EFFECT OF ELEVATED CARBON DIOXIDE LEVELS ON GROWTH AND BIOCHEMICAL COMPOSITION OF SELECTED MICROALGAE

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KUALA LUMPUR

2012

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DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF BIOTECHNOLOGY

INSTITUTE OF BIOLOGICAL SCIENCES
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2012

UNIVERSITI MALAYA

ORIGINAL LITERARY WORK DECLARATION

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Registration/Matric No: **SGF 090019**

Name of Degree: **Masters of Biotechnology**

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"):
EFFECT OF ELEVATED CARBON DIOXIDE LEVELS ON GROWTH AND BIOCHEMICAL COMPOSITION OF SELECTED MICROALGAE

Field of Study: **Algal Biotechnology**

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ABSTRACT

The fixation of carbon dioxide by microalgae plays a major role in mitigation of carbon dioxide in the atmosphere as it can reduce the "Greenhouse" effects. In this study, ten strains of microalgae from the University of Malaya Algae Culture Collection (UMACC) were used for selection of potential strains with high biomass, lipid, protein and carbohydrate productivity. The microalgae screened included freshwater and marine strains including one isolated from palm oil mill effluent (*Chlorella* UMACC 275). The microalgae strains were grown in flask cultures for 12 days in batch culture with continuous aeration from an aquarium air-pump. Of the microalgae screened, *Chlorella vulgaris* UMACC 001 produced highest biomass 240.67±5.77mg/l and lipid 38.67± 4.62% DW with 69.36±4.103% DW protein and 1.92±0.177% DW carbohydrate. The specific growth rate (μ) of *Chlorella vulgaris* UMACC 001 attained was $0.2433 \pm 0.0168 day^{-1}$. Besides, *Scenedesmus quadricauda* UMACC 041, *Chlorella* UMACC 275 and *Oocystis* UMACC 074 also produced relatively higher biomass, lipid, protein and carbohydrate content compared with other strains studied. All the four strains were grown at carbon dioxide levels ranging from 0.04%, 5% to10% for 10 days in batch culture on 12:12 h light-dark cycle. *Chlorella vulgaris* UMACC 001 showed maximum specific growth rate, $0.3707 \pm 0.0171 day^{-1}$ at 5% CO_2. It also produced significant biomass of 268.67±22.030mg/L ($p<0.05$), lipid 34.58±1.140%DW ($p<0.05$), protein 59.44±3.905% DW ($p<0.05$) and carbohydrate 1.37±0.104% DW ($p<0.05$) content. *Chlorella* UMACC 275 attained higher biomass 146±9.165mg/L and lipid content 31.05±5.703% DW ($p<0.05$) with 53.59±2.20% DW ($p<0.05$) protein and 1.85±0.137% DW ($p<0.05$) carbohydrate content at 10% CO_2 although the production was less than *Chlorella vulgaris* UMACC 001. *Scenedesmus quadricauda* UMACC 041 produced higher biomass 257.33±56.757mg/L ($p<0.05$) but lower lipid content 28.48±6.884%DW with 52.803±2.001% DW ($p<0.05$) protein and 1.19±0.030% DW

($p<0.05$) carbohydrate content at 5% CO_2. Therefore, *Chlorella vulgaris* UMACC 001 was shown to be a potential strain for mitigating carbon dioxide and as a feedstock for biofuel as it produces high amounts of lipid, protein and biomass.

ABSTRAK

Pengikatan karbon dioksida oleh mikroalga memainkan peranan yang paling penting dalam mengurangkan karbon dioksida dalam atmosfera bagi mengatasi kesan rumah hijau. Dalam penyelidikan ini, sepuluh strain dari Universiti Malaya Algae Culture Collection (UMACC) telah digunakan bagi memilih strain yang berpotensi menghasilkan jisimbio, kandungan lipid, protein dan karbohidrat yang tinggi. Semua strain daripada mikroalga air tawar, marin dan satu strain dari palm oil mill effluent (*Chlorella* UMACC 275). Semua strain telah dikulturkan dalam kelalang selama 12 hari dalam sistem „batch' dengan pembekalan udara yang berterusan dari pum akuarium. Berdasarkan strain yang diskrin, *Chlorella vulgaris* UMACC 001 mencatatkan jisimbio yang tinggi 240.67±5.77mg/l dan lipid yang tinggi 38.67±4.62%DW dengan 69.36±4.103% DW protein dan 1.92±0.177% DW karbohidrat. Kadar pertumbuhan *Chlorella vulgaris* UMACC 001 yang maksima adalah (μ) 0.2433±0.0168hari^{-1}. Selain, *Scenedesmus quadricauda* UMACC 041, *Chlorella* UMACC 275 dan *Oocystis* UMACC 074 juga menghasilkan jisimbio, kandungan lipid, protein dan karbohidrat yang tinggi berbanding dengan strain yang lain. Kesemua empat strain tersebut telah dirawat dengan tahap karbon dioksida yang berlainan dari 0.04%, 5% dan 10% selama 10 hari dalam kultur „batch' dengan sumber cahaya dalam kitar 12:12h cahaya:gelap. *Chlorella vulgaris* UMACC 001 menunjukkan kadar pertumbuhan spesifik yang maksimum pada 5% CO_2 0.3707±0.0171hari^{-1}. Ia juga menghasilkan jisimbio yang signifikan sebanyak 268.67±22.03mg/l ($p<0.05$) dan kandungan lipid sebanyak 34.58±1.14% DW ($p<0.05$) dengan 59.44±3.905% DW ($p<0.05$) protein dan 1.37±0.104% DW ($p<0.05$) karbohidrat. *Chlorella* UMACC 275 menghasilkan jisimbio yang tinggi 146±9.165mg/L and kandungan lipid 31.05±5.703% DW ($p<0.05$) dengan 53.59±2.20% DW ($p<0.05$) protein dan 1.85±0.137% DW ($p<0.05$) karbohidrat pada 10% CO_2 walaupun hasilnya kurang berbanding dengan *Chlorella vulgaris* UMACC

001. *Scenedesmus quadricauda* UMACC 041 telah menghasilkan jisimbio yang tinggi 257.33±56.757mg/L ($p<0.05$) tetapi kandungan lipid yang rendah 28.48±6.884% DW pada 5% CO_2 dengan kandungan protein 52.803±2.001% DW ($p<0.05$) dan karbohidrat 1.19±0.030% DW ($p<0.05$) content. Oleh itu, *Chlorella vulgaris* UMACC 001 menjadi strain yang berpotensi dalam mengurangkan kandungan karbon dioksida dan menghasilkan lipid, protein dan jisimbio yang tinggi.

