwater tropical microalgae species were cultivated in respectively Conway medium and BBM under uniform conditions. Figure 4.8 demonstrates how the fourteen different microalgae species varied in their protein composition, which ranged from 17.276±0.76 to 59.5123±0.78 % dry weight. The percentage of protein was significantly ($p < 0.05$) higher in *Navicula* sp. and lower in *Krichneriella* sp. Among *Nannochloropsis* sp., *Choricystis* sp., *Chromochloris* sp. and *Chlorobotrys* sp. there was no significant ($p < 0.05$) difference in protein content. Moreover, *Chlmydomona*s sp., *Navicula* sp. and *Oscillatoria* sp4 showed nearly similar percentage of protein content. The result showed that lipid content varied among those species (Figure 4.9). The total lipid content varied as 21.07±0.71, 14.13±0.18, 27.4±0.69, 20.33±0.81, 13.8±0.87, 22.2±0.50, 25.53±0.87, 21.86±0.53, 22.13±0.47, 23.13±0.07, 13±0.12, 9.07±0.07, 17.13±0.13% dry weight among *Chlamydomonas* sp., *Navicula* sp., *Gonyostomum* sp., *Nannochloropsis* sp., *Choricystis* sp., *Chromochloris* sp., *Tetraedron* sp., *Chlorobotrys* sp. *Coelastrella* sp., *Kirchneriella*

sp. and four strains of *Oscillatoria* sp. respectively. Lipid content was significantly ($p < 0.05$) higher in *Gonyoatomum* sp. and lower in *Oscillatoria* sp3. Total lipid content of *Chlamydomonas* sp., *Nannochloropsis* sp., *Chromochloris* sp., *Chlorobotrys* sp., *Coelastrella* sp., and *Kirchneriella* sp. showed no significance difference. Carbohydrate production in various microalgae also differ from species to species which is represented in Figure 4.10. *Chlamydomonas* sp., *Navicula* sp., *Gonyostomum* sp., *Nannochloropsis* sp., *Choricystis* sp., *Chromochloris* sp., *Tetraedron* sp., *Chlorobotrys* sp. *Coelastrella* sp., *Kirchneriella* sp. and four strains of *Oscillatoria* resulted in 7.49±0.46, 6.15±0.41, 13.56±0.48, 11.91.78±0.72, 14.41±0.52, 10.37±0.09, 9.69±0.82, 7.27±0.37, 18±0.06, 11.09±0.06, 17.04±0.08, 14.89±0.32 and 7.49±0.14 % dry weight of carbohydrate, respectively. Significantly ($p < 0.05$) maximum and minimum amount of carbohydrate were detected in *Kirchneriella* sp. and *Navicula* sp. respectively. Together with this, similar kind of carbohydrate content was detected in *Chlamydomonas* sp., *Navicula* sp., *Coelastrella* sp and *Oscillatoria* sp4.

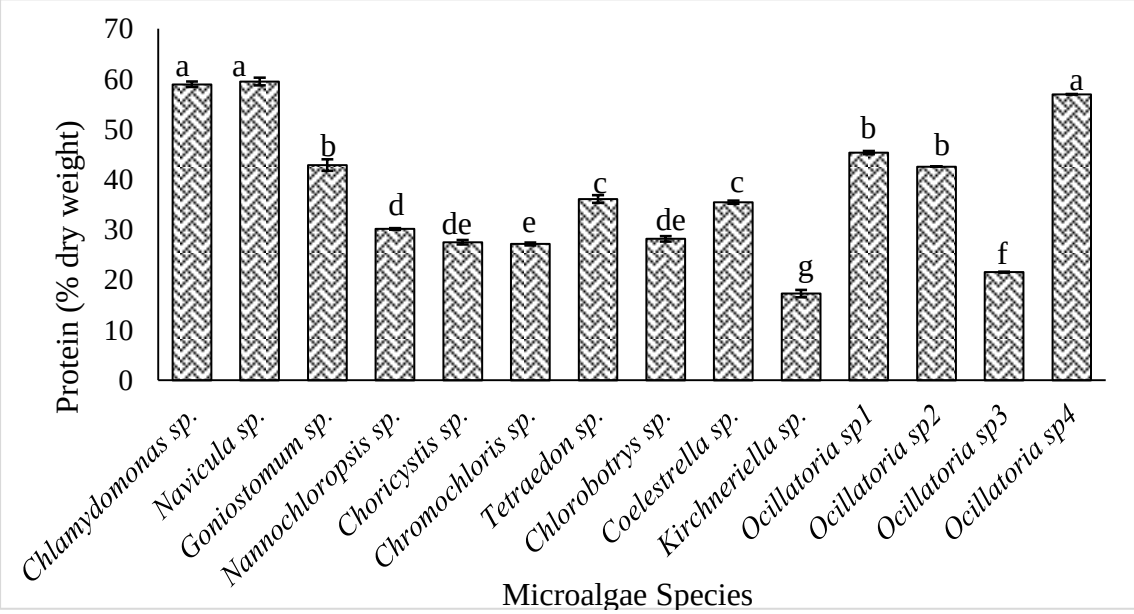


Figure 4.5: Protein content (% dry weight) (mean ± SE) of isolated freshwater and marine microalgae cultured in respectively BBM and Conway Medium. Values with the different letters within each series indicate significant differences ($p < 0.05$) among the species.

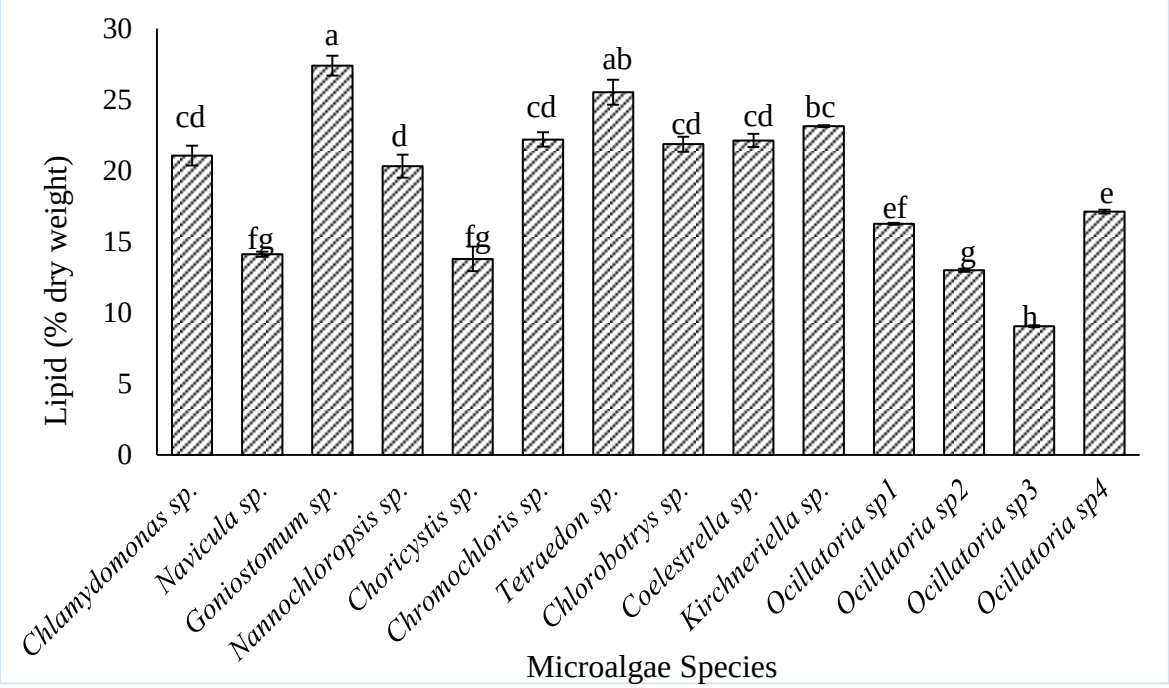


Figure 4.6: Lipid content (% dry weight) (mean ± SE) of isolated freshwater and marine microalgae cultured in respectively BBM and Conway Medium. Values with the different letters within each series indicate significant differences ($p < 0.05$) among the species.

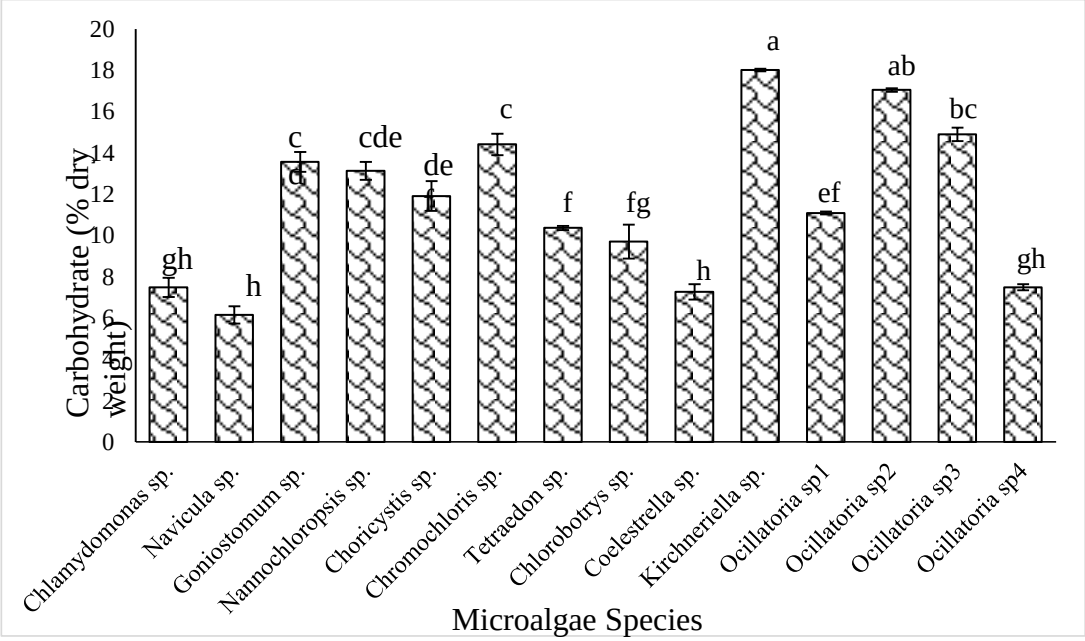


Figure 4.7: Carbohydrate content (% dry weight) (mean ± SE) of freshwater and marine microalgae cultured in respectively BBM and Conway Medium. Values with the different letters within each series indicate significant differences ($p < 0.05$) among the species.

4.8 Fatty Acid Profile

In this study, table 4.6 and 4.7 showed fatty acid content of marine and freshwater microalgae, respectively. Highest amount of SAFA was found in *Oscillatoria* sp1 and lowest was *Nannochloropsis* sp. On the other hand, lowest amount of PUFA was found in *Oscillatoria* sp1(6.20±1.30 ppm)and highest was recorded in *Navicula* sp. (673.2±16.32). In addition, *Navicula* sp. showed highest amount of n-6 PUFA, where lowest was found in *Choricystis* sp. Along with this, *Gonyostomum* sp. and *Chromochloris* sp. showed lowest and highest amont of n-3 PUFA in this study, respectively. But, no significant differencence was found in Methyl icosa-5,8,11,14,17-pentaenoateand Methyl Docosahexanoate content of microalgal species under this experiment

Table 4.8: Fatty acid content of selected isolated marine microalgae

Carbon	**Fatty Acid Methyl Esters**	***Chlamydomonas* sp.**	***Navicula* sp.**	***Gonyostomum* sp.**	***Nannochloropsis* sp.**	***Oscillatoria* sp1**	***Oscillatoria* sp2**
		Conc (ppm)					
C8:0	Methyl Octanoate	2.04±2.01	1.52±0.04	1.63±0.02	1.11±0.03	0.27±0.26	0.02±0.00
C10:0	Methyl Decanoate	1.39±1.37	1.16±0.04	1.47±0.02	0.87±0.02	0.46±0.01	3.57±0.02
C12:0	Methyl Laurate	5.86±5.85	4.27±0.06	5.05±0.02	3.85±0.06	3.30±0.06	0.23±0.00
C13:0	Methyl Tridecanoate	1.11±0.01	0.01±0.00	0.40±0.00	0.48±0.00	0.27±0.01	5.31±0.00
C14:0	Methyl Myristate	1.07±0.06	0.05±0.00	0.17±0.02	0.11±0.00	0.06±0.00	4.18±1.49
C16:0	Methyl Palmitate	4.30±0.06	2.51±2.13	2.22±0.03	3.08±2.71	14.32±0.68	9.37±0.14
C18:0	Methyl Stearate	4.71±0.04	6.95±0.15	0.57±0.26	1.76±0.14	13.85±0.70	7.59±6.04
C20:0	Methyl Arachidate	2.62±0.92	4.31±0.08	9.73±1.96	4.31±0.99	1.36±0.09	0.59±0.00
C17:0	Methyl Heptadecanoate	3.99±0.02	3.53±0.00	4.24±0.02	1.29±1.28	0.97±0.00	1.55±0.04
C21:0	Methyl Heneicosanoate	0.34±0.08	0.05±0.02	0.02±0.01	0.11±0.11	0.22±0.02	0.27±0.02
C22:0	Methyl Behenate	2.68±0.17	0.02±0.00	0.003±0.01	0.003±0.00	3.91±0.05	0.89±0.01
C23:0	Methyl Tricosanoate	ND	ND	0.18±0.1	0.05±0.05	ND	ND
C24:0	Methyl Lignocerate	ND	ND	ND	ND	ND	ND
	ΣSAFA	**30.11±10.2^{abc}**	**24.38±2.47^{abc}**	**25.67±2.22^{abc}**	**17.01±2.44^{bc}**	**39.00±1.18^{a}**	**33.55±4.48^{ab}**
C16:1	Methyl Palmitoleate	6.05±0.34	1.02±0.08	8.19±0.04	6.60±0.51	6.43±0.03	1.97±0.05
C18:1	Methyl Oleate	0.26±0.00	2.64±0.05	0.31±0.00	0.47±0.00	0.15±0.01	0.78±0.01
C20:1	Methyl cis-11-eicosenoate	0.004±0.00	0.004±0.00	0.01±0.00	0.09±0.00	0.01±0.01	0.03±0.01
C22:1	Methyl Erucate	0.51±0.00	0.09±0.09	0.19±0.12	1.00±0.01	0.13±0,02	0.35±0.02
C24:1	Methyl Nervonate	0.07±0.00	0.09±0.04	0	0.01±0.01	0.004±0.00	0.05±0.01
	ΣMUFA	**6.89±0.34^{c}**	**3.84±0.18^{c}**	**8.70±0.07^{c}**	**8.18±0.5^{c}**	**6.73±0.01^{c}**	**3.18±0.08^{c}**
C18:2n-6	Methyl Linoleate	213.99±10.12	667.59±16.48	321.63±9.51	2.11±0.16	0.77±0.05	233.01±1.13
C20:3n-6	Methyl 11-14-17- Eicosatrienoate	2.04±0.07	1.98±0.09	0.24±0.03	1.91±0.03	0.44±0.03	0.22±0.09

Carbon	**Fatty Acid Methyl Esters**	***Chlamydomonas* sp.**	***Navicula* sp.**	***Gonyostomum* sp.**	***Nannochloropsis* sp.**	***Oscillatoria* sp1**	***Oscillatoria* sp2**
		Conc (ppm)					
C20:4n-6	Methyl Arachidonate	5.73±0.20	0.21±0.07	2.28±0.09	0.77±0.63	0.04±0.01	0.10±0.03
	Σn6-PUFA	**221.76±0.39^{cd}**	**669.78±16.3^{a}**	**323.15±9.57^{b}**	**4.79±0.81^{f}**	**1.25±0.07^{f}**	**233.33±1.07^{d}**
C18:3n-3	Methyl Linolenate	0.49±0.02	3.20±0.00	1.26±0.10	2.47±0.40	0.89±0.03	1.23±0.00
C20:5n-3	**Methyl icosa-5,8,11,14,17-pentaenoate**	**0.72±0.09**	**0.003±0.00**	**0.60±0.02**	**2.55±2.14**	**2.58±0.05**	**2.60±0.05**
C22:5n-3	Methyl Docosapentaenoate	0.90±0.02	0.01±0.00	0.12±0.12	1.41±0.05	1.32±1.32	0.00±0.00
C22:6n-3	**Methyl Docosahexanoate**	**0.37±0.02**	**0.25±0.01**	**0.10±0.00**	**0.19±0.01**	**0.17±0.13**	**0.15±0.03**
	Σn3-PUFA	**2.49±0.08^{c}**	**3.46±0.01^{c}**	**2.08±0.24^{c}**	**6.62±2.49^{abc}**	**4.96±1.37^{bc}**	**3.98±0.02^{c}**
	ΣPUFA	**224.24±10.39^{cd}**	**673.2±16.32^{a}**	**326.23±9.33^{b}**	**11.41±1.67^{f}**	**6.20±1.30^{f}**	**237.31±1.05^{de}**
	Σn3/ Σn6	**0.01±0.00^{c}**	**0.01±0.00^{c}**	**0.01±0.00^{c}**	**1.38±0.78^{bc}**	**3.97±1.32^{b}**	**0.02±0.00^{c}**
	DHA/EPA	**0.51±0.04^{b}**	**98.4±16.75^{a}**	**0.16±0.00^{b}**	**0.07±0.21^{b}**	**0.06±0.05^{b}**	**0.06±0.01^{b}**
	SAFA/TUFA	**0.13±0.05^{c}**	**0.04±0.00^{c}**	**0.07±0.01^{c}**	**0.87±0.22^{b}**	**3.02±0.40^{a}**	**0.14±0.02^{c}**
	SAFA/TFA	**0.12±0.04^{d}**	**0.03±0.00^{d}**	**0.07±0.01^{d}**	**0.46±0.06^{bc}**	**0.75±0.02^{a}**	**0.12±0.01^{d}**
	TUFA/TFA	**0.88±0.04^{a}**	**0.97±0.00^{a}**	**0.93±0.01^{a}**	**0.54±0.06^{bc}**	**0.25±0.02^{d}**	**0.88±0.01^{a}**

Fatty acid values are expressed as mean of the duplicates. Here, **SAFA** means Saturated Fatty Acids, **MUFA**= Monounsaturated fatty acids, **n6-PUFA**= ω-6 polyunsaturated fatty acids, **n3-PUFA**= ω-3 polyunsaturated fatty acids, **DHA**= Docosahexaenoic acid, **EPA=** Eicosapentaenoic acid, **TUFA**= Total unsaturated fatty acids, **TFA**= Total fatty acids.