AKNOWLEDGEMENTS

I would like to extend my sincere gratitude to my supervisor Professor Phang Siew Moi who gave me continuous support, guidance, encouragement and immense knowledge throughout this study. Her guidance helped me at all times during the research and writing of this thesis.

Besides, I would like to express my gratitude to my co-supervisor Professor Chu Wan Loy who guided and supported me throughout this research work. I wish to express my gratitude to my second co-supervisor Dr. Harrison Lau from the Malaysian Palm Oil Board (MPOB) for his encouragement and support in completing this project.

My sincere thanks go to my seniors and fellow labmates Teoh Ming Li, Kak Emy, Vejesri and Lalitha for their guidance, cooperation and moral support for me to complete this research work. I wish to express my sincere gratitude to my fiancee Mr.Suntaresan Unnikrishnan for his continuous support and encouragement to complete my thesis.

Last but not least I would like to express my heartfelt gratitude to my beloved mother Madam Jevaratanam for her love, continuous encouragement and moral support that drives me to complete this research work successfully. I also wish to thank my friends and relatives for their encouragement to me all the time in striving towards this achievement.

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LIST OF SYMBOLS AND ABBREVIATIONS

ºC	Degree celcius
% dry weight	percentage of dry weight
µm	Microgram
µL	Microliter
mg	Milligram
g	gram
g/l	gram per liter
ml	milliliters
L	Liters
mg/l	Milligram per liter
psi	Per square inch
rpm	revolutions per minute
nm	Nanometer
µ, day $^{-1}$	Specific growth rate
M	Molar (mol/dm^{-3})
ml/min	Milliliter per minute
v/v	Volume per volume
ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
BBM	Bold's Basal Medium
CO_2	Carbon Dioxide
CCM	Carbon Concentrating Mechanism
DIC	Dissolved inorganic carbon
DW	Dry weight
FAME	fatty acid methyl esters

MUFA	Monounsaturated Fatty Acids
OD_{620nm}	Optical Density at 620nm
POME	Palm Oil Mill Effluent
PUFA	Polyunsaturated Fatty Acids
RNAi	Ribonucleic Acid interference
S.D	Standard Deviation
SFA	Saturated Fatty Acids
TFA	Total Fatty Acids
UMACC	University Malaya Algae Culture Collection

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Global warming has become one of the threatening issues among populations worldwide as a result of human activities. This phenomenon is linked to the increase in the atmospheric concentrations of carbon dioxide, nitrogen and sulfur oxides, methane and other greenhouse gases with carbon dioxide constituting the major component. The rise of global temperature has effects on humans and the environment (Bilanovic et al., 2009). Since carbon dioxide is the major greenhouse gas, it is crucial to mitigate the high concentrations of the gas in the atmosphere as a way of reducing the greenhouse effect.

Various physiochemical techniques such as dry absorption, adsorption and membrane separation have been used to reduce carbon dioxide levels in the atmosphere (Lee et al., 2000). Besides, other methods such as carbon dioxide recovery and storage, alternative power and fuel consumption have been examined for the sequestration of carbon dioxide in atmosphere to overcome the greenhouse effect (Yue and Chen, 2005). However, these approaches are less effective due to the high cost and also high energy consumption. Therefore, biological approaches become good alternative in order to mitigate excess carbon dioxide in the atmosphere. Algae serve as an important resource to mitigate the carbon dioxide in the atmosphere and hence reduce the greenhouse effect (Eduardo et al., 2010).

Algae are eukaryotic organisms that can convert energy from solar into chemical energy by the process of photosynthesis. It is known that algae act as the largest primary producer in terms of biomass (Matsunaga et al, 1999). Algae are highly capable of removing carbon dioxide by the process of biofixation that can reduce the carbon dioxide emission and oxygen recovery. In addition, the fixed carbon dioxide into the microalgal cell wall leads to the production of valuable products in the algal biomass (Sung et al., 1999). According to Kaur and Bhatnagar (2002), algae have the ability to capture about ten times of energy from the sun compared to the terrestrial plants enabling the microalgae towards higher carbon dioxide consumption.

There are freshwater and marine microalgae which have high photosynthetic efficiency and possess high cell division rate compared to terrestrial plants. Therefore, they absorb more carbon dioxide by fixation to produce higher biomass at increased growth rates (Mata et al., 2010; Parker, 2009). There are various carbon dioxide sources that may be used for the fixation process which include industrial waste gases, emitted gas from power plants and from the atmosphere (Eduardo et al., 2010). Some microalgae have higher potential to survive and are capable of fixing carbon dioxide under extreme conditions such as low pH and acidic gases compared to the terrestrial plants that exhibit fatal effects at these conditions (Kurano et al., 1995).

Algae can accumulate high amounts of lipids that are capable of producing high amounts of oil, which subsequently can be converted into biodiesel (Chisti, 2008). Due to the energy crisis worldwide, biodiesel has gained much attention as a substitute for non- renewable fuels such as petroleum (Huang et al., 2010). In other words, algae may also be a source of biofuel and renewable energy that can be reliable in the future. The integration of CO_2 biofixation by algae and the use of the algal biomass as a feedstock for biodiesel production is an attractive approach in the biomitigation of greenhouse gases.

In this study, selected microalgae from the University of Malaya Algae Culture Collection (UMACC) were assessed for their growth and biochemical composition at elevated levels of carbon dioxide.

1.2 RESEARCH QUESTIONS

This present study is designed to answer the following research questions:

i) Can the tropical microalgae grow at the elevated carbon dioxide levels?

ii) Will growth at elevated carbon dioxide levels result in a change in the biochemical composition (protein, lipid and carbohydrate) of the microalgae?

1.3 OBJECTIVES OF STUDY

To answer the research questions, the following objectives were formulated:

i) To profile the growth and biochemical composition of selected microalgae from the University of Malaya Algae Culture Collection (UMACC)

ii) To measure the growth of selected algae at elevated concentrations of carbon dioxide

iii) To profile the biochemical composition of selected algae species grown at elevated carbon dioxide levels

1.4 RESEARCH APPROACH

Stage 1

Selection of ten microalgae from UMACC:

a) *Chlorella vulgaris* Beijerinck UMACC 001
b) *Scenedesmus quadricauda* UMACC 041
c) *Oocystis* UMACC 074
d) *Scenedesmus obtusus* UMACC 100
e) *Chlorococcum miniatum* UMACC 102
f) *Chlorella vulgaris* Beijerinck UMACC 103
g) *Chlorella vulgaris* UMACC 245
h) *Chlorella vulgaris* UMACC 253
i) *Chlorella* UMACC 255
j) *Chlorella* UMACC 275 (from POME)

Stage 2

a) Preparation of medium for cultivation of microalgae
b) Maintenance of stock culture
c) Preparation of inoculum

Stage 3

Analysis of microalgae based on
a) growth parameters: OD_{620nm}, cell count, chlorophyll-a content, carotenoid content, specific growth rate, pH and biomass production

b) biochemical composition: protein, carbohydrate, lipid and distribution of total fatty acids

Selection of microalgae based on biomass, lipids and specific growth rate

Stage 4

a) Growth of selected microalgae at different levels of carbon dioxide (0.04%, 5% and 10%)

b) Analysis of microalgae based on
i) growth parameters: optical density OD_{620nm}, cell count, chlorophyll-a content, carotenoid content, specific growth rate, pH, amount of dissolves carbon dioxide and biomass production

ii) biochemical composition: protein, carbohydrate, lipid and fatty acids composition

↓

Stage 5

a) Data analysis
b) Discussion and answer to the research questions

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION OF ALGAE

Algae are known as the most primitive and fast-growing plants in worldwide and they are an important source of useful biomass. Algae do not have morphological features such as roots, stems and non-sterile covering of reproductive cells. Algae have chlorophyll-a as the primary photosynthetic pigment for them to convert light energy into chemical energy by the process of photosynthesis (Brennan and Owende 2010). According to Kaur and Bathnagar (2002), algae have the capability of trapping energy from sunlight ten times higher than the terrestrial plants. However, some of the species are not able to photosynthesis but live in a parasitic form. Algae are found in a wide range of habitats including marine and freshwaters, deserts and even in snow and ice.

Due to its ability to inhabit different environments, they have produced a wide array of bioactive compounds. Based on the systemic classification, this specialized group of species can be divided into the following nine major groups: Chlorophyceae (green Algae), Phaeophyceae (brown algae), Pyrrophyceae (dinoflagellates), Rhodophyceae (red algae) and Chrysophyceae (yellow-green algae) (Irina and John, 2006). Generally, algae can be divided into two major groups known as microalgae and macroalgae or seaweed. It is reported that there are about 8000 species of green microalgae, from both marine and fresh water system (Parker, 2009).

2.2 MACROALGAE

Generally, the multicellular marine alga or marine plant is well defined as seaweed that only habitats marine waters. Seaweeds are multicellular macroalgae that are non-vascular photosynthetic organisms. The main habitat of seaweeds is coastal region of oceans especially within rocky intertidal and reef like habitats. They like to attach to the rock on the bottom of the ocean (Demirbas and Fatih, 2011; Rorrer and Cheney, 2004).

Macroalgae can be divided into three major groups that including Heterokontophyta (brown algae), Rhodophyta (red algae) and Chlorophyta (green algae) based on their pigmentation. The habitat of seaweeds may include extreme environments whereby they have to compete for light, nutrients and space with each other and other marine species. Due to this adverse condition, the seaweeds develop various chemical defense mechanisms for them to survive among their competitors and the predators (Rorrer and Cheney, 2004). According to Fuller et al, (1992), the bioactive chemicals or compounds that are released by the seaweeds for their defense have large pharmacological significance. For example, the red alga *Portieria hornemannii* contain halomon which possesses anti tumor activity.

Besides that, macroalgae have been widely used in bioremediation especially in waste water treatment that are economically viable. Macroalgae culture has been proposed as an alternative for waste treatment in open water systems with studies conducted in countries like United States, China and Japan (Zhou et al., 2006).

Apart from that, the cell wall of seaweeds consists of high amounts of phycocolloids, type of polysaccharides that provide antioxidant, antitumor and anticoagulant activities. It is also known that the polysaccharides like agar, carrageenan and alginate are commercially used in food and cosmetics industries (Cardozo et al., 2007).

According to Escrig and Muniz (2000), brown algae and red algae have been widely used in the food industry because they are highly capable of producing dietary fiber such as laminarans, cellulose, fucan and alginates.

2.2 MICROALGAE

Microalgae are known generally as the unicellular photosynthetic organisms that can absorb solar energy from sunlight, converting carbon dioxide and water into algal biomass and other metabolites (Figure 2.1) (Hemaiswarya et al., 2011). It is estimated that over 40,000 species of microalgae have been identified and yet to be identified (Hu et al., 2008). Another study stated that about 200,000-800,000 species exist of which about 35,000 species are described (Tabatabaei et al., 2011). This wide array of specialized species inhabits diverse ecological habitats such as freshwater, brackish, marine and saline environments. They are good in adapting with a range of temperatures and pH, and unique nutrient availabilities (Hu et al., 2008).