Table 4.9: Fatty acid content of selected isolated freshwater microalgae

Carbon	Fatty Acid	*Choricystis* sp.	*Chromochloris* sp.	*Tetraedron* sp.	*Chlorobotrys* sp.	*Coelestrella* sp.	*Kirchneriella* sp.	*Oscillatoria* sp3	*Oscillatoria* Sp4
	Methyl Esters			**Conc (ppm)**					
C8:0	Methyl Octanoate	1.59±0.06	0.06±0.00	1.17±0.00	0.73±0.01	1.47±0.01	1.26±0.02	0.03±0.00	0.02±0.00
C10:0	Methyl Decanoate	0.01±0.00	1.18±0.05	0.44±0.43	0.48±0.00	0.50±0.49	1.02±0.01	1.16±1.15	3.57±0.02
C12:0	Methyl Laurate	2.60±0.03	5.89±0.08	3.64±0.00	1.99±0.03	6.37±0.11	4.88±0.05	0.02±0.00	0.23±0.00
C13:0	Methyl Tridecanoate	0.15±0.14	0.53±0.01	0.54±0.00	0.28±0.00	0.42±0.01	0.49±0.00	1.89±0.03	5.31±0.00
C14:0	Methyl Myristate	0.02±0.00	0.17±0.00	0.06±0.00	0.08±0.01	1.31±0.04	0.14±0.00	0.74±0.22	4.18±1.49
C16:0	Methyl Palmitate	2.97±0.01	5.28±0.09	3.47±1.32	6.56±0.10	5.76±1.01	6.66±0.09	1.33±0.04	9.37±0.14
C18:0	Methyl Stearate	10.50±0.38	3.13±0.15	0.45±0.38	2.67±0.03	0.23±0.01	0.09±0.01	2.53±0.10	7.59±6.04
C20:0	Methyl Arachidate	2.07±0.07	1.87±1.17	7.97±0.08	9.98±0.76	4.17±0.31	5.20±1.13	3.49±0.36	0.59±0.00
C17:0	Methyl Heptadecanoate	0.79±0.00	0.08±0.07	1.74±0.00	2.79±0.02	0.08±0.01	0.02±0.01	1.93±1.92	1.55±0.04
C21:0	Methyl Heneicosanoate	0.02±0.00	4.43±0.18	0.02±0.01	0.04±0.00	1.51±1.5	2.16±0.91	2.32±2.08	0.27±0.02
C22:0	Methyl Behenate	3.57±0.05	1.13±0.03	0.003±0.00	2.75±0.05	1.66±0.06	ND	1.76±0.06	0.89±0.01
C23:0	Methyl Tricosanoate	ND	ND	ND	0.06±0.01	ND	ND	ND	ND
C24:0	Methyl Lignocerate	ND	ND	ND	ND	ND	ND	ND	ND
	ΣSAFA	**24.29±0.33^{abc}**	**23.73±1.4^{abc}**	**19.50±0.58^{bc}**	**28.41±0.76^{abc}**	**23.48±0.36^{abc}**	**21.92±1.86^{abc}**	**17.20±1.07^{c}**	**33.55±4.48^{ab}**
C16:1	Methyl Palmitoleate	6.26±2.77	20.48±5.97	7.71±3.73	35.72±0.50	10.79±2.2	6.02±0.22	12.24±0.51	1.97±0.05
C18:1	Methyl Oleate	0.08±0.07	0.83±0.01	0.16±0.15	0.47±0.02	0.5±0.00	0.38±0.00	0.53±0.00	0.78±0.01
C20:1	Methyl cis-11-eicosenoate	0.01±0.00	0.08±0.01	0.004±0.00	0.15±0.15	0.01±0.00	0.01±0.01	0.17±0.00	0.03±0.01
C22:1	Methyl Erucate	0.06±0.01	1.12±0.2	0.07±0.01	0.26±0.00	0.29±0.01	0.10±0.03	0.00±0.00	0.35±0.02
C24:1	Methyl Nervonate	0.02±0.02	0.05±0.03	0.01±0.01	0.01±0.00	0.02±0.01	0.02±0.01	0.09±0.04	0.05±0.01
	ΣMUFA	**6.42±2.68^{c}**	**22.57±5.9^{b}**	**7.95±3.89^{c}**	**36.61±0.37^{a}**	**11.6±2.18**	**6.53±0.24^{bc}**	**13.04±0.47^{bc}**	**3.18±0.08^{c}**

Carbon	Fatty Acid	*Choricystis* sp.	*Chromochloris* sp.	*Tetraedron* sp.	*Chlorobotrys* sp.	*Coelestrella* sp.	*Kirchneriella* sp.	*Oscillatoria* sp3	*Oscillatoria* Sp4
		Conc (ppm)							
C18:2n-6	Methyl Linoleate	0.71±0.09	159.87±0.33	0.74±0.05	238.3.30±7.8	337.22±0.38	236.08±0.99	0.66±0.44	233.01±1.13
C20:3n-6	Methyl 11-14-17-Eicosatrienoate	0.17±0.05	0.89±0.02	0.55±0.01	0.89±0.02	1.4485±0.12	0.84±0.11	3.22±0.05	0.22±0.09
C20:4n-6	Methyl Arachidonate	0.32±0.01	0.13±0.01	0.87±0.02	2.27±0.00	3.313±0.04	1.99±0.07	0.47±0.00	0.10±0.03
	Σn6-PUFA	**1.20±0.02^{f}**	**160.89±0.7^{e}**	**2.16±0.04^{f}**	**241.45±7.7^{c}**	**341.98±0.30^{b}**	**238.92±1.17^{c}**	**4.35±0.48^{f}**	**233.33±1.07^{cd}**
C18:3n-3	Methyl Linolenate	10.71±0.05	14.29±0.33	2.17±0.25	1.99±0.03	4.67±0.02	4.47±1.77	7.22±0.41	1.23±0.00
C20:5n-3	**Methyl icosa-5,8,11,14,17-pentaenoate**	**2.87±0.02**	**1.03±0.7**	**2.13±1.96**	**0.46±0.03**	**1.86±0.6**	**1.07±0.13**	**0.02±0.01**	**2.60±0.05**
C22:5n-3	Methyl Docosapentaenoate	0.64±0.02	0.01±0.12	1.09±1.09	1.04±0.01	0.01±0.01	3.66±3.65	0.01±0.00	0.00±0.00
C22:6n-3	**Methyl Docosahexanoate**	**0.12±0.00**	**0.16±0.00**	**0.11±0.00**	**0.12±0.03**	**0.11±0.01**	**0.25±0.25**	**0.16±0.01**	**0.15±0.03**
	Σn3-PUFA	**14.33±0.08^{ab}**	**15.48±0.82^{a}**	**5.51±3.29^{abc}**	**3.60±7.75^{abc}**	**6.64±0.65^{c}**	**9.45±5.31^{abc}**	**7.41±0.41^{abc}**	**3.98±0.02^{bc}**
	ΣPUFA	**15.53±0.11^{f}**	**176.37±1.16^{e}**	**7.67±3.25^{f}**	**245.06±7.75^{c}**	**348.63±0.94^{b}**	**155.87±4.14^{c}**	**11.76±0.08^{f}**	**237.31±1.05^{cd}**
	Σn3/ Σn6	**11.98±0.16^{a}**	**0.10±0.00^{c}**	**2.56±1.58^{bc}**	**0.02±0.00^{c}**	**0.02±0.00^{c}**	**0.06±0.02^{c}**	**1.70±0.29^{bc}**	**0.02±0.00^{c}**
	DHA/EPA	**0.04±0.00^{b}**	**0.15±0.01^{b}**	**0.05±0.32^{b}**	**0.27±0.00^{b}**	**0.06±0.01^{b}**	**0.24±0.27^{b}**	**7.69±3.96^{b}**	**0.06±0.01^{b}**
	SAFA/TUFA	**1.11±0.12^{b}**	**0.12±0.01^{c}**	**1.25±0.01^{b}**	**0.14±0.00^{c}**	**0.07±0.00^{c}**	**0.13±0.01^{c}**	**0.69±0.05^{bc}**	**0.14±0.02^{c}**
	SAFA/TFA	**0.52±0.03^{b}**	**0.11±0.01^{d}**	**0.56±0.00^{b}**	**0.12±0.05^{d}**	**0.06±0.00^{d}**	**0.12±0.01^{d}**	**0.41±0.02^{c}**	**0.12±0.01^{d}**
	TUFA/TFA	**0.47±0.03^{c}**	**0.89±0.01^{a}**	**0.44±0.00^{c}**	**0.88±0.05^{a}**	**0.94±0.00^{a}**	**0.88±0.01^{a}**	**0.59±0.02^{b}**	**0.88±0.01^{a}**

Fatty acid values are expressed as mean of the duplicates. Here, **SAFA** means Saturated Fatty Acids, **MUFA**= Monounsaturated fatty acids, **n6-PUFA**= ω-6 polyunsaturated fatty acids, **n3-PUFA**= ω-3 polyunsaturated fatty acids, **DHA**= Docosahexaenoic acid, **EPA=** Eicosapentaenoic acid, **TUFA**= Total unsaturated fatty acids, **TFA**= Total fatty acids.

4.11 Amino Acid Profile

Quantitative and qualitative determination of amino acid were shown in Table 4.6. In this study, non-essential amino acids were found than the essential amino acids. Highest amount of total essential amino acid content was found in *Teraedron* sp. (41.23%) and *Nannochloropsis* sp. (33.04 %) showed lowest total essential amino acid content under this study.

Table 4.10 Amino acid content (%) of isolated marine microalgae.

Compound Name (570 nm)	**Types**	***Chlamydomonas Sp.***	***Navicula sp.***	***Gonyostomum sp.***	***Nannochloropsis Sp.***
			Amount (%)		
Histidine	EAA	4.70	5.39	4.08	4.32
Isoleucine	EAA	1.97	2.96	2.21	2.73
Leucine	EAA	7.34	7.15	8.36	0
Lysine	EAA	6.84	3.69	5.71	5.17
Methionine	EAA	2.06	3.21	1.66	2.55
Phenylalanine	EAA	3.84	3.75	4.15	3.73
Threonine	EAA	4.72	5.47	5.91	6.37
Tyrosine	EAA	3.73	3.26	3.52	3.81
Valine	EAA	3.04	3.85	4.05	4.36
	ΣEAA	**38.24**	**38.73**	**39.65**	**33.04**
Alanine	NEAA	11.30	10.51	10.25	12.69
Arginine	NEAA	5.67	5.97	6.34	6.98
Aspartic acid	NEAA	12.33	13.44	12.28	12.26
Glutamic acid	NEAA	13.51	15.66	13.48	14.07
Glycine	NEAA	6.70	6.21	7.49	7.02
Cysteine	NEAA	0.15	0.28	0.03	0.44
Serine	NEAA	5.47	5.61	5.48	5.94
Proline	NEAA	6.62	3.59	4.98	7.57
	ΣNEAA	**61.75**	**61.27**	**60.33**	**66.97**

EAA: Essential Amino Acid, NEAA: Non-Essential Amino Acid

Table 4.11 Amino acid content (% amino acid) of isolated freshwater microalgae.

Compound Name (570 nm)	**Types**	***Choricystis sp.***	***Chromo chloris sp.***	***Tetraedron sp***	***Chlorobotrys Sp***	***Coelastrella sp.***	***Chlorobotrys sp***
				Amount(%)			
Histidine	EAA	5.27	3.57	4.45	5.44	3.83	5.44
Isoleucine	EAA	2.07	2.10	2.21	1.74	2.04	1.74
Leucine	EAA	7.41	7.54	8.35	7.57	8.12	7.57
Lysine	EAA	6.25	4.70	6.19	6.89	5.83	6.89
Methionine	EAA	1.49	1.57	1.54	1.30	1.82	1.30
Phenylalanine	EAA	3.80	4.15	4.11	4.03	3.86	4.03
Threonine	EAA	5.55	5.34	5.63	5.82	6.36	5.82
Tyrosine	EAA	3.83	3.62	3.88	3.67	4.13	3.67
Valine	EAA	3.90	3.73	4.87	3.88	3.77	3.88
	ΣEAA	**39.57**	**36.32**	**41.23**	**40.34**	**39.76**	**36.76**
Alanine	NEAA	10.35	15.12	9.14	8.76	9.20	8.76
Arginine	NEAA	5.26	5.48	5.34	6.00	4.86	6.00
Aspartic acid	NEAA	12.34	11.48	12.05	12.41	12.88	12.41
Glutamic acid	NEAA	13.41	12.78	13.58	13.89	14.11	13.89
Glycine	NEAA	8.40	7.77	7.59	7.25	7.41	7.25
Cysteine	NEAA	0.09	0.08	0.48	0.05	0.15	0.05
Serine	NEAA	5.66	5.59	5.59	5.79	6.13	5.79
Proline	NEAA	4.93	5.39	4.99	5.48	5.49	5.48
	ΣNEAA	**60.44**	**63.69**	**58.76**	**59.63**	**60.23**	**63.24**

EAA: Essential Amino Acid, NEAA: Non-Essential Amino Acid

Table 4.6. Amino acid content (% amino acid) of isolated marine and freshwater cyanobacteria.

Compound Name (570 nm)	**Types**	***Oscillatoria sp1***	***Oscillatoria sp2***	***Oscllatoria sp3***	***Oscillatoria sp4***
			Amount(%)		
Histidine	EAA	4.19	0	4.72	3.26
Isoleucine	EAA	2.83	3.02	2.05	2.80
Leucine	EAA	7.98	7.73	7.52	7.58
Lysine	EAA	4.54	4.23	6.05	3.64
Methionine	EAA	1.85	2.25	2.17	1.95
Phenylalanine	EAA	3.37	4.01	3.88	3.60
Threonine	EAA	5.40	5.80	5.23	5.95
Tyrosine	EAA	3.76	4.10	3.64	4.05
Valine	EAA	3.68	4.22	3.65	3.84
	ΣEAA	**37.6**	**35.36**	**38.91**	**36.67**
Alanine	NEAA	12.65	9.91	12.50	12.99
Arinine	NEAA	7.07	6.01	6.22	7.09
Aspartic acid	NEAA	12.12	13.36	11.64	13.23
Glutamic acid	NEAA	15.06	17.98	13.91	13.75
Glycine	NEAA	5.71	6.99	6.44	6.28
Cysteine	NEAA	0.07	1.72	0.21	0.12
Serine	NEAA	5.99	5.93	5.82	6.31
Proline	NEAA	3.74	2.75	4.35	3.58
	ΣNEAA	**62.41**	**64.65**	**61.09**	**63.35**

EAA: Essential Amino Acid, NEAA: Non-Essential Amino Acid

Chapter 5: Discussion

5.1 Water quality parameters of the sampling sites

Macro and micro nutrients availability plays a significant role on the growth and biochemical composition where sufficient amount of nutrients mainly nitrogen, phosphorus is mandatory to achieve optimum growth rates in microalgal cells (Xia et al., 2013). The growth rate reduced when the metabolic requirements and supplied nutrients are not balanced properly (Zarrinmehr, 2019). Some other factors like temperature, light, salinity, pH etc. also play a major rule in growth and biochemical compositions of microalgae (Yeh and Chang, 2012). According to Santhosh and Singh (2007) pH should be between 6.5 and 9.0, which were observed from all sampling site. Generally, DO is maximum in mid - afternoon due to photosynthesis and minimum in the early morning due to highest respiration and decomposition than photosynthesis (CWC, 2019). However, DO >5mg/L is essential to support good fish production (Bhatnagar and Singh, 2010) and DO of all sampling sites under this experiment were in optimal range. Temperature of the both marine and freshwater sampling sites was in ideal level where the optimum growth temperature is mostly between 20 and 30˚C for most marine microalgae (Chisti, 2008). Optimum phosphorus concentration for microalgae is between 0.001 g/L to 0.179 g/L (Roopnarain et al., 2014), where TAN concentration must be less than 0.5 mg/L and desirable range of nitrite-nitrogen is 0-1 mg/L (Stone and Thomforde, 2004). In the entire sampling site, nitrogen and phosphorus concentration was in ideal range that is required for plankton growth.