There are four main classes of microalgae namely diatoms (Bacillariophyta), green algae (Chlorophyta), blue green algae and golden algae(Chrysophyta). These organisms can be divided into two main groups which are the benthic filamentous species and the phytoplankton. The habitat of microalgae is both freshwater and marine water systems (Demirbas and Fatih, 2011). The size of the microalgae is known to be less than 20µm (Khoo et al., 2011). Many marine microalgae have the ability to remove metals such as cadmium and are widely used in bioremediation of carbon dioxide (Matsunaga et al., 1999). Microalgae are highly capable of generating biomass that only uses carbon dioxide as a carbon source and convert it into the valuable biochemical known as lipids or natural oil (Widjaja, 2009).

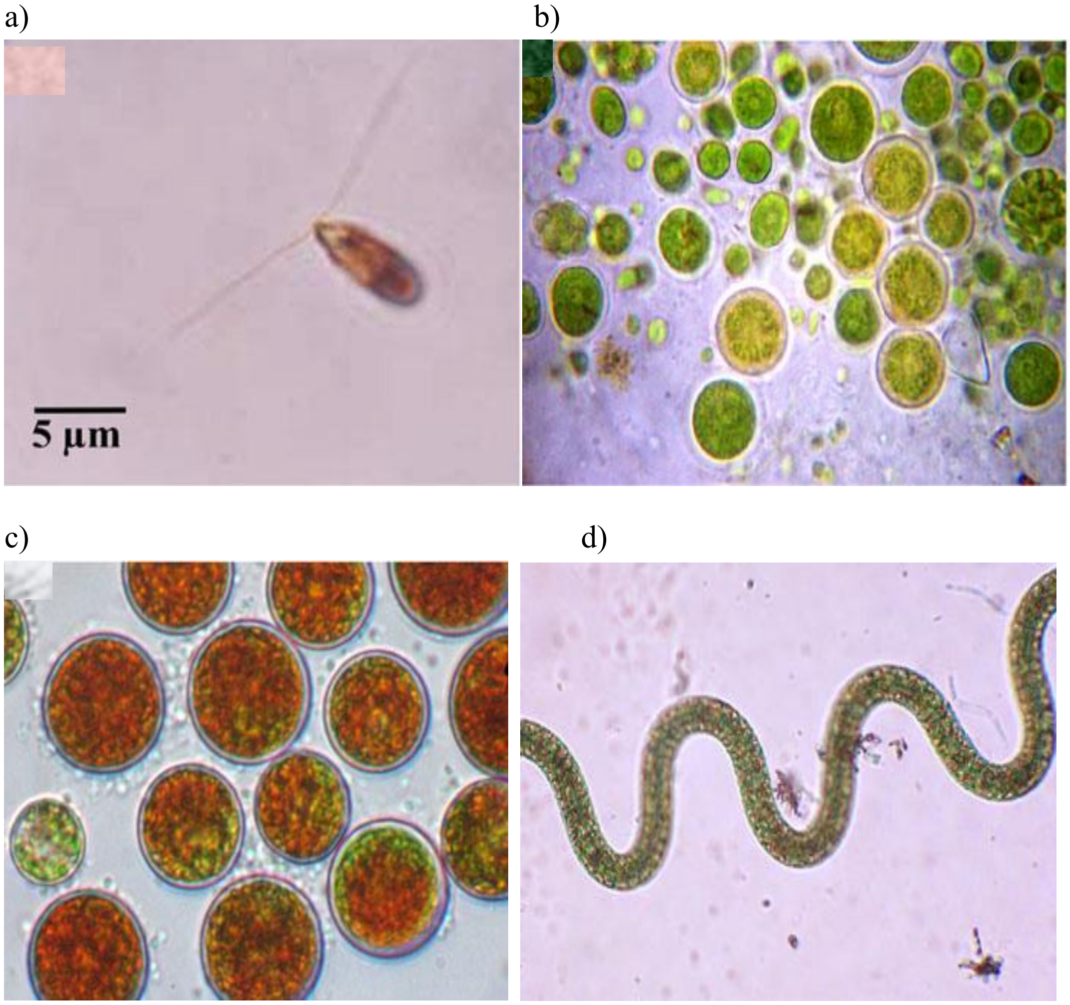


Figure 2.1: Examples of microalgae a) *Dunaliella* b) *Chlorella*
c) *Haematococcus* d) *Spirullina*

(Source: Hemaiswarya et al., 2011)

Chlorella is a common microalga that is widely used for many purposes. *Chlorella* is a single eukaryotic cell that exhibits well defined nucleus structure as in Figure 2.1 (b). *Chlorella* is a spherical and non-motile green alga that has the size within the range of 2.0–10.0 µm in diameter. *Chlorella* inhabits both fresh and marine water systems. Some species of *Chlorella* can be found in moist soil, surface of tree trunks, water pots and damp walls while others live symbiotically with other organisms. *Chlorella parasitica* is one of the species that lives symbiotically in the cells of *Paramecium* and *Hydra* (Phukan et al., 2011).

Besides that, *Scenedesmus* is another microalgae that been studied for its commercial and medical purposes. It is a ubiquitous organism that can be found in freshwater lakes and rivers. It consists of more than 200 species and almost 1200 strains have been identified. (Kim et al., 2007).

2.3 GROWTH PHASE OF MICROALGAE

As microalgae are microscopic, they possess the type of cell cycle as the other microbial cells. The growth cycle of microalgae has four different phases including lag phase, exponential phase, stationary phase and death phase (Figure 2.2) in a batch culture system. The time required for each phase would depend on the initial cell concentration, concentration and constituents of the nutrient medium, pH, temperature and other factors.

During the lag phase which is the initial phase of the cell cycle, the cells begin to adapt to the environment or the culture condition and there is no cell production. The second phase is the logarithmic or exponential phase where cells rapidly reproduce giving an increase the number of cells. This leads to an increase in the biomass concentration. The gradient of the exponential phase is used to calculate the specific growth rate (μ) of the microorganism (Najafpour, 2007).