5.2 Characterization of isolated microalgae

In Bangladesh, huge numbers of algal species were reported to occur in freshwater, brackish water and marine habitats (Ahmed et al., 2008) that could be a potential source of feed for aquaculture, biofuel production, pharmaceuticals and nutraceuticals industry. Characterization of isolated microalgae was done according to John et al. (2002) and Belcher and Swale (1976) and isolated microalgae was *Chlamydomonas* sp., *Navicula* sp., *Gonyostomum* sp., *Nannochloropsis* sp., *Choricystis* sp., *Chromochloris* sp., *Tetraedron* sp., *Chlorobotrys* sp. *Coelastrella* sp., *Kirchneriella* sp. *Oscillatoria* sp1, *Oscillatoria* sp2, *Oscillatoria* sp3 and *Oscillatoria* sp4.

5.3 Growth phases of isolated microalgae

In case of *Chlamydomonas* sp. previous study done by Puzanskiy et al. (2018) showed differences in growth phases of the current study due variation in the nutritional composition of the culture media or different strains from the different environment. In case of *Choricystis* sp., growth curve determined by Praharyawan et al. (2020) was found slightly different from the present study, though lag phase was found similar. The variation in stationary phase was found due to different culture media used in the experiment. Duong et al. (2015) reported similar days of lag phase of *Tetraedron* sp. to the present study. But variation in the exponential, stationary and onset of death phase varied due to different growth parameters of the culture period. The growth pattern (lag, exponential and stationary phase) of *Nannochloropsis* sp. in this study was slightly different from the previous study done by Ermavitalini et al. (2019). Combined treatment media of Indole 3-acetic acid (IAA) and 6-Benzyl Amino Purine (BAP) was used in that previous study for cultivation of *Nannochloropsis* sp. The differences in the performances observed can be linked to different growth media used in *Nannochloropsis* sp. growth. Moreover, cell density was slightly higher than the earlier study found 4.877×10^7 cells mL^{-1} in Conway media reported by Khatoon et al. (2014). The differences observed can be linked to different strains from different environment. No previous study has been found in case of other species under this experiment.

5.4 Specific growth rate (SGR), cell duplication time, cell doublings per day and cell density on harvest of isolated freshwater microalgae

In Bangladesh, a very limited attention has been paid to the indigenous microalgae and its potentialities. Some world wide data are available regarding the SGR of *Nannochloropsis* sp. and *Tetraedron* sp. SGR of *Tetraedron* sp. reported by Duong et al. (2015) showed similar value to the current study. Yustinadiar et al. (2020) reported almost similar growth rate in case of *Nannochloropsis* sp. isolated from marine habitat and resulted about a growth rate of 0.25/day in stationary phase and 0.55/day on day 1 at 0.75:0.25 hr flashing light treatment in Walne medium. No previous study has been found in case of other species. The microalga *Choricystis* sp. showed lower cell duplication time compared to the other microalgae strains. Microalgae growth characteristics vary from species to species.

Moreover cell doublings per day was also varied among the species because of the variation in lag phase, log phase and stationary phase of individual species. Due to variation in growth rate which depend not only microalgae species but also culture environments. In different growth phases, microalgae growth rate differ due to the variation in several activity like in lag phase it adapts to the culture condition such as medium, pH, temperature and lighting (Krishnan et al., 2015). And then start to undergo rapid cell division and the cell of the culture will increase gradually in exponential order, as enzymes and metabolites needed for cell division are available here (Prayitno, 2016). After which, stationary phase taken place when the equal rate of the cell division and cell death occur due to depletion of nutrients in the medium (Krishnan et al., 2015). Along with this, *Gonyostomum* sp. resulted highest cell number on harvest because smaller size species grow rapidly than the larger ones because of their large surface or volume ratio of smaller sized cells which simplify assimilation of nutrients at comparatively faster rate (Phatarpekar et al., 2000).

5.5 Volumetric, areal and lipid productivity of isolated freshwater microalgae

The biomass production of microalgae is a mere function of the instantaneous growth rate and algal cell concentration in culture (Daniel and Srivastava. 2016). In the present study, different microalgae showed variation in biomass productivity which can be justified as productivity of microalgae is also influenced by the microalgal strain used and the characteristics of the environment, where it grows (Mercado et al., 2020). Moreover, lipid productivity of microalgae also varied among species as it depends on biochemical composition of microalgae, nutrient content of culture media and different stress factors. Microalgae cells accumulated a larger quantity of lipid when they were subject to unfavorable culture conditions (Lucas-Salas et al., 2013). In case of marine *Nannochloropsis* sp. strains, Daniel and Srivastava (2016) detected about 1088.44 g m^{-3} d^{-1} mean volumetric productivity, 10.10 g m^{-2} d^{-1} mean areal productivity from 30 mm thickness of thick tubular photobioreactor which was higher from the findings of the current study. Very less data has been recorded regarding the biomass and productivity of microalgal species under this study. Lipid productivity of *Tetraedron* sp. reported by Duong et al. (2015) was found lower than the current study. Based on the outcomes of the current study, *Gonyostomum* sp., *Nannochloropsis* sp., *Chromochloris* sp., Chlorobotrys

sp. and *Kirchneriella* sp. can be utilized a potential source of biofuel production as they showed higher lipid productivity

5.6 Pigments content of isolated freshwater and marine microalgae

5.6.1 Chlorophyll

According to Lavin (2000), chlorophyll a is the primary pigment, and the others such as chlorophyll b and c serve as accessory pigments. Present study resulted higher chlorophyll a content than chlorophyll b in case of each species under this study. Total chlophyll content (Chlorophyll a and b) of *Chlamydomonas* sp. determined by Darwish et al. (2020) showed higher chlorophyll content than the present study. The variation in the result was found due to different culture medium used for cultivation and other factors such as alteration of light intensity and the light regime (Pandey et al., 2013). TAP media (Tris-Acetate-Phosphate) was used in the previous study done by Darwish et al. (2020) and the growth condition was 16:8 h alternating light: dark cycle, 23◦C and 100 rpm with ambient CO2 level. On the other hand, *Chlamydomonas* sp. was cultured in Conway media. The present study reported higher amount of chlorphyll-b content from *Chlamydomonas* sp. In case of *Navicula sp.,* González-Vega et al. (2021) optimized the growing condition for pigment production, where 0.44mol/L 0f $NaNO_3$, 40 PSU salinity, 3.5 days culture and methanol as a solvent were found as optimum growth conditions. The current study resulted higher chlorophyll a production than the previous study done by González-Vega et al. (2021). The variation in the result was observed due to the variations in the amounts of culture nutrients and other factors such as light, temperature, water quality, and cell extraction method. Moreover, chlorophyll concentration also varies based on the solvent (Wellburn, 1994). In the present study, *Navicula* sp. showed lower chlorophyll a content compared to other microalgae under this study. Previous study done by Fakhri et al. (2017) reported that, *Nannochloropsis* sp. showed different Chlorophyll-a content at different light intensities such as 4.977±0.095 µg/mL at 1500 lux, 6.520±0.049 µg/mL at 3000 lux and 8.304±0.248 µg/mL at 4500 lux in 28 °C temperature with photoperiod 24: 0 and continuous aeration in Walne medium. But the present study, *Nannochloropsis* sp. showed higher chlorophyll-a content than Fakhri et al. (2017) study which due to varieties in the nutrient composition of the culture medium (Oo et al., 2017). In the present study,

chlorophyll a content of *Coelastrella* sp. was found higher than the earlier work done by Iyer et al. (2015). But the chlorophyll b content was almost similar to the earlier work done by Iyer et al. (2015). Earlier work done by Kulandaivel et al. (2007) resulted higher chlorophyll a content of *Oscillatoria* sp. than four strains of *Oscillatoria* sp. under the present study. The difference in the result was found due to variation in the use of different culture media (Oo et al., 2017). In the previous study by Kulandaivel et al. (2007) synthetic media and dairy effluent were used for cultivation. On the other hand, BBM and Conway medium were used for culturing freshwater and marine water strain of *Oscillatoria* sp., respectively. In case of other species under this experiment, no previous study on pigment content has been found. But, some species in the current study displayed almost same chlorophyll contents to other microalgal species. In case of *Chromochloris* sp., chlorphyll-a, and b content of the present study was almost similar to the chlorophyll a and b content of *Chlorella vulgaris* reported by Singh et al. (2017). On the other hand, chlorophyll a of *Tetraedron* sp. was almost similar as *Ankistrodesmus falcatus* in Bold's Basal medium at 702 lux light intensity in a 12-day culture period (Ogbonna et al., 2021). Moreover, Chlorophyll content in *Choricystis* sp. in the current study was almost similar with the study done by Donghui et al. (2016) on *Dunaliella tertiolecta* at the N: P of 16:1 during the period of cultivation in the urea containing Tk medium. As no previous study has been done on *Choricystis* sp. pigment content, our study concluded that, *Choricystis* sp. have more chlorophyll-a content than *Navicula* sp. reported here. Moreover, no earlier work has been found on pigment content of *Gonyostomum* sp., *Chlorobotrys* sp. and *Kirchneriella* sp. Also, chlorophyll content of those microalgal species under this experiment didn't match with chlorophyll content of other microalgal species. From the present study, it can be concluded that, *Chlamydomonas* sp., *Nannochloropsis sp.*, *Chromochloris* sp., *Tetraedron* sp., *Chlorobotrys* sp., and *Coelastrella* sp. *sp.* can widely be used as a great source of chlorophyll.

5.6.2 Carotenoid

The present study reported higher amount of carotenoid content from *Chlamydomonas* sp. Almost similar amount of carotenoid content was reported by Darwish et al. (2020). Previous study done by González-Vega et al. (2021) optimized the growing condition for pigment production from *Navicula* sp., where the optimum growth conditions were found

as 0.44mol/L 0f $NaNO_3$, 40 PSU salinity, 3.5 days culture and methanol as a solvent. The current experiment resulted higher carotenoid production than the previous study which indicates variation in the culture media was responsible for the variation in the result (Islam et al., 2021). Moreover, the solvent used for extraction can also be a probable reason for the differences in the findings (Rise et al., 1994). Carotenoid contents of *Nannochloropsis* sp. vary at various light condition such as 2.830±0.014 μg/mL at 1500 lux, 3.518±0.018 μg/mL at 3000 lux and 3.892±0.016 μg/mL at 4500 lux in 28 °C temperature with photoperiod 24: 0 and continuously aeration in Walne medium (Fakhri et al., 2017). But in the current study, *Nannochloropsis* sp. resulted lower carotenoid content than Fakhri et al. (2017) detected earlier. Differences in light intensity significantly influenced the total carotenoid contents and increased with the increment of light intensity in *Nannochloropsis* sp. (Fakhri et al., 2017). Earlier work done by Iyer et al. (2015) resulted higher carotenoid content of *Coelastrella* sp. than the current study. The difference in the result was found due to variation in the environmental parameters and chlorophyll concentrations (Techetel and Ruppel, 1992). In addition, carotenoids biosynthesis can also directly affected by biomass content (Velichkova, 2014). Moreover, Carotenoid content of four strains of *Oscillatoria* sp. under this experiment was found almost similar to the carotenoid content determined by Dharma et al. (2017). In this study, four strains of Oscillatoria sp. revealed lower levels of carotenoid than all other species combined, with Oscillatoria sp2 having the lowest value among the four strains. In case of other species under this experiment, no previous study on pigment content has been found. But, some species in the current study displayed almost same carotenoid content to other microalgal species. There was no previous study on pigment content of *Chromochloris* sp. However, *Dunaliella salina* contain similar amount of carotenoid in 0.7M salt concentration in Conway medium reported by Khatoon et al. (2020). Earlier study reported about 4.9mg/L carotenoid content in *Scenedesmus almeriensis* at 30°C (Sanchez et al., 2008) which was almost similar to the carotenoid content of *Chlorobotrys* sp. and *Kirchneriella* sp. under the current study. Carotenoid content of *Gonystomum* sp. and *Tetraedron* sp. in this study was found almost equivalent with the carotenoid content of *Pectinodesmus* sp. determined by Eze et al. (2021) in flask condition. Marzorati et al. (2020) had earlier opined that *Spirulina* sp. contained about 3.5 ± 0.2 $mg.g^{-1}$ of total carotenoid content. However, in the current study,

Choricystis sp. showed almost same amount of carotenoid content. Present study also concludes that, *Chlamydomonas* sp., *Chromochloris* sp., *Chlorobotrys* sp., *Coelastrella* sp. and *Kirchneriella* sp. have high carotenoid content and huge potentiality to contribute in human and animal food industry.

5.6.3 Phycobiliproteins

In an earlier study Zuorro et al. (2021) opined that *Oscillatoria* sp. grown in optimized BG-11 media at 28 °C with a light: dark cycle of 12:12 h at 100 μmol m^{-2} s^{-1} for 15 days resulted with values of 15.21, 3.95, and 1.89 (% w/w), phycocyanin, allophycocyanin and phycoerythrin, respectively. In the present study, phycocyanin, allophycocyanin and phycoerythrin content of four strains of *Oscillatoria* sp. were found higher than the earlier study, though phycoerythrin content of *Oscillatoria* sp3 was lower than the earlier study. The differences in the findings may occur due to different culture environment, as growth and pigment production of microalgal species are are affected by environmental change (Chen et al., 2011). According to a prior study by Montero-Lobatoa et al. (2020), Chroococcidiopsis sp. produced 204 mg g-1 of phycobiliproteins under a light intensity of 10 mol photons m-2 s-1, with a relative abundance of 40.9% for phycocyanin, 23.3% for phycoerythrin, and 35.8% for allophycocyanin. Additionally, Nostoc species, Arthrospira platensis, and Porphyridium purpureum were found in industrial wastewater with highest concentrations of 199 mg/g DW, 303 mg/g DW, and 93 mg/g total phycobiliproteins, according to Arashiro et al. (2020). In this experiment, *Nannochloropsis* sp. showed higher phycocyanin, allophycocyanin and phycoerythrin production than the earlier work done by Islam et al. (2021). The variation may occur due to variation of light exposure during culture period. No previous study has been reported on phycobilliprotein detetmination of *Chlamydomonas* sp., *Navicula* sp., *Gonystomum* sp., *Choricystis* sp., *Chromochloris* sp., *Tetraedron* sp *Chlorobotrys* sp., *Coelastrella* sp. and *Kirchneriella* sp. However, compared to cyanobacterial strains, very little total phycobiliprotein was found in *Chlamydomonas* sp., *Navicula* sp., *Gonystomum* sp., *Nannochloropsis* sp., *Choricystis* sp., *Chromochloris* sp., *Tetraedron* sp *Chlorobotrys* sp., *Coelastrella* sp. and *Kirchneriella* sp. (Zuorro et al., 2021; Montero-Lobatoa et al., 2020; Arashiro et al., 2020), as the light harvesting pigments phycobiliprotein commonly found in cyanophyceae and cryptophyceae (Glazer, 1994). Additionally, according to Graham and Wilcox (2000), Chlorophyta (Green Microalgae)

are stated to contain primarily chlorophyll-a, b, and b-carotene, prasinoxanthin, siphonaxanthin, and astaxanthin and those microalgae in the current study all belong to the Chlorophyceae class. In this experiment, it can be concluded that among fourteen species of microalgae, four strains of *Oscillatoria* sp. belongs to cyanophyceae showed higher phycobiliprotein content than ten other green and brown microalgal species.