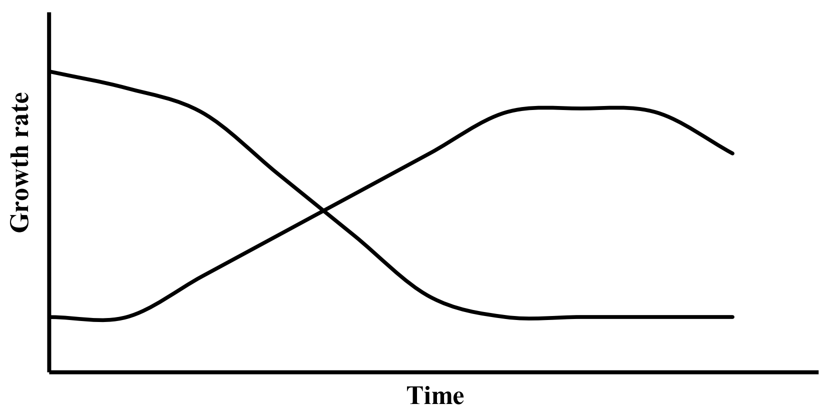


Figure 2.2: Growth phase and nutrient concentration of algae in batch culture (Source: Mata et al., 2010)

The next phase is the stationary phase, where growth rate remains constant. According to Espinosa et al, (2008), the production of secondary metabolites like fatty acids, carbohydrates and pigments of microalgae is highest at stationary phase. Lastly, the cell enters the death phase that causes a decline in the growth curve, due to the depletion of nutrients, light limitation due to auto shading and presence of inhibitory organic compounds secreted by aging cells (Yates and Smotzer, 2007).

2.4 BIOCHEMICAL COMPOSITION OF MICROALGAE

Microalgae are rich in nutritional compounds that have attracted attention for commercial applications. Microalgae are generally easy to culture, requiring only sunlight and carbon dioxide to produce chemical components. Microalgae produces three main organic substances namely lipid, protein and carbohydrate. Other high value products are chlorophyll, carotenoids, vitamins and other compounds (Figure 2.3) (Singh and Gu, 2010; Zepka et al., 2008; Otles and Pire, 2001).

The biochemical composition of microalgae varies between species (Table 2.1) and according to culture conditions (Harun et al., 2010; Um and Kim, 2009; Fernandez et al., 2006). It may depend on the growth phase, culture medium composition and abiotic factors including irradiance and temperature. The main biochemical components in microalgae are protein content (30-40% of total dry weight), lipid (10-20% of total dry weight) and carbohydrate content (5-15% of total dry weight). Under extreme growth conditions, they are highly capable of producing valuable lipid compounds such as wax esters, sterols and hydrocarbons, as well as prenyl derivatives such as tocopherols, carotenoids, terpenes, quinines (Hu et al., 2008).

The high protein content attained by microalgal species is one of the main reasons to consider them as alternative sources of protein for consumption. Microalgae are capable of producing a wide range of amino acids that are beneficial to human and animals.

Besides, the carbohydrate production in microalgae can be obtained in the form of starch, glucose, sugars and other polysaccharides. The lipid in microalgae is made of glycerol, sugars or bases esterified to saturated or unsaturated fatty acids (Spolaore et al., 2006). Microalgae consume organic and inorganic sources for the accumulation of lipid in their cell wall that can be different from one species to another. Many microalgae are capable of producing lipid within the range of 30 to 60% of the dry cell weight. During limited nitrogen condition, the cellular content in the thylakoid declines, activates acyl hydrolase and stimulates the hydrolysis of phospholipids. Nitrogen limitation causes diacylglycerol acyltransferase to be activated and converts acyl-CoA to triglyceride and hence increases the lipid productivity (Xin et al., 2010). Besides, microalgae are rich of polyunsaturated fatty acids such as eicosa pentaenoic acid and docosahexaenoic acid that can be used for various purposes.

Two decades ago, *Chlorella, Spirulina* and *Dunalliela* were the only genera that were utilized for their nutritional value. They have high protein content which is about 50-60% of the total dry weight and various amino acids, essential fatty acids, vitamins and pigments. Since then, the demand for the quality nutrition in microalgae increased compared to the conventional nutrient sources (Zepka et al., 2008). There were 46 large scale factories in Asia that produced more than 1000 kg of microalgae for nutritional purposes by the year 1986. The commercial production of *Dunaliella salina* as a source of β-carotene became the third major microalgal industry that was established by Western Biotechnology (Spolaore et al., 2006).

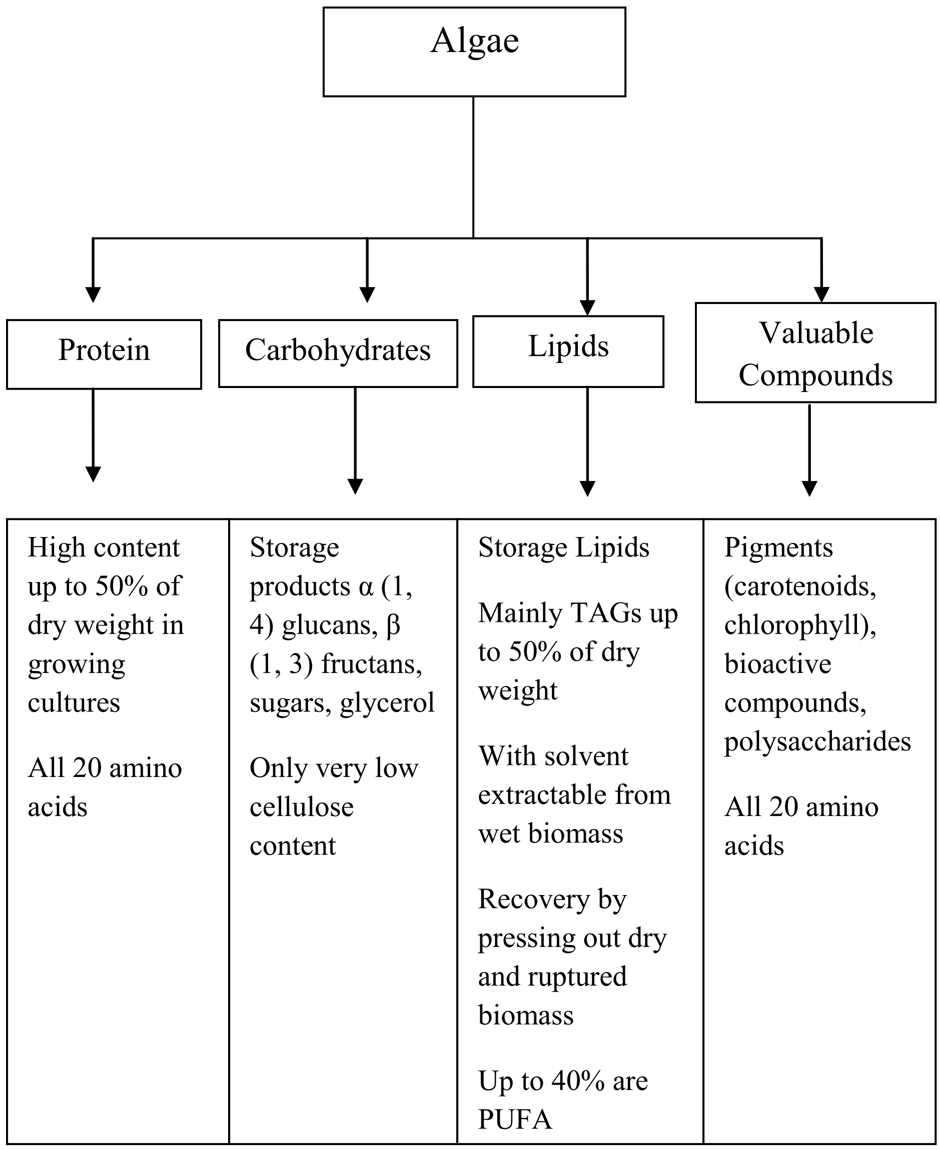


Figure 2.3: Biochemical components of typical microalgae
(Source: Singh and Gu, 2010)

Table 2.1: Biochemical composition of various microalgae on a % dry matter basis

Species	Protein	Carbohydrate	Lipid	Nucleic Acid
Scenedesmus obliquus	50–56	10–17	12-14	3-6
Scenedesmus quadricauda	47	-	1.9	-
Scenedesmus dimorphus	8–18	21–52	16-40	-
Chlamydomonas rheinhardii	48	17	21	-
Chlorella vulgaris	51–58	12–17	14-22	4-5
Chlorella pyrenoidosa	57	26	2	-
Spirogyra	6–20	33–64	11-21	-
Dunaliella bioculata	49	4	8	-
Dunaliella salina	57	32	6	-
Euglena gracilis	39–61	14–18	14-20	-
Prymnesium parvum	28–45	25–33	22-39	1-2
Tetraselmis maculata	52	15	3	-
Porphyridium cruentum	28–39	40–57	9-14	-
Spirulina platensis	46–63	8–14	4-9	2-5
Spirulina maxima	60–71	13–16	6-7	3-4.5
Synecoccus	63	15	11	5
Anabaena cylindrica	43–56	25–30	4-7	-

(Source: Um and Kim, 2009; Harun et al., 2010)

2.5 APPLICATIONS OF MICROALGAE

Generally, microalgae produce a wide range of high value compounds that are important in nutrition, pharmacology and medicine. The compounds include vitamin C, biotin, metabolites that consist of pharmacological values such as anticholesterolemic, antitumor, immunomodulatory, antibacterial and antimycotic (Converti et al., 2009). Among the factors influencing promotes to the nutritional value of microalgae are size and shape, digestibility, biochemical composition, enzymes, toxins and the requirements of animal feeding on the alga (Hemaiswarya et al., 2011).

Since microalgae have the ability to grow under extreme conditions, they can be manipulated and can be stimulated to produce more biomass that can be utilized as human and animal supplements (Morais and Costa, 2007). It is proved that dried microalgae consist of high value of nutritious protein content that is safe for human consumption. Apart from this, the protein from microalgae have gained attention than the conventional protein sources to be added in food stuffs due to its high cost production (Becker, 2007). The dried algal biomass is sold in human health food market in the form of tablets and capsules for consumption (Demirbas and Fatih, 2011). Since 1960s, Japan was the first country to introduce the commercial application of microalgae for various purposes as the *Chlorella* was the first species been studied and applied (Varfolomeev and Wasserman, 2011).

Beside capsules, the microalgae are also found in the snack foods, pastas, candy bar or gums and beverages for the natural food colour. There are four main microalgae strains used for commercial purposes which include *Arthrospira*, *Chlorella*, *Dunaliella salina* and *Aphanizomenon flos-aquae*. *Arthrospira* contains high protein and other nutritive value that promotes the alleviation of hyperlipidemia, suppression of hypertension, protection against renal failure, promotes the growth of intestinal *Lactobacillus* and reduces of high serum glucose level. In addition, *Chlorella* consists of β-1, 3-glucan that acts as an immunostimulator and reduces lipids in blood. Other than that, this *Chlorella* also promotes healing wounds, prevention of atherosclerosis, hypercholesterolemia and antitumor activity. *Dunaliella salina* is exploited for its β-carotene content that can reach 14% of dry weight (Spolaore et al., 2006). It is used in baking industry and also the biomass used for animal and fish fodder (Varfolomeev and Wasserman, 2011). Apart from that, microalgae are rich in chlorophyll content that performs chelating agent activity and can be used in ointment, pharmaceutical purposes such as liver recovery and ulcer treatment, as well as for cell repair, increasing amount of hemoglobin in blood and promotes cell growth (Singh and Gu, 2010).

For animal feed, the microalgae are widely used for poultry animals, pets and aquaculture species. It has been reported that about 30% of the world algal production is used for animal feed purposes (Becker, 2007; Spolaore et al., 2006). *Hypnea cervicornis* and *Cryptonemia crenulata* are rich sources of protein that have been used in shrimp feed to improve the cell growth. It is also used as nutritional feed for tilapia to promote growth. Another microalga *Porphyridium* is used as high nutritional feed for the metabolism of chicken, known to reduce the cholesterol level of egg yolk by 10% (Singh and Gu, 2010). In addition, the higher PUFA content in microalgae such as eicosapentaenoic acid, arachidonic acid and docosahexaenoic acid enhances the quality of the animal feed. *Nannochloropsis* species are widely used as a food in aquaculture and have been identified for the commercial production of eicosapentaenoic acid (Hemaiswarya et al., 2011).