5.7 Proximate composition of isolated microalgae

The present study showed that the protein, lipid and carbohydrate content in *Chlamydomonas* sp. was significantly higher in Conway Medium. A study done by Darwish et al. (2020) where the protein content of *Chamydomonas* sp. was found 46.9% DW (Dry weight). According to Kliphuis et al. (2012), in a study where the biomass composition of *Chlamydomonas* sp. as% of DW under various energy inputs were assessed, the protein content of *Chamydomonas* sp. ranged between 37 and 42%. In the present study, the protein content was found 58.96% DW. The variation in the protein content can be occurred due to different growth conditions. In a previous study variation in the protein content of *Chlamydomonas* sp. under different growth conditions was observed (Boyle & Morgan 2009). In the present study, *Chlamydomonas* sp. showed a lipid content of 21.07% and the percentage is close to the study done by Darwish et al. (2020) (24.7%). As high oil yield of many microalgae species has been utilized in biofuel production, this species may have potential in this area (Hussain et al., 2017). In this study, *Chlamydomonas* sp. showed lower carbohydrate content (7.4902 %). The total amount of carbohydrates in microalgae varies depending on the species, the growth environment and cultivation period throughout the cell cycle. Green microalgae typically comprise 20% (DW) of carbohydrates, of which half is starch. Algal carbohydrates are good source of dietary fibre and also offer some health benefits (Wells et al.,2017). S0, this species can be used as a potential source of healthy food. The protein content (59.51%) of *Navicula* sp. in this study was higher than a previous study done by Fimbres et al. (2015) where biochemical composition of *Navicula* sp. cultured at different light intensities and wavelength was assessed. Though lipid content is lower, carbohydrate content is close to that study. Compared to an earlier study published by Khatoon et al. (2014), where Nannochloropsis sp. had about 44.3% protein and 32% lipid in 30ppt salinity (dry basis), the protein and lipid content of current study is lower. It is possible that the variances are

caused by the fact that the biochemical composition of microalgae differs with various compositions of media and growing conditions (Chen et al., 2011). Due to its higher protein and lipid content *Nannochloropsis* sp. can be extensively used as animal feed in aquaculture (Rodolfi et al., 2003). On the other hand, no previous data has been found regarding biochemical composition of *Gnoyostomum* sp. In this present study, the protein and lipid content of *Gnoyostomum* sp. cultured in Conway media was respectively 42.86 % and 27.4 %. In a previous study, it was reported that microalgae containing high protein can be used as a potential source of animal feed (Bleakley and Hayes 2017). Hence, *Gnoyostomum* sp. can be considered as a potential source of feed industry. The protein content of *Tetraedron* sp. was found higher in current study than the experiment done by Duong et al. (2015).The result of the present study was supported by Thepsuthammarat et al. (2023) cliamed that *Coelastrella* sp. contain 33.91 % protein. But higher lipid and lower carbohydrate content was found in this study which wasn't supported by that study. No previous study has been reported regarding biochemical composition of *Choricystis* sp., *Chromochloris* sp. and *Chlorobotrys* sp. However, the lipid and protein con tent of these species are higher. So, these species can be used as a potential source of feed of fish and other animals. In the present study, protein content of three strains of *Oscillatoria* was higher than the experiment done by Rasheedy et al., (2017), but protein content of third strain of *Oscillatoria* (*Oscillatoria* sp3) was lower than that. Simillarly except third strain, other three strains of *Oscillatoria* showed higher lipid content than that previous study. But, carbohydrate content of four strains in the current study were lower than that. These variations may occur due to the changes in nutrient concentration or growth conditions (Converti et al., 2009). Environmental elements including light, salinity, and the availability of nutrients have a significant impact on the growth rate and biochemical composition of microalgae, and these factors vary from species to species (Garca et al., 2012).

6.7 Fatty acid composition:

High amounts of -3 PUFAs are produced by microalgae, which are also prospective sources of specific fatty acids using large-scale cultivation techniques (Irmak & Arzu, 2020). PUFAs have recently attracted a lot of interest due to their multiple uses in pharmaceuticals and nutraceuticals. In this present study, *Chlamydomonas* sp. showed significantly ($p<0.005$) higher amount of PUFA. SAFA of *Chlamydomonas* sp. in this study was found higher than a previous study done by Darwish et al. (2020). Moreover, unsaturated fatty acid content was found higher than the saturated fatty acid, where the previous study showed almost similar amout of unsaturated and saturated fatty acid content. The variation in the result may occur due to difference in the species. Moreover, differences in the findings may also happen due to variation in the nutrient composition of the culture media (Siaut et al., 2011). A previous study done by Hasan et al. (2022) resulted higher amount of SAFA and n-6 PUFA of *Nannochloropsis* sp. than the current study which due to the varieties in the growth condition. In case of *Choricystis* sp., Menezes et al. (2015) reported similar amount of SAFA to the current study. But, PUFA was found higher than the present study which due to different media used for cultivation. Menezes et al. (2015) used WC media foe the growth where BBM was used in the present study. Moreover, Tetraedeon sp. showed lower SAFA and higher PUFA than an earlier work done by Duong et al. (2015). According to Ahlgren *et al*. 1992, cyanobacteria can produce more omega-3 fatty acids than green algae. In the present study, PUFAs were found higher in case of Strain 2 M2 (25) and Strain 4 Fw2. The data analysis showed that long-chain fatty acids (LCFAs; longer than 12 carbons) made up almost 92% of the total fatty acid levels in the *Oscillatoria* sp. species studied. Medium-chain fatty acids (MCFAs; 8 to 12 carbons) made up about 12% of the overall fatty acids. In contrast, *Oscillatoria* sp. did not contain any short-chain fatty acids (SCFAs; less than 6 carbons). Irmak & Arzu (2020) revealed that *Oscillatoria* sp. contain 34.74% SFA, 60.63% MUFA, 4.3% PUFA. In this study, Strain 1 M1 (20) shows similarity with the author's data in case of SFA, PUFA and have high variations in case of MUFA and which may be due to the species variations. The similarity of Strain 1 M1 (20) can be due to the planktonic nature of the both algae. Mundt *et al.* (2003) also narrated that fatty acids prevented the development of the Gram-positive bacteria *Bacillus subtilis, Micrococcus flavus* and *Staphylococcus aureus* in an agar plate diffusion test.

Thus, rich fatty acid composition may also provide the antimicrobial properties in *Oscillatoria* species.

6.7 Amino acid composition:

The total amino acids composition represents both protein constituents, free amino acids and/or amino acid salts. Protein quality is valued based on 8 essential amino acids, namely, methionine, leucine, isoleucine, lysine, phenylalanine, threonine, tryptophan and valine (Lourenço et al., 2002). In the current study total sum of non-essential amino acid was found higher than the total sum of essential amino acids. Total sum of essential amino acids of *Chlamydomonas* sp. was found lower (38.24 mg/g) than a study done by Darwish et al. (2020). Moreover, in this study non-essential amino acids of *Chlamydomonas* sp. was found higher than essential amino acids. Amino acid content of *Nannochloropsis* sp. in this study was found lower than the study done by Hasan et al. (2022) and these changes may occur due to variations in the growth condition. However, that previous study showed higher amount of non-essential amino acids than the essential amino acids like the present study. Higher amount of total sum of essential amino acids were recorded in *Tetraedron* sp. under this study. Less report has found has been found regarding amino acid profile of *Tetraedron* sp and other species under this study. Present study revealed that non-essential amino acids are found higher than essential amino acids in *Oscillatoria*. Strain 3 Fw1 possess highest essential fatty acids followed by Strain 1 M1 (20), Strain 4 Fw2 and Strain 2 M2 (25). On the contrary, Strain 2 M2 (25) and Strain 3 Fw1 showed highest and lowest percentage of non-essential fatty acid respectively. Non-essential fatty acids such as alanine, aspartic acid and were found prevalent in *Oscillatoria* sp. Strain 2 M2 (25) showed highest value of both glutamic acid (17.98%) and aspartic acid (13.36%) while Strain 4 Fw2 showed highest value of alanine (12.99%). Leucine and threonine were abundant in case of essential amino acids in all the *Oscillatoria* strains. Highest leucine (7.98%) and threonine (5.95%) value were obtained from Strain 1 M1 (20) and Strain 4 Fw2 respectively. Metcalf *et al.* (2021) indicated the abundance of amino acids in cyanobacterial food items and found higher percentage of glutamic acid (4.39%) followed by tyrosine (3.65%), tryptophan (2.98%), aspartic acid (2.3%), alanine (2.07), arginine (1.71%), valine (1.59%), threonine (1.4%) etc. which are lower than the result obtained by

this study. It can be due to the food processing loss of amino acid and also due to the species variations.

Chapter 6: Conclusion

Considering the findings obtained from the present study it can be concluded that, SGR varied among different growth phases of microalgae. *Chlamydomonas* sp. *Nannochloropsis* sp., *Tetraedron* sp., *and Coelastrella* sp showed higher quantity quantity of protein and lipid content and their importance in fish or animal feed industry and fuels production. Moreover, *Chioricystis* sp. showed lowest cell duplication time and *Navicula* sp. resulted highest cell doublings per day which can be utilized for mass production of microalgae. Along with this, *Tetraedron* sp. accumulated much higher lipid in a day and can be as a potential species for biodiesel production. Interestingly, *Chlamydomonas* sp., *Chromochloris* sp. and *Chlrobotrys* sp. showed higher carotenoid and chlorophyll content and confirms their potentiality in pigment production and utilization of those microalgae in as natural food coloring agent.

Chapter 7: Recommendations and Future Prospects

Chlamydomonas sp., *Navicula* sp., *Gonyostomum* sp. *Coelastrella* sp., *Oscillatoria* sp1, *Oscillatoria* sp2 and *Oscillatoria* sp4 showed a good quantity of protein but it could not represent the digestibility of microalgal protein as human and fish feed.. Along with this, *Chlamydomonas* sp., *Chromochloris* sp., *Chlorobotrys* sp., *Coelastrella* sp., and *Kirchneriella* sp. showed higher carotenoid content. Moreover, *Chlamydomonas* sp., *Nannochloropsis* sp., *Chromochloris* sp., *Tetraedron* sp., and *Chlorobotrys* sp. showed higher chlorophyll content, where four strains of Oscillatoria sp. showed higher chlorophyll content compared to those microalgal species. But pigment (chlorophyll and carotenoid) utilization by fish and other animal required to determine to identify the microalgal pigment potentiality as natural food coloring agent. Therefore, future research attempts may include the followings:-

- Digestibility of isolated microalgae to utilize as fish or animal feed;
- Pigment utilization capability by fish or other aquatic animal to strengthen the aquaculture industry;

Future prospects

- This study may establish a complete isolation procedure of microalgae
- Will provides a complete profile of fourteen different marine and freshwater microalgae isolated from Bangladesh
- Current results can be used as a basis for the manipulation of microalgae for high protein, lipid and pigments production
- Current results also show the potential use of microalgae in feed industry and biodiesel production by utilizing the amino acid and fatty acid profile of microalgal species
- May contribute to choose potential strains that possess fast growth, suitable pigments, proximate composition, fatty acid and amino acid profile to boost up the aquaculture, pharmaceuticals and nutraceuticals industry

References

Ahlgren G, Gustafsson IB, Boberg M. 1992. Fatty acid content and chemical composition of freshwater microalgae 1. Journal of phycology. 28(1): 37-50.

Ahmed ZU, Begum ZNT, Hassan MA, Khondker M, Kabir SMH, Ahmad M, Ahmed ATA, Rahman AKA, Haque EU. 2008. Encyclopedia of flora and fauna of Bangladesh. vol. 6. angiosperms: Dicotyledons (Acanthaceae-Asteraceae). Asiatic Society of Bangladesh, Dhaka. pp 408.

Alfasane MA, Chowdhury MMK, Mehnaz M. 2019. Molecular characterization and new reports of two green algae from Bangladesh. Bangladesh Journal of Plant Taxonomy. 26(1): 39–45.

Al-Hoqani U, Young R, Purton S. 2016. The biotechnological potential of *Nannochloropsis*. Perspect. Phycol. 4: 1-15.

Andersen RA, Brett RW, Potter D, Sexton JP. 1998. Phylogeny of the Eustigmatophyceae based upon 18S rDNA, with emphasis on *Nannochloropsis*. Protist. 149(1): 61-74.

Andreeva A, Budenkova E, Babich O, Sukhikh S, Ulrikh E, Ivanova S, Prosekov A, Dolganyuk V. 2021. Production, purification, and study of the amino acid composition of microalgae proteins. Molecules. 26: 2767.

Arashiro LT, Boto-Ordóñez M, Van Hulle SWH, Ferrer I, Garfí M, Rousseau DPL. 2020. Natural pigments from microalgae grown in industrial wastewater. Bioresource Technology. 303: 122894.

Aris H, Su L, Roland H, Michael DK. 2010. Chlorophyll extraction from microalgae: A review on the process engineering aspects. International Journal Of Chemical Engineering. 391632: 1–11.

Aytenfisu AH, Yang M, MacKerell AD. 2018. Drude polarizable force field for glycosidic linkages involving pyranoses and furanoses. Journal of Chemical Theory and Computation. 14: 3132–3143.

Ba´saca-Loya GA, Valdez MA, Enrı´quez-Guevara EA, Gutierrez-Milla´n LE, Burboa MG. 2009. Extraction and purification of B-phycoerythrin from the red microalga *Rhodosorus marinus, Ciencias marinas*. International Journal Of Marine Science. 35: 359–368.

Barclay W, Apt K. 2013. Strategies for Bioprospecting Microalgae for Potential Commercial Applications. In: Richmond A., Hu Q, editors. Handbook of Microalgal Culture: Applied Phycology and Biotechnology. Wiley-Blackwell, UK. pp. 69–79.

Becker W. 2004. Microalgae in human and animal nutrition. In: Richmond A, editors. Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science Ltd, Cambridge. pp. 312–351.

Belcher H, Swale E. 1976. A beginner's guide to freshwater algae. London, HMSO. pp. 47.

Bellinger EG, Sigee DC. 2010. Freshwater algae: identification, enumeration and use as bioindicators. JohnWilley & Sons, Ltd. DOI:10.1002/9780470689554

Bennett A, Bogorad L. 1973. Complementary chromatic adaption in a filamentous blue-green alga. Journal of Cell Biology. 58: 419–435.

Bhagavathy S, Sumathi P. 2012. Purification and characterization of carotenoids from green algae *Chlorococcum humicola* by HPLC-NMR and LC-MS-APCI. Biomedicine and Preventive Nutrition. 2: 276–282.

Bhatnagar A, Singh G. 2010. Culture fisheries in village ponds: a multilocation study in Haryana, India. Agriculture and Biology Journal of North America. 1(5): 961–968.

Bleakley S, Hayes M. 2017. Algal proteins: extraction, application, and challenges concerning production. Foods. 6(5): 33.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification, Canadian Journal of Biochemistry and Physiology. 37(8): 911–917.

Bohlin K. 1901. Étude sur la flore algologique d'eau douce des Açores (Vol. 27, No. 4). Norstedt PA, Söner.

Borowitzka MA. 2013. *Dunaliella*: biology, production, and markets. In: Richmond A., Hu Q, editors. Handbook of microalgal culture: applied phycology and biotechnology. Wiley-Blackwell, UK. pp. 359–368.

Bory de Saint-Vincent JBGM. 1822. Bacillarées. Dictionnaire Classique d'Histoire Naturelle. 2: 127–129.

Boyle NR, Morgan JA. 2009. Flux balance analysis of primary metabolism in *Chlamydomonas reinhardtii*. BMC systems biology. 3:1-14.

Britannica T. 2016. *Oscillatoria*. Encyclopedia Britannica. Available from: https://www.britannica.com/science/Oscillatoria

Burlew JS. 1953. Algal culture from laboratory to pilot plant. Algal culture from laboratory to pilot plant.

Chen M, Tang H, Ma H, Holland TC, Ng KS, Salley SO. 2011. Effect of nutrients on growth and lipid accumulation in the green algae *Dunaliella tertiolecta*. Bioresource Technology. 102: 1649–1655.

Chisti Y. 2007. Biodiesel from microalgae. Biotechnology Advances. 25: 294–306.

Chisti Y. 2008. Biodiesel from microalgae beats bioethanol. Trends in Biotechnology. 26(3): 126–131.

Chiu SY, Kao CY, Tsai MT, Ong SC, Chen CH, Lin CS. 2008. Lipid accumulation and CO_2 utilization of *Nannochloropsis oculata* in response to CO_2 aeration. Bioresource Technology. 100: 833–838.

Chodat R. 1922. Matériaux pour l'histoire des algues de la Suisse.

Choong YJ, Yokoyama H, Matsumura Y, Lam MK, Uemura Y, Dasan YK, Kadir WNA, Lim JW. 2020. The potential of using microalgae for simultaneous oil removal in wastewater and lipid production. Journal of Environmental Science and Technology. 17: 2755–2766.

Converti A, Casazza AA, Ortiz EY, Perego P, Del Borghi M. 2009. Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. Chemical Engineering and Processing: Process Intensification. 48(6): 1146–1151.

Cupp EE. 1943. Marine plankton diatoms of the west coast of North America.

CWC. 2019. Effect of time and temperature on do levels in river waters. ministry of jal shakti department of water resources, river development & Ganga rejuvenation, Central Water Commission, June 2019.

Da Silva D, Sant'Anna CL, Tucci A, Comas A. (2013). New planktic species of *Kirchneriella* Schmidle (Chlorophyceae, Selenastraceae) from Brazilian freshwaters. Brazilian Journal of Botany. 36: 153-157.