Furthermore, microalgae are used as natural dyes that can be used in food industry, pharmaceutical industry, cosmetics industry, and textile industry. The dyes from microalgae are non toxic and renewable. Phycocyanin from microalgae is widely used in the food industry especially in ice cream, candies, nonalcoholic beverages, dietary foodstuffs, cosmetics industry, and pharmaceutical industry. Microalgae are also exploited for cosmetic purposes such as regenerating creams for face and lotions, sun protection creams, shampoos and hair masks. The extract from *Chlorella vulgaris* was found to stimulate the synthesis of skin collagen and can help to regenerate fibers and wrinkle smoothing. Another microalgae species *Arthrospira* that is rich in protein is used to reduce skin senescence (Varfolomeev and Wasserman, 2011).

Nowadays, environmental issues have become one of the most threatening to the public. The release of industrial and municipal wastewaters to the environment causes the most pollution. This phenomenon threatens the public especially the people living in rural areas. Basically, the wastewater consists of large amount of waste materials such as nitrogen, phosphorus and metals that can lead to eutrophication. Algal cells become the primary choice in treating wastewater by the process of bioremediation. All these materials can be utilized as nutrient sources for growth of the algal cells which can then treat the wastewater (Brennan and Owende, 2010).

Since this approach has mutual benefits, algal cells become the choice for bioremediation. It is known that *Scenedesmus*, *Phormidium*, *Botryococcus*, *Chlorella vulgaris*, *Chlamydomonas* and *Spirulina* are the most widely used for the treatment of wastewater (Brennan and Owende, 2010; Rawat et al., 2010). Among these species, *Chlorella vulgaris* has been reported as one of the highly potential strain for wastewater treatment. A study showed that *Chlorella vulgaris* has the ability to remove nitrogen in the form of ammonium ion and ammonia (Kim et al., 2007). Another study proved that *Chlorella vulgaris* are highly capable of removing colour or textile dye from textile wastewater about 60-69% (Lim et al., 2010). Besides that, *Ankistrodesmus braunii* and *Scenedesmus quadricauda* have been studied for their ability to remove phenol from olive oil mill wastewater. It was proved that 50% of phenol that can cause phytotoxic effects can be removed (Pinto et al., 2003).

The liquid sludge waste in the production of crude palm oil is known as palm oil mill effluent (POME). It is rich in organic matter and highly polluting. Microalgae culture is able to reduce the environmental pollution from POME as the microalgae have a role in self purification of natural waters. Microalgae are capable of reducing the inorganic components like nitrates and orthophosphate where it can utilize the components for their growth. For example, *Isochrysis* culture reduced 46% of nitrate and 83% of orthophosphate from the POME (Vairappan and Yen, 2008). A recent study stated that *Scenedesmus obliquus* was used to treat brewery wastewater to produce biomass. Generally, this species is versatile and effective in removing contaminants from wastewater where it can utilize the contaminants as nutrients for their growth (Mata et al., 2011).

Industrial wastes that consist of large amounts of metals including cadmium, cuprum, chromium and plumbum can cause serious environmental pollution. Microalgae have the ability to detoxify the polluting metals by the process of biosorption, adsorption and bioaccumulation. For example, *Chlorella* is widely used as metal resistant microalgae in industrial effluent as it can remove toxic metals such as chromium. *Spirulina platensis* can remove cadmium by adsorption (Rehman et al., 2007). Another study reported that *Botryococcus braunii* was used to remove nitrate and phosphate from sewage and produced compounds rich in hydrocarbons. *Scenedesmus obliquus* is capable of removing 98% of phosphorus and 100% of ammonium from urban wastewater in stirred cultures (Brennan and Owende, 2010).

This indicates that microalgae may be widely used for the removal of residual nutrients, toxic metals and other contaminants in wastewater as it is a potential source and contribute to the bioremediation purposes.

2.6 MITIGATION OF CARBON DIOXIDE BY MICROALGAE

Excessive emission of carbon dioxide has become the main threatening issue in the world leading to greenhouse effects. It is vital to reduce the level of carbon dioxide in the atmosphere (Mata et al., 2010). Based on the previous studies, mitigation of carbon dioxide can be achieved in two methods namely chemical and biological methods. The chemical approach seems to be less effective as it consumes more energy and high cost. Therefore, biological approach that uses microalgae has become the promising substitute to overcome this problem. This approach attracted much attention as it is environmental friendly and economically viable (Mata et al., 2010; Eduardo et al., 2010).

Generally, microalgae utilize the same type of resources such as water, sunlight, nutrients and carbon dioxide as the terrestrial plants. However, they can use up the resources more efficiently especially in capturing more carbon dioxide from atmosphere (Parker, 2009). Microalgae are known as the best candidate for the mitigation of carbon dioxide by means of microalgal photosynthesis. It has been proved that plants have the ability of capturing carbon dioxide about 3-6% of fossil fuels while microalgae are capable of capturing about 10-50 times for the process of photosynthesis (Chinnasamy et al., 2009). Microalgae that have high photosynthesis capacity can convert the inorganic carbon sources into potential biofuels, food, feed and high value bioactive compounds by autotrophic cultivation (Chisti, 2007). There are two main biological systems whereby the carbon dioxide can be reduced. They are including atmospheric carbon dioxide at very low concentration and higher concentration of discharged gases from heavy industries (Kurano et al., 1995). As a consequence of carbon fixation, the microalgae are able to produce other elements such as carbohydrates, proteins, lipids, chemicals and food from the algal biomass (Sung et al., 1999).

Recently, carbon dioxide is the main greenhouse gas that increased drastically due to human activities and industrialization threatening global warming issue. Currently, the flux of carbon dioxide out of fossil fuels is about 600 times greater than that into fossil fuels. There are four major atmospheric reservoirs for carbon dioxide which includes vegetation and soil, fossil fuels, oceans, and ocean sediments (Keffer and Kleinheinz, 2002).The rising of global temperature not only leads to green house effects but also increases the temperature of surface air and subsurface ocean.