Daniel RS, Srivastava A. 2016. Cell productivity of *Nannochloropsis gaditana* CCAP 849/5 in varied reactor types with different culture light paths and light regimes. Journal of Algal Biomass Utilization. 7(1): 12–20.

Darwish R, Gedi MA, Akepach P, Assaye H, Zaky AS, Gray DA. (2020). *Chlamydomonas reinhardtii* is a potential food supplement with the capacity to outperform *Chlorella* and *Spirulina*. Applied Sciences. 10(19): 6736.

Dharma A, Sekatresna W, Zein R, Chaidir Z, Nasir N. 2017. Chlorophyll and total carotenoid contents in microalgae isolated from local industry effluent in West Sumatera, Indonesia. Der Pharma Chemica. 9(18): 9–11.

Diesing CM. 1866. Revision der Prothelminthen: Abtheilung: Mastigophoren:(vorgelegt in der Sitzung vom 5. October 1865). Gerold.

Donghui S, Xi B, Jing S. 2016. Characterization of the growth, chlorophyll content and lipid accumulation in a marine microalgae *Dunaliella tertiolecta* under different nitrogen to phosphorus ratios. Journal of Ocean University of China. 15 (1): 124–130.

Drouet F, Cohen A. 1935. The morphology of *Gonyostomum* semen from woods hole, Massachusetts. The Biological Bulletin. 68(3): 422-439.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. 1956. Colorimetric method for determination of sugars and related substances, Analytical Chemistry. 28: 350–356.

Duong VT, Ahmed F, Thomas-Hall SR, Quigley S, Nowak E, Schenk PM. 2015. High protein-and high lipid-producing microalgae from northern Australia as potential feedstock for animal feed and biodiesel. Frontiers in bioengineering and biotechnology.3: 53.

Duong VT, Ahmed F, Thomas-Hall SR, Quigley S, Nowak E, Schenk PM. 2015. High protein-and high lipid-producing microalgae from northern Australia as potential feedstock for animal feed and biodiesel. Frontiers in bioengineering and biotechnology. 3: 53.

Duong VT, Ahmed F, Thomas-Hall SR, Quigley S, Nowak E, Schenk PM. 2015. High protein-and high lipid-producing microalgae from northern Australia as potential feedstock for animal feed and biodiesel. Frontiers in bioengineering and biotechnology. 353.

Ehrenberg C. 1834. Dritter Beitrag zur Erkenntniss grosser Organisation in der Richtung des kleinsten Raumes. Berlin: Konigl. Akad. d. Wiss. 1833: 145-336.

Eriksen NT. 2008. The technology of microalgal culturing. Biotechnology Letters 30: 1525–1536.

Ermavitalini D, Nurhatika S, Rahayu AE, Arifiyanto A. 2019. Growth profile of *Nannochloropsis* sp. with combination effect of Indole 3-Acetic Acid (IAA) and 6-Benzyl Amino Purine (BAP). Bioscience Research. 16(3): 2402–2408.

Eze CN, Ogbonna IO, Aoyagi H, Ogbonna JC. 2022. Comparison of growth, protein and carotenoid contents of some freshwater microalgae and the effects of urea and

cultivation in a photobioreactor with reflective broth circulation guide on *Desmodesmus subspicatus* LC172266. Brazilian Journal of Chemical Engineering. 39: 23–33.

Fakhri M, Arifin NB, Hariati AM, Yuniarti A. 2017. Growth, biomass, and chlorophyll-a and carotenoid content of *Nannochloropsis* sp. strain BJ17 under different light intensities. Jurnal Akuakultur Indonesia. 16 (1): 15–21.

FAO. 1996. Manual on the production and use of live food for aquaculture. Lavens P and Sorgeloos P (Eds), FAO Fisheries Technical Paper: p 361.

Fernandez-Valenzuela S, Chávez-Ruvalcaba F, Beltran-Rocha JC, San Claudio PM, Reyna-Martínez R. Isolation and culturing axenic microalgae: Mini–review. The Open Microbiology Journal. 10: 15(1).

Fimbres-Olivarría D, López-Elías JA, Martínez-Córdova LR, Carvajal-Millán E, Enríquez-Ocaña F, Valdéz-Holguín E, Miranda-Baeza A. 2015. Growth and biochemical composition of *Navicula* sp. cultivated at two light intensities and three wavelengths.

Folch J, Lees M, Stanley GHS. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry. 226: 497–509.

Fott B. 1976. *Choricystis*, eine neue Gattung der Chlorococcales (Chlorophyceae). Algological Studies/Archiv für Hydrobiologie, Supplement Volumes. 382-388.

Ganguly S, Mohanty A, Mitra T, Mohanty S, Das BK, Mohanty BP. 2018. Nutrient profiling and evaluation of fish as a dietary component. Food Research International. 103: 21–29.

García N, López-Elías JA, Miranda A, Martínez-Porchas M, Huerta N, García A. 2012. Effect of salinity on growth and chemical composition of the diatom *Thalassiosira weissflogii* at three culture phases. Latin American Journal of Aquatic Research. 40(2): 435–440.

García N, López-Elías JA, Miranda A, Martínez-Porchas M, Huerta N, García A. 2012. Effect of salinity on growth and chemical composition of the diatom *Thalassiosira weissflogii* at three culture phases. Latin American Journal of Aquatic Research. 40(2): 435–440.

Gelfand VI. 1991. Bershadsky AD. Microtubule dynamics: mechanism, regulation, function. Annu Rev Cell Biol. 7: 93-116.

Glazer AN. 1994. Phycobiliproteins: A family of valuable widely used fluorophores. Journal of Applied Phycology. 6: 105–112.

Gomont M. 1892. Monographie des Oscillariées (Nostocacées homocystées), vol. ser. 7, vol. 15 of Annales des Sciences Naturelles, Botanique Series. Fortin.

Gong Y, Jiang M. 2011. Biodiesel production with microalgae as feedstock: from strains to biodiesel. Biotechnology Letters. 33: 1269–1284.

González-Vega RI, Cárdenas-López JL, López-Elías JA, Ruiz-Cruz S, Reyes-Díaz A, Perez-Perez LM, Del-Toro-Sánchez CL. 2021. Optimization of growing conditions for pigments production from microalga *Navicula incerta* using response surface methodology and its antioxidant capacity. Saudi Journal of Biological Sciences. 28(2): 1401-1416.

Graham L, Wilcox L. 2000. Algae. Prentice-Hall, Englewood Cliffs, NJ.

Granado-Lorencio F, Herrero-Barbudo C, AciénFernández G, Molina-Grima E, Fernández-Sevilla JM, Pérez-Sacristán B, Blanco-Navarro I. 2009. In vitro bioaccesibility of lutein and zeaxanthin from the microalgae *Scenedesmus almeriensis*. Food Chemistry. 114: 747–752.

Griffiths MJ, Van Hille RP, Harrison STL. 2010. Selection of direct transesterification as the preferred method for assay of fatty acid content of microalgae. Lipids. 45: 1053-1060.

Guillard RRL. 1995. Culture Methods. In: Manual on Harmful Marine Microalgae (eds. G.M. Hellegraeff, D.M. Anderson and A.D. Cembella). IOC Manuals and Guides No. 33. UNESCO. pp. 45-62.

Guiry MD, Guiry GM. 2020. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: *https://www.algae base.org.*

Guiry MD. 2007. The freshwater and terrestrial algae. Royal Irish Academy.

Hanagata N, Karube I, Chihara M. 1996. Bark-inhabiting green algae in Japan (1) *Scenedesmus komarekii* and *Coelastrella multistriata var. multistriata* (Scotiellocystoideae, Chlorellaceae, Chlorophyceae). The journal of Japanese botany. 71(2): 87-97.

Harris E. 2009. The genus *Chlamydomonas*.The *Chlamydomonas* source book. 1: 1-24.

Hasan, J, Islam Z, Rabby AF, Sonia SS, Aktaruzzaman M, Rahman T, Mahmud Y. 2022. Dataset describing the growth pattern, amino acid and fatty acid profile of five indigenous marine microalgae species of Bangladesh. Data in Brief. 45: 108643.

Hasle GR, Syvertsen EE. 1997. Marine diatoms. In: Tomas, C. R. (ed.) Identifying Marine Phytoplankton. 5-385.

Havlik I, Lindner P, Scheper T, Reardon KF. 2013. On-line monitoring of large cultivations of microalgae and cyanobacteria. Trends Biotechnology. 31 (7): 406–414.

Hibberd DJ. 1974. Observations on the cytology and ultrastructure of *Chlorobotrys regularis* (West) Bohlin with special reference to its position in the Eustigmatophyceae. British Phycological Journal. 9(1): 37-46.

Hibberd DJ. 1981. Notes on the taxonomy and nomenclature of the algal classes Eustigmatophyceae and Tribophyceae (synonym Xanthophyceae). Botanical journal of the linnean society. 82(2): 93–119

Hoham RW, Bonome TA, Martin CW, Leebens- Mack JH. 2002. A combined 18s rDNA and rbcL phylogenetic analysis of *Chloromonas* and *Chlamydomonas* (Chlorophyceae, Volvocales) emphasizing snow and other cold-temperature habitats1. Journal of phycology. 38(5): 1051-1064.

Hosikian A, Lim S, Halim R, Danquah MK. 2010. Chlorophyll extraction from microalgae: A review on the process engineering aspects, International Journal of Chemical Engineering, 391632: 1–11.

Hu X, Yang X, Li L, Wu Y, Lin W, Huang H, Yang SH. 2015. Antioxidant properties of microalgae protein hydrolysates prepared by neutral protease digestion. Applied Mechanics and Materials. 707: 149–153.

Huang WP, Sun H, Deng T, Razafimandimbison SG, Nie ZL, Wen J. 2013. Molecular phylogenetics and biogeography of the eastern Asian-eastern North American disjunct Mitchella and its close relative *Damnacanthus* (Rubiaceae, Mitchelleae). Botanical Journal of the Linnean Society. 171(2): 395–412.

Hussain F, Shah SZ, Zhou W, Iqbal M. 2017. Microalgae screening under CO_2 stress: Growth and micro-nutrients removal efficiency. Journal of Photochemistry and Photobiology B: Biology. 170: 91–98.

Hyka P, Lickova S, Přibyl P, Melzoch K, Kovar K. 2013. Flow cytometry for the development of biotechnological processes with microalgae. Biotechnology Advances. 31: 2–16.

Irmak ŞO, Arzu AB. 2020. Determination of the fatty-acid composition of four native microalgae species. GSC Advanced Research and Reviews. 4(1): 001-008.

Islam Z, Khatoon H, Rahman MR, Hasan J, Hasan SJ. 2021. Screening of natural pigments from indigenous marine microalgae isolated from different coastal aquafarms of Bangladesh. Bangladesh Journal of Veterinary and Animal Sciences. 9(2).

Islam Z, Khatoona H, Minhaz TM, Rahmana MR, Hasan S, Mahmud Y, Hossaina MS, Sarker J. 2021. Data on growth, productivity, pigments and proximate composition of indigenous marine microalgae isolated from Cox's Bazar Coast. Data in Brief. 35: 106860.

Iyer G, Nagle V, Gupte YV, Desai S, Iyer M, Moramkar N, Sawant V. 2015. Characterization of high carotenoid producing *Coelastrella oocystiformis* and its anti-cancer potential. Int. J. Curr. Microbiol. Appl. Sci. 4(10): 527-536.

Jadhavar PB, Papdiwal PB. 2016. *Tetraedrons* at Nathsagar water reservoir (Paithan), Maharashtra. Journal of Algal Biomass Utilization. 7(3): 65-67.

Jeffrey SW, Humphrey GF. 1975. New spectrophotometric equations for determining chlorphylls a, b, and c, in higher plants, algae and natural phytoplankton, Bhiochemie und Physiologie der Pflanzen. 167: 191–194.

Jenkins SH. 1982. Standard methods for the examination of water and wastewater, Water Research. 16(10): 1495–1496.

John DM, Whitton BA, Brook AJ. 2002. The Freshwater algal flora of the British Isles: an identification guide to freshwater and terrestrial algae, Cambridge University Press, 2002.

Kaewpintong K. 2004. Cultivation of *Haematococcus pluvialis* in airlift bioreactor. Master thesis in Chemical Engineering. Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University.

Kaufnerová V, Eliáš M. 2013. The demise of the genus *Scotiellopsis Vinatzer* (Chlorophyta). Nova Hedwigia. 97(3-4): 415-428.

Kawaguchi K. 1980. Microalgae Production Systems in Asia. In: Algae Biomass Production and Use. Shelef G, Soeder CJ, editors. Elsevier/North Holland Biomedical Press, Amsterdam. pp. 25-33.

Khan MI, Shin JH, Kim JD. 2018. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microbial Cell Factories. 17 (1): 36.

Khatoon H, Rahman NA, Banerjee S, Harun N, Suleiman SS, Zakaria NH, Lananan F, Hamid SHA, Endut A. 2014. Effects of different salinities and pH on the growth and proximate composition of *Nannochloropsis* sp. and *Tetraselmis* sp. isolated

from South China Sea cultured under control and natural condition. International Biodeterioration and Biodegradation. 95: 11–18.

Khatoon H, Yuan GTG, Mahmud AI, Rahman MR. 2020. Growth and carotenoid production of *Dunaliella salina* (Dunal) Teodoresco, 1905 cultured at different salinities. Asian Fisheries Science. 33: 207–212.

Kleinegris D, Janssen M, Brandenburg WA, Wijffels RH. 2009. The selectivity of milking of *Dunaliella salina*. Marine Biotechnology. 12: 14– 23.

Kliphuis AM, Klok AJ, Martens DE, Lamers PP, Janssen M, Wijffels RH. 2012. Metabolic modeling of *Chlamydomonas reinhardtii*: energy requirements for photoautotrophic growth and maintenance. Journal of applied phycology. 24: 253-266.

Kol E, Chodat F. 1934. Quelques algues nouvelles des sols et de la neige du Parc National Suisse, Engadine.

Komárek J. 1983. Chlorophyceae (Grunalgen) Ordnung Chlorococcales. Das Phytoplankton des SuBwassers. 1-1044.

Krishnan V, Uemura Y, Thanh NT, Khalid NA, Osman N, Mansor N. 2015. Three types of Marine microalgae and *Nannocholoropsis oculata* cultivation for potential source of biomass production. Journal of Physics. Conference series. 622: 012034.

Kulandaivel S, Prakash R, Anitha R, Arunnagendran N. 2007. Comparative studies on biochemical profile of *Spirulina platensis* and *Oscillatoria* sp. on synthetic medium and dairy effluent. J. Pure Appl. Microbiol. 1: 109-112.

Kützing FT. 1845. Phycologia germanica, di Deutschlands Algen in bundigen Beschreibungen. Wilh. Köhne.

Laing I. 1991. Cultivation of marine, unicellualar algae. Laboratory Leaflet Number. 67: 1–32.

Lamers PP, Janseen M, De-Vos RCH, Bino RJ, Wijffels RH. 2012. Carotenoid and fatty acid metabolism in nitrogen-starved *Dunaliella salina*, a unicellular green microalga. Journal of Biotechnology. 162: 21–27.

Lavens P, Sorgeloos P. 1996. Manual on the production and use of live food for aquaculture. Rome: Food and Agriculture Organization of the United Nations.

Lavin PLS. 2000. Nitrogenio organic intracellular: Extracao, papel dosiologico e acumilacao por microalgas maerinhas, em cultivos estanques," (Doctoral dissertation, Dissertacao Universidate Federal Fluminense, Niteroi, Rio de Janeiro).

Lebret, K., Fernández Fernández, M., Hagman, C. H., Rengefors, K., & Hansson, L. A. (2012). Grazing resistance allows bloom formation and may explain invasion success of Gonyostomum semen. *Limnology and Oceanography*, *57*(3), 727-734.

Lourenço SO. 2006. Cultivation of microalgae: principle of applications. São Carlos: Rima.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry. 193: 265–275.

Lubián, L. M., Montero, O., Moreno-Garrido, I., Huertas, I. E., Sobrino, C., González-del Valle, M., & Parés, G. (2000). Nannochloropsis (Eustigmatophyceae) as source of commercially valuable pigments. *Journal of Applied Phycology*, *12*, 249-255.

Lucas-Salas LM, Castrillo M, Martínez D. 2013. Effect of dilution rate and water reuse on biomass and lipid production of *Scenedesmus obliquus* in a two-stage novel photobioreactor. Bioresource Technology. 143: 344–352.

Mathivanan K, Elumalai S, Kanna GR. 2015. Screening, extraction and estimation of phycobiliproteins from cyanobacteria. Golden Research Thoughts. 4(8): 1–4.

Menezes RS, Soares AT, Lopes RG, Magnotti C, Derner RB, Mori CC, Antoniosi Filho NR. 2015. Evaluation of fatty acid composition of the microalgae *Choricystis minor var. minor* according to two different nutrient feeding strategies. Journal of Renewable and Sustainable Energy. 7(4).

Mercado I, Álvarez X, Verduga M, Cruz A. 2020. Enhancement of biomass and lipid productivities of *Scenedesmus* sp. cultivated in the wastewater of the dairy industry. Processes. 8: 1458.

Metcalf JS, Dunlop RA, Banack SA, Souza NR, Cox PA. 2021. Cyanotoxin analysis and amino acid profiles of cyanobacterial food items from Chad. Neurotoxicity Research. 39(1): 72-80.

Michael A. 2018. Morphology of microalgae. Microalgae in Health and Disease Prevention. 2018: 23-72

Moheimani NR. 2005. The culture of Coccolithophorid algae for carbon dioxide bioremediation. Ph.D thesis. Murdoch University.

Moore S, Stein WH. 1951. Chromatography of amino acids on sulfonated polystyrene resins. Journal of Biological Chemistry. 192, 663-681.

Mundt S, Kreitlow S, Jansen R. 2003. Fatty acids with antibacterial activity from the cyanobacterium Oscillatoria redekei HUB 051. Journal of Applied Phycology. 15: 263-267.

Nascimento MDLS, Rizza AA, Di Palma MDLA, Dublan GL, Salerno LM, Rubio L, Curatti. 2015. Cyanobacterial biological nitrogen fixation as a sustainable nitrogen fertilizer for the production of microalgal oil. Algal Res.12: 142–148.

Nayek S, Choudury IH, Jaishee N, Roy S. 2014. Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. Research Journal of Chemical Sciences. 4(9): 63–69.

Ogbonna JC, Nweze NO, Ogbonna CN. 2021. Effects of light on cell growth, chlorophyll, and carotenoid contents of *Chlorella sorokiniana* and *Ankistrodesmus falcatus* in poultry dropping medium. Journal of Applied Biology and Biotechnology. 9(2): 157–163.

Pandey VD, Pandey A, Sharma V. 2013. Biotechnological applications of cyanobacteria phycobiliproteins. International Journal of Current Microbiology and Applied Sciences. 2(9): 89 – 97.

Park D, Jagtap S, Nair SK. 2014. Structure of a PL17 family alginate lyase demonstrates functional similarities among exotype depolymerases. Journal of Biological Chemistry. 289: 8645–8655.

Parsons T, Maita Y, Lalli C. 1984. A manual of chemical and biological methods for seawater analysis. Oxford: Pergamon Press. 122(5): 570.

Perumal P, Prasath BB, Santhanam P, Ananth S, Devi AS, & Kumar SD. 2012. Isolation and culture of microalgae. In Workshop on advances in aquaculture technology. 166: 181.

Phang SM, Chu WL. 1999. University of Malaya, Algae culture Collection, Catalogue of Strains. Institute of Post Graduate Studies and Research, University of Malaya, Kuala Lumpur, Malaysia. pp. 77.

Phatarpekar PV, Sreepada RA, Pednekar C, Achuthankutty CT. 2000. A comparative study on growth performance and biochemical composition of mixed culture of *Isochrysis galbana* and *Chaetoceros calcitrans* with monocultures. Aquaculture. 181: 141–155.

Phukan MM, Chutia RS, Konwar BK, Kataki R. 2011. Microalgae *chlorella* as a potential bio-energy feedstock. Applied Energy. 88: 3307–3312.

Pickett-Heaps JD. 1972. Cell division in *Tetraedron*. Annals of Botany. 36(4): 693-701.

Ponis E, Probert I, Véron B, Mathieu M, Robert R. 2006. New microalgae for the Pacific oyster Crassostrea gigas larvae. Aquaculture. 253: 618–627.

Praharyawan S, Rahman DY, Susilaningsih D. 2020. The enhancement of growth, biomass production and lipid productivity of microalgae *Choricystis* sp. LBB13-AL045 by the addition of hot water extract of its dried biomass. In IOP Conference Series: Earth and Environmental Science (Vol. 457, No. 1, p. 012071). IOP Publishing.

Prayitno J. 2016. Pola Pertumbuhandan Pemanenan biomassa dalam fotobioreaktor mikroalga untuk penangkapan karbon. Jurnal Teknologi Lingkungan. 17(7): 45–52.

Prescott GW. 1951. Algae of the western Great Lakes area. Cranbrook Institute of Science. Bull. 31: 135-137.

Probert I, Klaas C. 1999. Microalgae culturing. Retrieved February 2, 2017, from INA: The International Nannoplankton Association: http://ina.tmsoc.org/ codenet/culturenotes.htm.

Puzanskiy R, Tarakhovskaya E, Shavarda A, Shishova M. 2018. Metabolomic and physiological changes of *Chlamydomonas reinhardtii* (Chlorophyceae, Chlorophyta) during batch culture development. Journal of Applied Phycology. 30: 803-818.

Rasheedy AGMSH, Farahat MADAZ, Mohammed TA. 2017. The differential efficiency of *Chlorella vulgaris* and *Oscillatoria* sp. to treat the municipal wastewater. Journal of Biology, Agriculture and Healthcare.7: 22.

Ratha SK, Rao PH, Govindaswamy K, Jaswin RS, Lakshmidevi R, Bhaskar S, Chinnasamy S. 2016. A rapid and reliable method for estimating microalgal biomass using a moisture analyser. Journal of Applied Phycology. 28(3): 1725–1734.

Reyes y, Chenard G, Aranda DG, Mesquita C, Fortes M, Ora J, Baceller L. 2012. Biodiesel production by hydroesterification of microalgal biomass using hetergenous catalyst. National Science. 4: 778–783.

Rise M, Cohen E, Vishkautsan M, Arad S. 1994. Accumulation of secondary carotenoids in *Chlorella zofingiensis*. Jornal on Plant Physics. 144: 287-292.

Rodolfi L, Zittelli GC, Barsanti L, Rosati G, Tredici MR. 2003. Growth medium recycling in *Nannochloropsis* sp. mass cultivation. Biomolecular Engineering. 20(4–6): 243–248.

Roth MS, Cokus SJ, Gallaher SD, Walter A, Lopez D, Erickson E, Niyogi KK. 2017. Chromosome-level genome assembly and transcriptome of the green alga

Chromochloris zofingiensis illuminates astaxanthin production. Proceedings of the National Academy of Sciences. 114(21): E4296-E4305.

Safi C, Charton M, Pignolet O, Silvestre F, Vaca-Garcia C, Pontalier P. 2013. Influence of microalgae cell wall characteristics on protein extractability and determination of nitrogen-to-protein conversion factors. Journal of Applied Phycology. 25: 523–529.

Sanchez JF, Fernandez JM, Acien FG, Rueda A, Perez-Parra J, Molina E. 2008. Influence of culture conditions on the productivity and lutein content of the new strain *Scenedesmus almeriensis*. Process Biochemistry. 43: 398–405.

Santhosh B, Singh NP. 2007. Guidelines for water quality management for fish culture in Tripura, ICAR Research Complex for NEH Region, Tripura Center, Publication no. 29.

Santos-Ballardoa DU, Rossib S, Hernándezd V, Gómezd RV, Rendón-Uncetad MC, Caro-Corralesa J, Valdez-Ortiz A. 2015. A simple spectrophotometric method for biomass measurement of important microalgae species in aquaculture. Aquaculture. 448: 87–92.

Sassenhagen I, Rengefors K, Richardson TL, Pinckney JL. 2014. Pigment composition and photoacclimation as keys to the ecological success of *Gonyostomum* semen (R aphidophyceae, S tramenopiles). Journal of phycology. 50(6): 1146-1154.

Schmidle W. 1893. Beiträge zur Algenflora des Schwarzwaldes und der Rheinebene. Naturforschende Gesellschaft zu Freiburg iB.

Shaish A, Ben-Amotz A, Avron M. 1992. Biosynthesis of β-carotene in *Dunaliella*. Carotenoids Part A: chemistry, separation, quantitation, and antioxidation. Methods Enzymology. 213: 439–444.

Shubert E, Gärtner G. 2015. Nonmotile coccoid and colonial green algae. In Freshwater Algae of North America (pp. 315-373). Academic Press.

Siaut M, Cuiné S, Cagnon C, Fessler B, Nguyen M, Carrier P, Peltier G. 2011. Oil accumulation in the model green alga *Chlamydomonas reinhardtii*: characterization, variability between common laboratory strains and relationship with starch reserves. BMC biotechnology. 11(1): 1-15.

Siegelman H, Kycia J. 1978. In: Algal Bili-Proteins: Handbook of Psychological Method, Cambridge University Press, Cambridge. pp. 71–79.

Singh P, Kumari S, Guldhe A, Misra R, Rawat I, Bux F. 2016. Trends and novel strategies for enhancing lipid accumulation and quality in microalgae. Renew Sustain Energy Reviews. 55: 1–16.

Singh R, Birru R, Sibi G. 2017. Nutrient removal efficiencies of *Chlorella vulgaris* from urban wastewater for reduced eutrophication. Journal of Environmental Protection Science. 8(1): 1–11.

Starr RC. 1954. Reproduction by zoospores in *Tetraedron bitridens*. American Journal of Botany. 17-20.

Stein J. 1980. Handbook of Phycological methods. Culture methods and growth measurements. Cambridge University Press. pp. 448.

Stoyneva MP, Descy JP, Balagué V, Compère P, Leitao M, Sarmento H. 2012. The queer *Tetraëdron minimum* from Lake Kivu (Eastern Africa): is it a result of a human impact?. Phytoplankton responses to human impacts at different scales. 273-283.

Sukkrom K, Bunnag B, Powtongsook S, Siangdung W, Pavasant P. 2016. Biomass and lipid enhancement in *Ankistrodesmus* sp. cultured with reused and minimal nutrients media. Preparative Biochemistry and Biotechnology. 46(5): 467–473.

Tarin NJ, Ali NM, Chamon AS, Mondol MN, Rahman MM, Aziz A. 2016. Optimizing *Chlorella vulgaris* and *Anabaena variabilis* growth conditions for use as biofuel feedstock. Journal of the Asiatic Society of Bangladesh Science. 42(2): 191–200.

Techetel BW, Ruppel HG. 1992. Lipid Bodies in Eremosphaera viridis De Bary (Chlorophyceae). Plant and Cell Physics. 31(1): 41-48.

Teresa MM, Antonio AM, Nidia S, and Caetano. 2010. Microalgae for biodiesel production and other applications: A review. Renewable and Sustainable Energy Reviews. 14: 217–232.

Thepsuthammarat K, Reungsang A, Plangklang P. 2023. Microalga *Coelastrella* sp. cultivation on unhydrolyzed molasses-based medium towards the optimization of conditions for growth and biomass production under mixotrophic cultivation. Molecules. 28(8):3603.

Thompson PA, Levasseur ME, Harrison PJ. 1989. Light-limited growth on ammonium vs. nitrate: what is the advantage for marine phytoplankton? Limnology and Oceanography. 34: 1014–1024.

Tompkins J, DeVille MM, Day JG, Turner MF. 1995. Culture collection of algae and protozoa. Catalogue of strains. 1-4.

Tschaikner A, Ingolic E, Gärtner G. 2007a. Observations in a new isolate of *Coelastrella terrestris* (REISIGL) HEGEWALD & HANAGATA (Chlorophyta, Scenedesmaceae) from alpine soil (Tyrol, Austria). Phyton. 46:237-245.

Tschaikner A, Ingolić E, Stoyneva MP, Gärtner G. 2007b. Autosporulation in the soil alga *Coelastrella terrestris* (Chlorophyta, Scenedesmaceae, Scenedesmoideae). Phytol. Balc. 13:29-34.

Uzunov BA, Stoyneva MP, Gärtner G, Kofler W. 2008. First record of *Coelastrella* species (Chlorophyta: Scenedesmaceae) in Bulgaria. Berichte des naturwissenschaftlichen-medizinischen Verein Innsbruck. 95: 27-34.

Velichkova K. 2014. Effect of different nitrogen sources on the growth of microalgae *Chlorella vulgaris* cultivation in aquaculture wastewater. Agricultural Science and Technology. 6(3): 337-340.

Vílchez C, Forján E, Cuaresma M, Bédmar F, Garbayo I, Vega JM. 2011. Marine carotenoids: biological functions and commercial applications. Marine Drugs. 9: 319–333.

Villarruel-Lopez A, Ascencio F, Nuno K. 2017. Microalgae, a potential natural functional food source—a review. Polish Journal of Food and Nutrition Sciences. 67: 251–263.

Wahidin S, Idris A, Shaleh SRM. 2013. The influence of light intensity and photoperiod on the growth and lipid content of microalgae *Nannochloropsis* sp. Bioresource Technology. 129: 7–11.

Wang Q, Lu Y, Xin Y, Wei L, Huang S, Xu J. 2016. Genome editing of model oleaginous microalgae *Nannochloropsis* spp. by CRISPR/Cas9. Plant. 88: 1071–1081.

Wellburn AR. 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal on Plant Physics. 144(3): 307-313.

Wells ML, Potin P, Craigie JS, Raven JA, Merchant SS, Helliwell KE, Brawley SH. 2017. Algae as nutritional and functional food sources: revisiting our understanding. Journal of applied phycology. 29:949-982.

White DA, Pagarette A, Rooks P, Ali ST. 2013. The effect of sodium bicarbonate supplementation on growth and biochemical composition of marine microalgae cultures. Journal of Applied Phycology. 25: 153–165.

Wiedenmann J. 2008. Marine proteins. In: Walsh PJ, Smith SL, Fleming LE, Solo-Gabriele HM, Gerwick WH, editors. Oceans and human health. risks and remedies from the sea. Academic Press, St. Louis, MO. pp. 469–495.

Xia A, Cheng J, Lin R, Lu H, Zhou J, Cen K. 2013. Comparison in dark hydrogen fermentation followed by photo hydrogen fermentation and methanogenesis between protein and carbohydrate compositions in *Nannochloropsis oceanica* biomass. Bioresource Technology. 138: 204–13.

Yang X, Liu P, Hao Z, Shi J, Zhang S. 2011. Characterization and identification of freshwater microalgal strains toward biofuel production. BioResources. 7: 686–695.

Yeh KL, Chang JS. 2012. Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga *Chlorella vulgaris* ESP-31. Bioresource Technology. 105: 120–127.

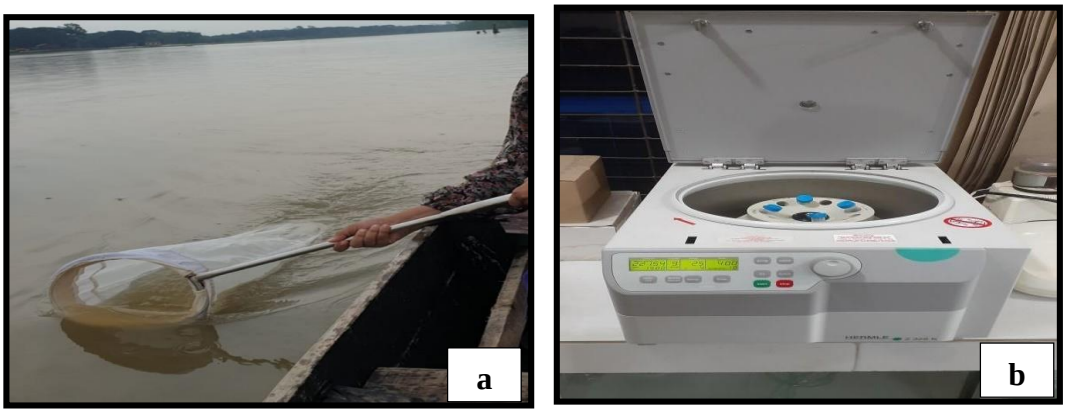
Yustinadiar N, Manurung R, Suantika G. 2020. Enhanced biomass productivity of microalgae *Nannochloropsis* sp. in an airlift photobioreactor using low-frequency flashing light with blue LED. Bioresources and Bioprocessing. 7: 43.

Zarrinmehr MJ, Farhadian O, Heyrati FP, Keramat J, Koutra E, Kornaros M, Daneshvar E. 2019. Effect of nitrogen concentration on the growth rate and biochemical composition of the microalga, *Isochrysis galbana*. The Egyptian Journal of Aquatic Research. 46(2).

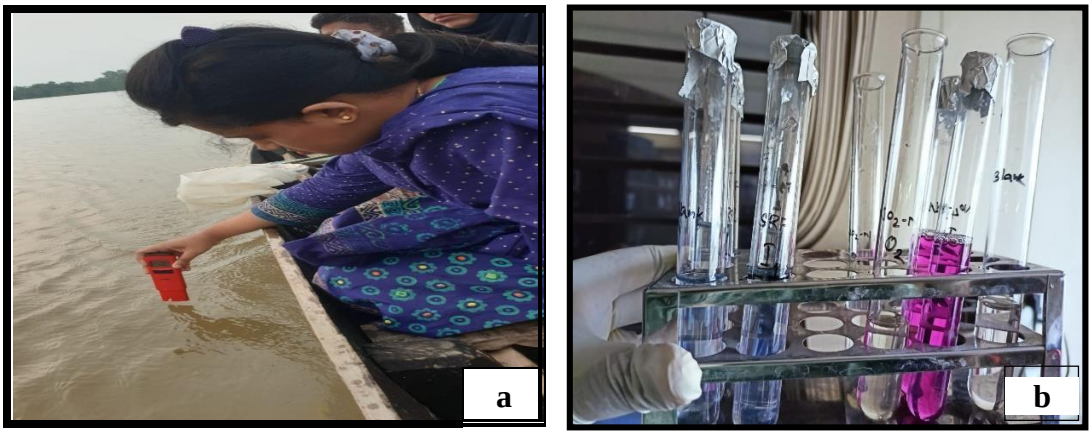
Zidarova R, Temniskova D, Dzhambazov B. 2009. Karyological and endosymbiotic notes on two *Choricystis* species (Trebouxiophyceae, Chlorophyta). Biologia. 64(1): 43-47.

Zuorro A, Leal-Jerez AG, Morales-Rivas LK, Mogollón-Londoño SO, Sanchez-Galvis EM, García-Martínez JB, Barajas-Solano AF. 2021. Enhancement of phycobiliprotein accumulation in Thermotolerant *Oscillatoria* sp. through media optimization. ACS Omega. 6(16): 10527–10536.

Appendices

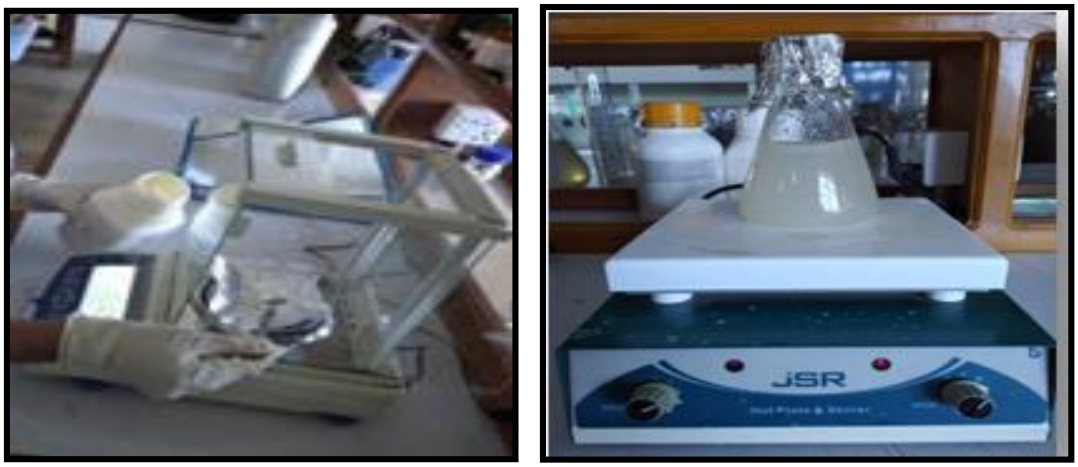


Appendix A: (a) Water sample collection, (b) Sample concentration

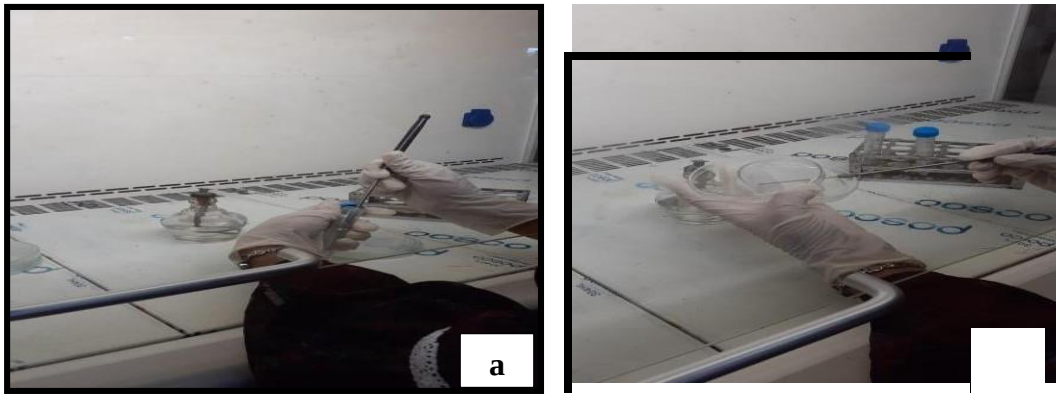


Appendix B: Determination of water quality parameter

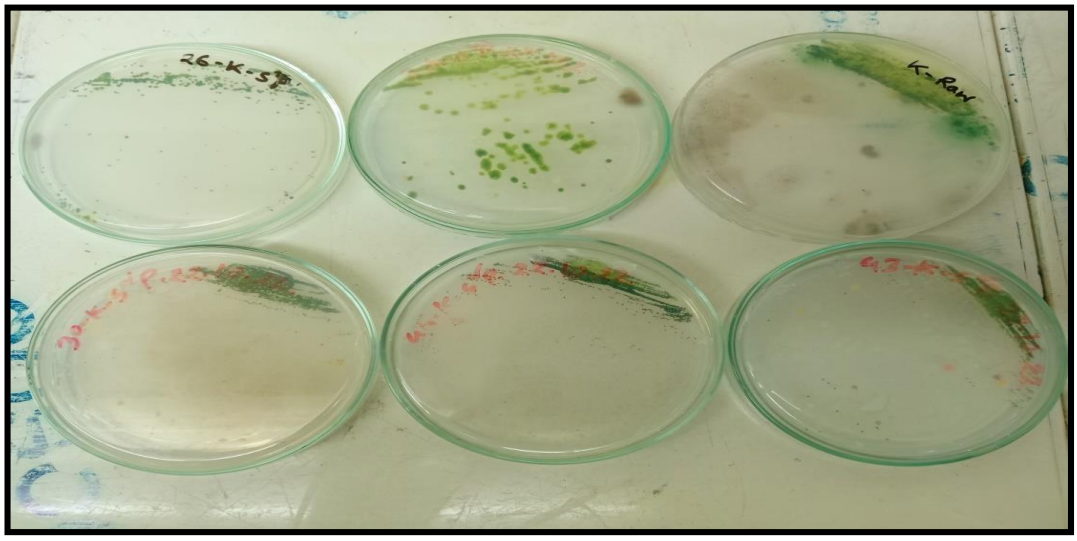
(a) Physical parameter (b) Chemical parameter



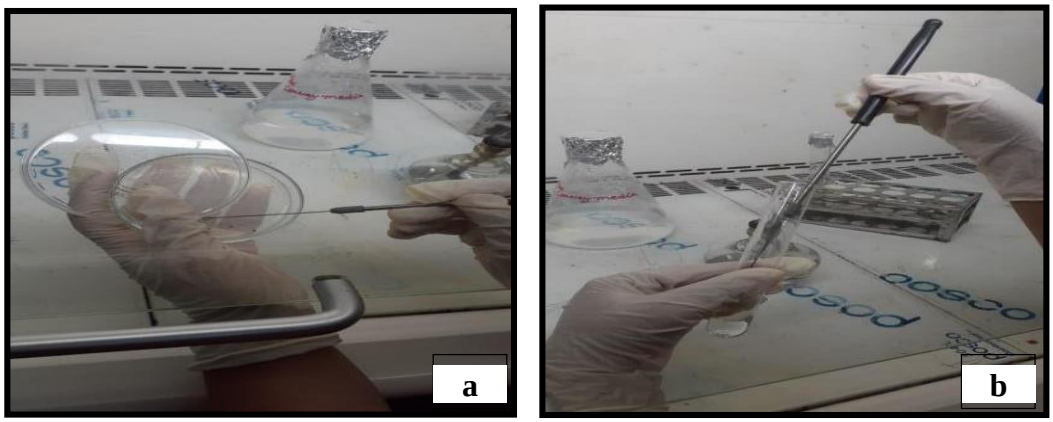
Appendix C: Agar media preparation



Appendix D: (a) Sample inoculation (b) Streaking in agar plates

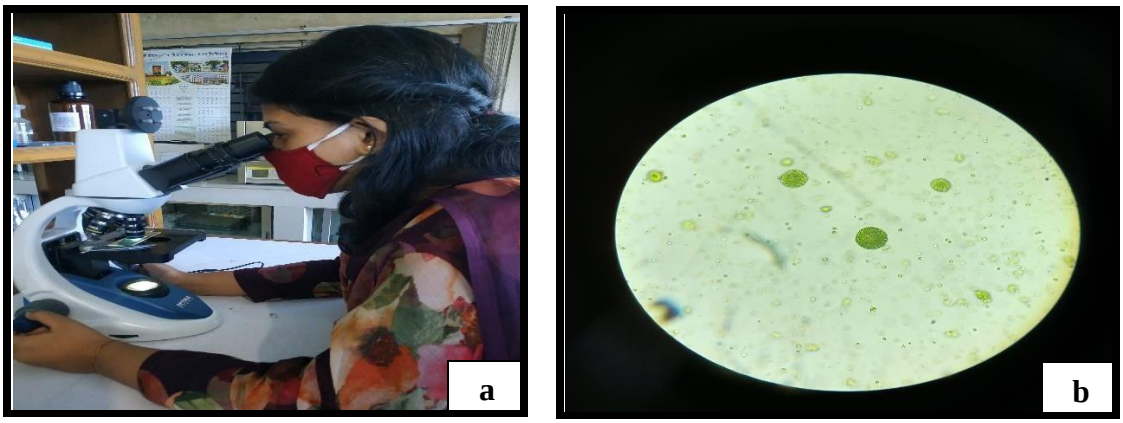


Appendix E: Observation of microalgal growth on agar plates



Appendix F: Microalgae isolation

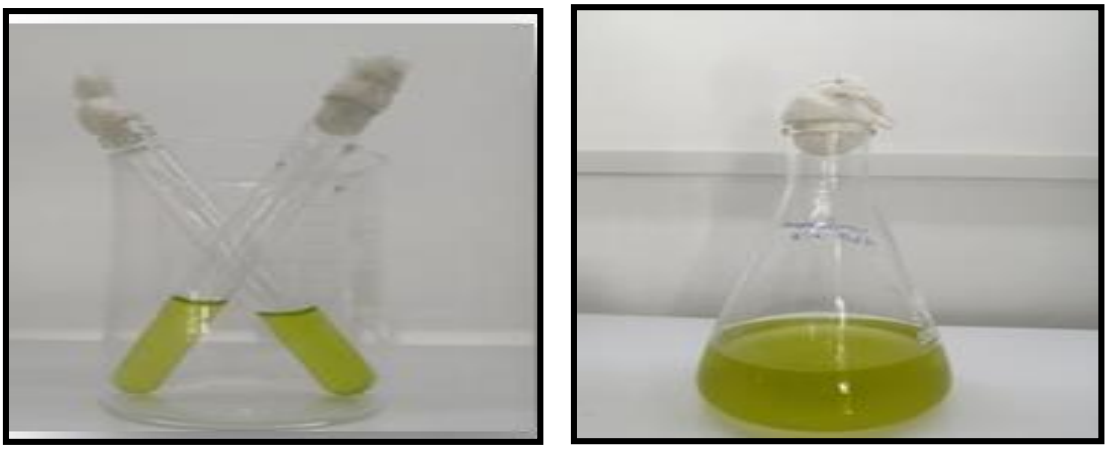
(a) Picking single colony from agar plate (b) Transferring single colony to the test tube



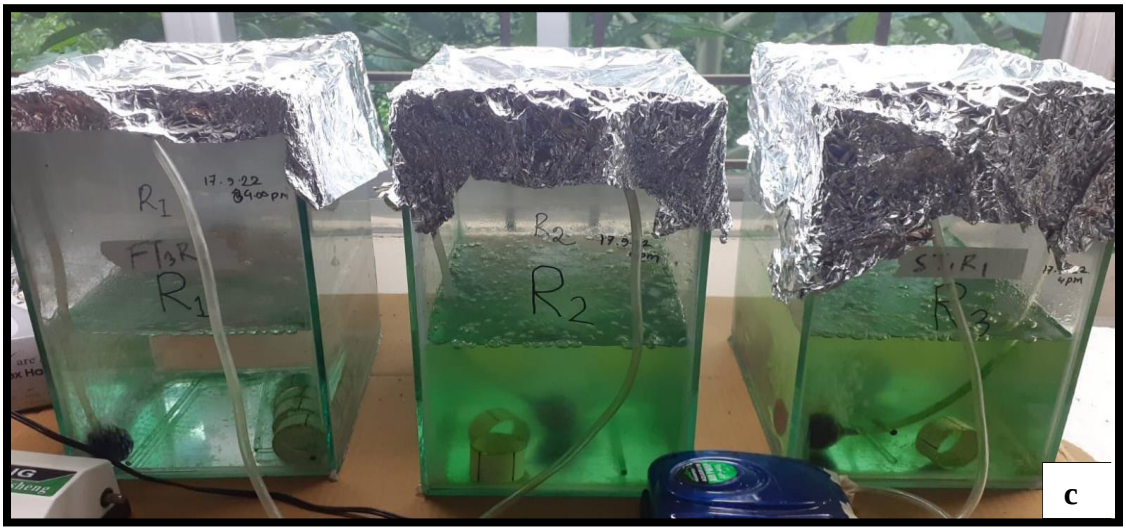
Appendix G: Morphological identification of microalgae

(a) Microscopic observation (b) Microscopic view of isolated microalgae

Appendix H: Preparation of culture media

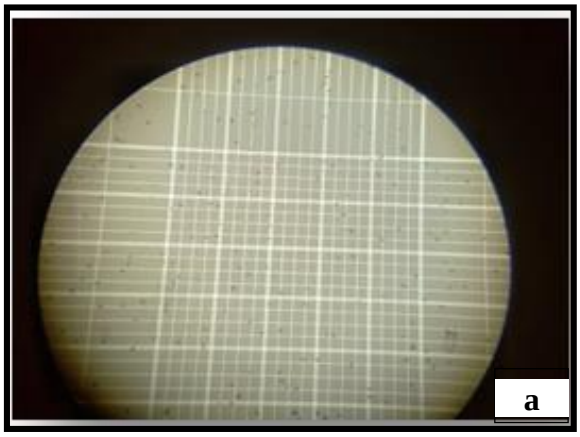


Appendix I: Culture and maintenance of single isolated microalgae



Appendix J: Growth curve determination of isolated microalgae

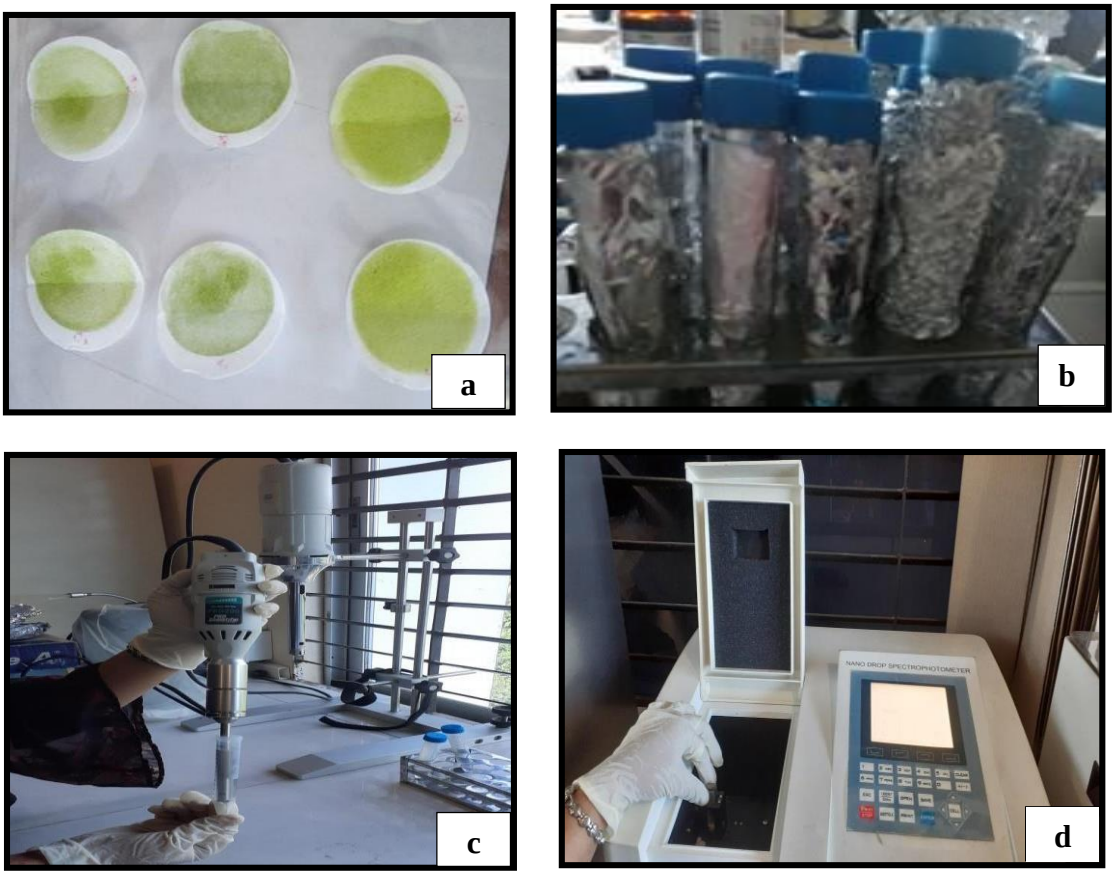
(a) Green microalgae (b) Brown microalgae (c) Cyanobacteria



Appendix K: Determination of (a) cell density (b) and optical densit

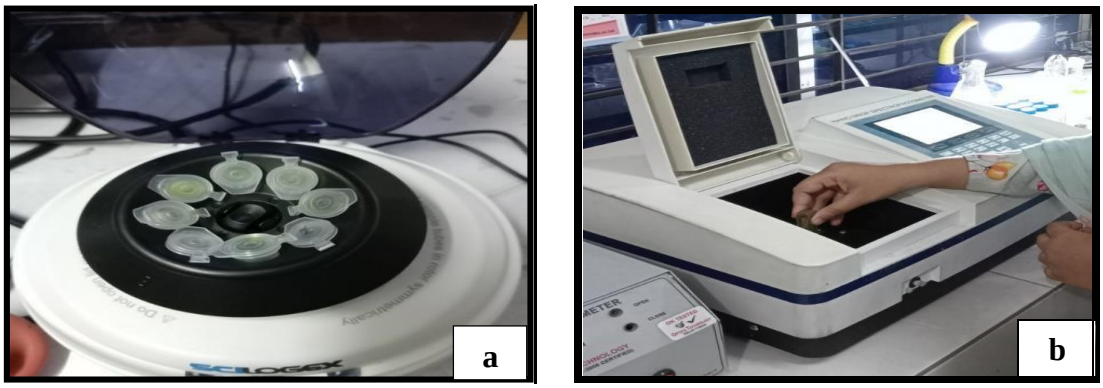
Appendix L: **(a) Mass culture of green microalgae (b) Mass culture of brown microalgae and (c) filamentous cyanobacteria using substrate (d) Substrate used in mass culture**

Appendix M: **Determination of biomass**



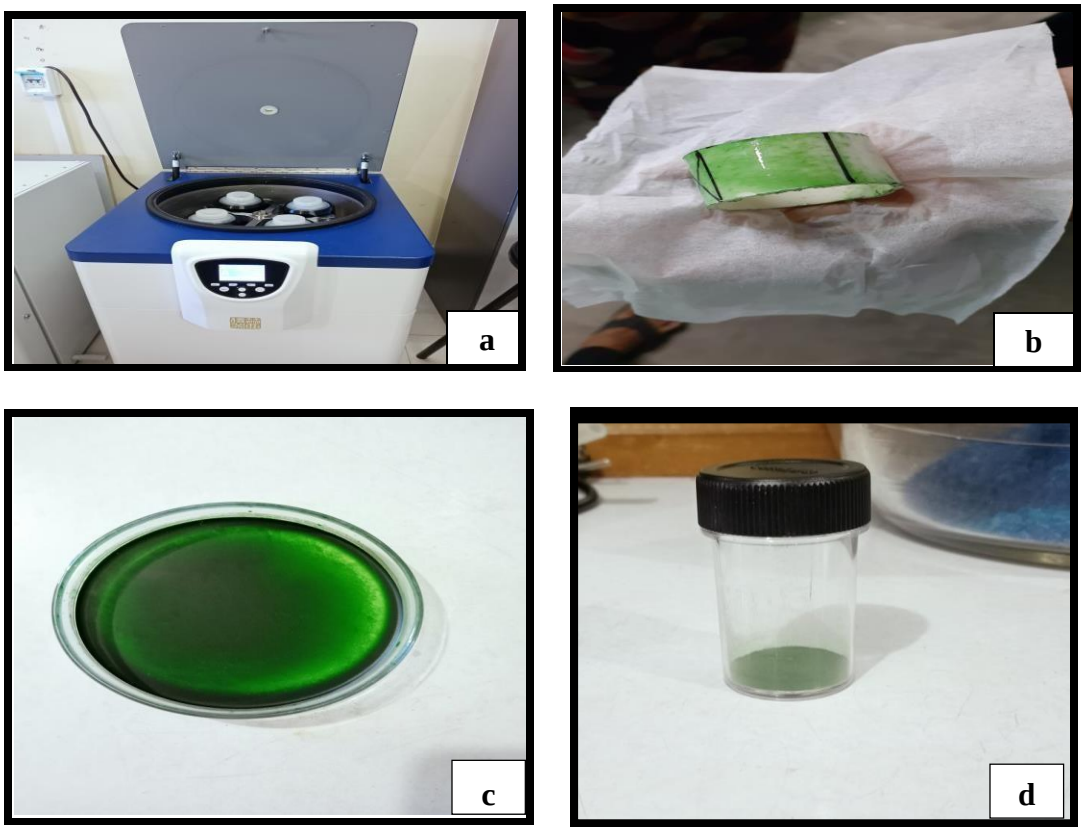
Appendix N: Determination of chlorophyll content

(a) Filtered sample on filter paper (b) Keeping filter paper in acetone (c) Homogenization of sample (d) Determination of chlorophyll through optical density



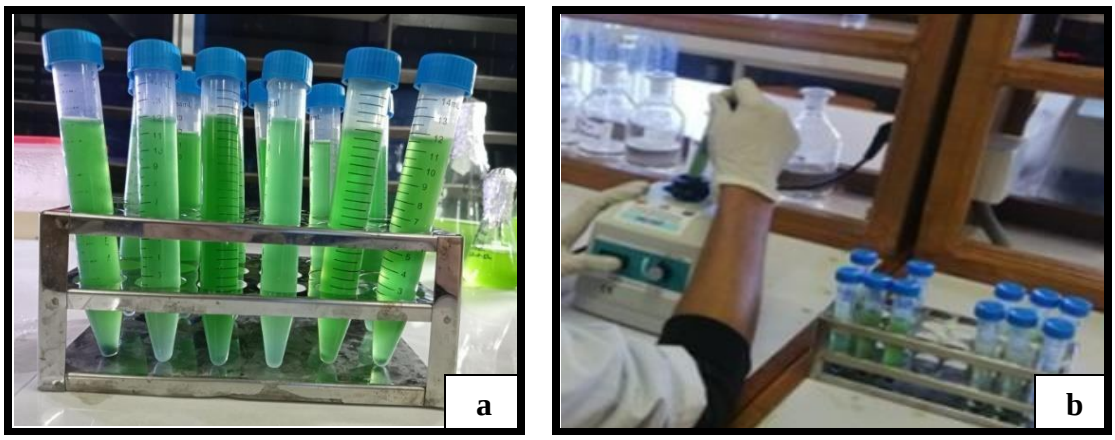
Appendix O: Determination of carotenoid content

(a) Centrifugation of sample (b) Determination of absorbance through spectrophotometer

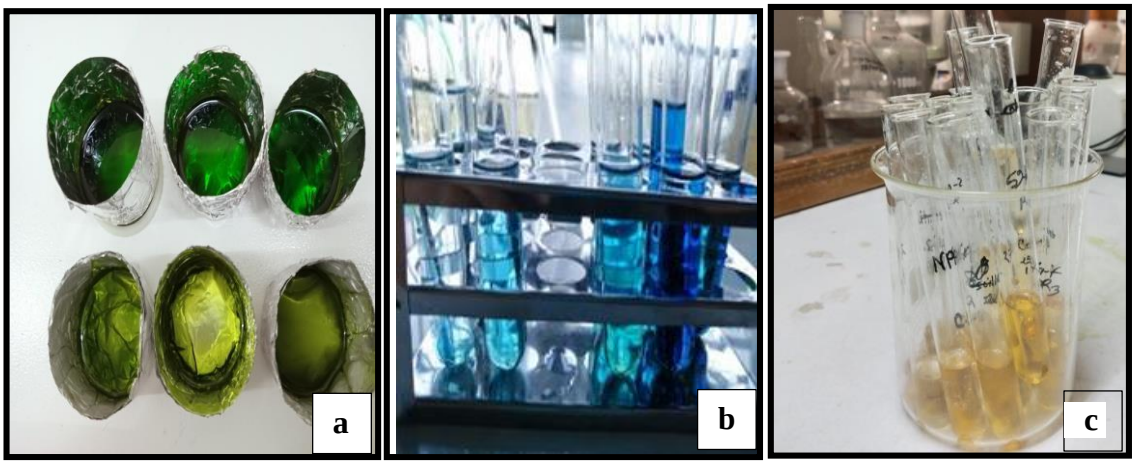


Appendix P: Biomass extraction

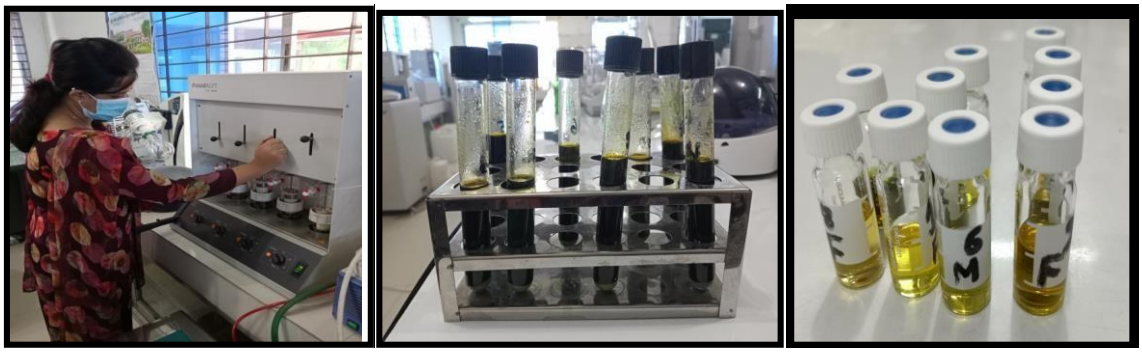
(a) Harvesting of microalgae (b) Harvesting from substrate (c) Dried biomass (d) Algae powder



Appendix Q: Determination of phycobiliprotein content (a) soaking of sample in phosphate buffer (b) Mixing using vortex mixture



Appendix R: Determination of lipid (a), protein (b) and carbohydrate (c) content



Appendix S: Determination of fatty acid content

Appendix 1: One way analysis of variance examining the difference in cell density on stationary phase of the isolated microalgae.

	Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Cell Density (cells/ml×10^7)	Between Groups	1440.658	9	160.073	1066.775	0
	Within Groups	3.001	20	0.15		
	Total	1443.659	29			

Appendix 2: One way analysis of variance examining the chlorophyll-a and b content of isolated microalgae.

	Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Chl-a	Between Groups	1881.796	13	144.754	12467.14	0
	Within Groups	0.325	28	0.012		
	Total	1882.121	41			
Chl-b	Between Groups	11.879	8	1.485	157.123	0
	Within Groups	0.17	18	0.009		
	Total	12.049	26			

Appendix 3: One way analysis of variance examining the difference in carotenoid content of isolated microalgae.

	Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Carotenoid	Between Groups	142.821	13	10.986	308.404	0
	Within Groups	0.997	28	0.036		
	Total	143.818	41			

Appendix 4: One way analysis of variance examining the difference in phycobiliprotein content of isolated microalgae.

	Source of Variation	Sum of Squares	Df	Mean Square	F	Sig.
Phycocyanine	Between Groups	25178.66	13	1936.82	35024.29	0
	Within Groups	1.548	28	0.055		
	Total	25180.2	41			
Allophycocyanin	Between Groups	1818.389	13	139.876	10039.67	0
	Within Groups	0.39	28	0.014		
	Total	1818.779	41			
Phycoerythrin	Between Groups	61.156	13	4.704	670.878	0
	Within Groups	0.196	28	0.007		
	Total	61.353	41			
Total Phycobiliprotein	Between Groups	42039.5	13	3233.807	71081.7	0
	Within Groups	1.274	28	0.045		
	Total	42040.77	41			

Appendix 5: One way analysis of variance examining the difference in proximate composition (protein, lipid and carbohydrate) of isolated microalgae.

	Source of Variation	Sum of Squares	Df	Mean Square	F	Sig.
Protein	Between Groups	7361.743	13	566.288	673.384	0
	Within Groups	22.706	27	0.841		
	Total	7384.449	40			
Lipid	Between Groups	1082.77	13	83.29	97.388	0
	Within Groups	23.947	28	0.855		
	Total	1106.716	41			
Carbohydrate	Between Groups	549.86	13	42.297	76.718	0
	Within Groups	15.437	28	0.551		
	Total	565.297	41			

Appendix 6: One way analysis of variance examining the difference in volumetric productivity of isolated microalgae.

	Source of Variation	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Volumetric Productivity(mg/L/Day)	Between Groups	4190.588	13	322.353	67694.24	0
	Within Groups	0.133	28	0.005		
	Total	4190.722	41			

Appendix 7: One way analysis of variance examining the difference in areal productivity of isolated microalgae.

	Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Areal Productivity ((mg/cm2/day)	Between Groups	41.906	13	3.224	67694.24	0
	Within Groups	0.001	28	0		
	Total	41.907	41			

Appendix 8: One way analysis of variance examining the difference in lipid productivity of isolated microalgae.

	Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Lipid Productivity (mg/L/Day)	Between Groups	255.078	13	19.621	322.046	0
	Within Groups	1.706	28	0.061		
	Total	256.784	41			

Appendix 9: One way analysis of variance examining the difference in cell duplication time of isolated microalgae.

	Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
cell duplication time (Day)	Between Groups	3.512	13	0.27	146787.5	0
	Within Groups	0	28	0		
	Total	3.512	41			

Appendix 10: One way analysis of variance examining the difference in Cell doublings per day of isolated microalgae.

	Source of Variation	Sum of Squares	Df	Mean Square	F	Sig.
Cell doublings per day (K)	Between Groups	0.873	9	0.097	6.859	0
	Within Groups	0.283	20	0.014		
	Total	1.156	29			

Appendix 11: One way analysis of variance examining the difference in SGR of isolated microalgae.

	Source of Variation	Sum of Squares	Df	Mean Square	F	Sig.
SGR	Between Groups	0.826	13	0.064	134153.2	0
	Within Groups	0	28	0		
	Total	0.826	41			

Appendix 12: One way analysis of variance examining the fatty acid content of isolated microalgae.

	Source of Variation	Sum of Squares	Df	Mean Square	F	Sig.
ΣSAFA	Between Groups	1014.374	13	78.029	4.165	0.006
	Within Groups	262.311	14	18.737		
	Total	1276.685	27			
ΣMUFA	Between Groups	2026.621	13	155.894	17.203	0
	Within Groups	126.869	14	9.062		
	Total	2153.49	27			
Σn6-PUFA	Between Groups	910753.18	13	70057.937	924.826	0
	Within Groups	1060.536	14	75.753		
	Total	911813.716	27			

Methyl icosa-5,8,11,14,17-pentaenoate	Between Groups	29.927	13	2.302	1.829	0.138
	Within Groups	17.62	14	1.259		
	Total	47.547	27			
Methyl Docosahexanoate	Between Groups	0.143	13	0.011	0.951	0.533
	Within Groups	0.162	14	0.012		
	Total	0.305	27			
Σn3-PUFA	Between Groups	500.221	13	38.479	5.568	0.002
	Within Groups	96.755	14	6.911		
	Total	596.976	27			
ΣPUFA	Between Groups	896013.498	13	68924.115	866.521	0
	Within Groups	1113.577	14	79.541		
	Total	897127.075	27			
Σn3/ Σn6	Between Groups	274.084	13	21.083	30.215	0
	Within Groups	9.769	14	0.698		
	Total	283.852	27			
DHA/EPA	Between Groups	18929.271	13	1456.098	36.164	0
	Within Groups	563.694	14	40.264		
	Total	19492.965	27			
SAFA/TUFA	Between Groups	18.145	13	1.396	43.46	0
	Within Groups	0.45	14	0.032		
	Total	18.595	27			
SAFA/TFA	Between Groups	1.462	13	0.112	107.524	0
	Within Groups	0.015	14	0.001		
	Total	1.476	27			
TUFA/TFA	Between Groups	1.462	13	0.112	107.524	0
	Within Groups	0.015	14	0.001		

	Total	1.476	27			

Brief bio-data of the student

Proma Dey is the daughter of Pritish Chandra Dey and Krishna Kali Mazumdar was born and grown up in Kaptai, Rangamati. She passed the Secondary School Certificate Examination in 2013 from Narangiri Govt. High School, followed by Higher Secondary Certificate Examination in 2015 from Chittagong Engineering University School & College. She graduated in 2020 with B.Sc Fisheries (Hons.) degree from Faculty of Fisheries, Chattaogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. She is now a candidate for the degree of MS under the Department of Aquaculture, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.