Many approaches have been applied to control the level of increasing carbon dioxide in the atmosphere. The first approach is to increase the efficiency of energy conversion. The second approach is to use energy sources that are lower in carbon or are carbon free and the most reliable approach is the sequestering of carbon dioxide (Keffer and Kleinheinz, 2002). There are few techniques have been studied for carbon sequestering such as deep ocean injection or increasing the amount of dissolved carbon dioxide in the ocean. Besides that, reduction of carbon dioxide into more permanent geological formations has also been suggested but not tested. There are also few chemical approaches studied but complication arises with these approaches such as environmental safety, long term effect and not cost effective comparing with other techniques (Keffer and Kleinheinz, 2002).

Though many physical and chemical techniques been have applied for the sequestration of carbon dioxide, yet a biological approach becomes more effective as it is environmental friendly and cheap. Initially, terrestrial plants were used to mitigate the carbon dioxide level. Later, microalgae seem to be the better choice in order to reduce the increasing concentration of CO_2 in the atmosphere. It is because microalgae are able to withstand extreme condition and has efficient fixation of carbon dioxide through the process of photosynthesis (Chiu, 2009; Keffer and Kleinheinz, 2002).

Microalgae have high potency to reduce the emission of carbon dioxide by adsorbing carbon dioxide from combustion gaseous. The flue gas from thermal power station becomes one of the main sources of CO_2 to cultivate microalgae as the source is almost free (Sforza et al., 2011). Generally, flue gas contains high amount of carbon dioxide up to 10-20% and other gases such as sulfur compounds and nitrogen oxide that comes from burning process which can inhibit the growth of microalgae. However, if nitrogen oxide dissolves in water, it can form ions such as nitrate that act as nutrient source for microalgal growth. While the accumulation of sulphite in solution enables the cells to grow (Westerhoff et al., 2010).

2.6.1 Carbon Metabolism in Microalgae

There are three main cultivation modes for microalgae in utilization of carbon source namely autotrophic, heterotrophic and mixotrophic mode (Garcia et al., 2001). In autotrophic mode, microalgae utilize inorganic carbon as CO_2 as carbon source with the availability of light source. For heterotrophic condition, organic compounds are used for growth where the algal cells obtain energy from the organic carbon source. The basic culture medium composition for heterotrophic cultures is similar to the autotrophic culture with the addition of organic carbon source such as acetate and glucose. Some microalgae can possess mixotrophic condition that uses both CO_2 and organic carbon simultaneously for assimilation. The most common cultivation is autotrophic condition as microalgae are photosynthetic and many microalgae are especially efficient solar energy convertors. Besides, this method is inexpensive as it uses CO_2 as carbon source and can reduce the level of CO_2 in the atmosphere by fixation (Garcia et al., 2001).

Microalgae being single celled organisms that carry out autotrophy also undergo C3 carbon fixation just like other plants. The microalgae utilize carbon dioxide by means of passive transport and active transport through the plasma membrane. In passive transport, the CO_2 can directly enter the cells through plasma membrane and in active transport ATP is used to transport CO_2 in the form of ions known as dissolved inorganic carbon into the cell (Hende et al., 2012).

In aqueous condition, the carbon dioxide is present in the form of dissolved inorganic carbon (DIC) such as HCO^3-, CO_3^{2-} and the consumption of CO_2 has the ability to change the pH of the medium (Velea et al., 2009). As the carbon dioxide gas dissolves in water, it can dissociate into few species of inorganic carbon source such as aqueous CO_2 (CO2(aq)), carbonic HCO^3-, CO_3^{2-} and CO_2 that make up the total DIC that follows Henry's Law as follows:

$$CO_2\,(aq) + H_2O \leftrightharpoons H_2CO^3 \leftrightharpoons H+ + HCO^{-3} \leftrightharpoons 2H+ + CO_2^{-3}$$

The presence of carbon species alters the pH of the culture medium as the pH greatly influences the photosynthetic activity of microalgae where they can grow better at alkaline pH. The bicarbonate (HCO_3^-) and other carbon species cause the changes in pH that can increase the pH of the culture medium. Bicarbonate becomes the dominant species in aqueous medium with the pH between 6.35 to 10.32, the most common in microalgae cultures (Hende et al., 2012). All the carbon species diffuse very slowly and are converted by hydrating, dehydrating and proton reactions that alter the pH of the water environment. Yet it has high solubility in gas- liquid transfer compared to oxygen. (Buehner et al., 2009; Velea et al., 2009).

The main inorganic sources for photosynthesis by microalgae are in the form of HCO_3^- and CO_2. Rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase) is the first enzyme in the Calvin cycle that assimilates CO_2 by converting it into 3-phosphoglycerate (3-PGA). Microalgae have a low CO_2-binding capacity of rubisco and fix more oxygen by oxygenase activity of rubisco that depends on the CO_2:O_2 ratio. Therefore, elevated levels of CO_2 allow the microalgae to possess carbon concentrating mechanism (CCM). High levels of CO_2 activate CCM that increases carbon species surrounding rubisco. It allows rubisco to fix more carbon species that can increase the carboxylating activity and repress the oxygenating activity in CCM. This allows increased photosynthesis rate and photosynthetic electron transport between photosystem I and photosystem II (Figure 2.4). (Hende et al., 2012; Zeng et al., 2011; Yang and Gao, 2003; Buey and Orus, 2001). For microalgae that uses CCM, actively transport carbon species across the cell membrane. Changes in plant metabolism such as reduction in stomata activity, reduction in photorespiration and dark respiration and increase of the C/N ratio occur with increased CO_2 (Papazi et al., 2008). For some microalgae, they can directly utilize the CO_2 as they do not use CCM and convert HCO_3^- to CO_2 using extracellular enzyme, carbonic anhydrase at cell surface. It allows the CO_2 enter the cells by active transport or diffusion (Hurd et al., 2009; Cuaresma et al., 2006).

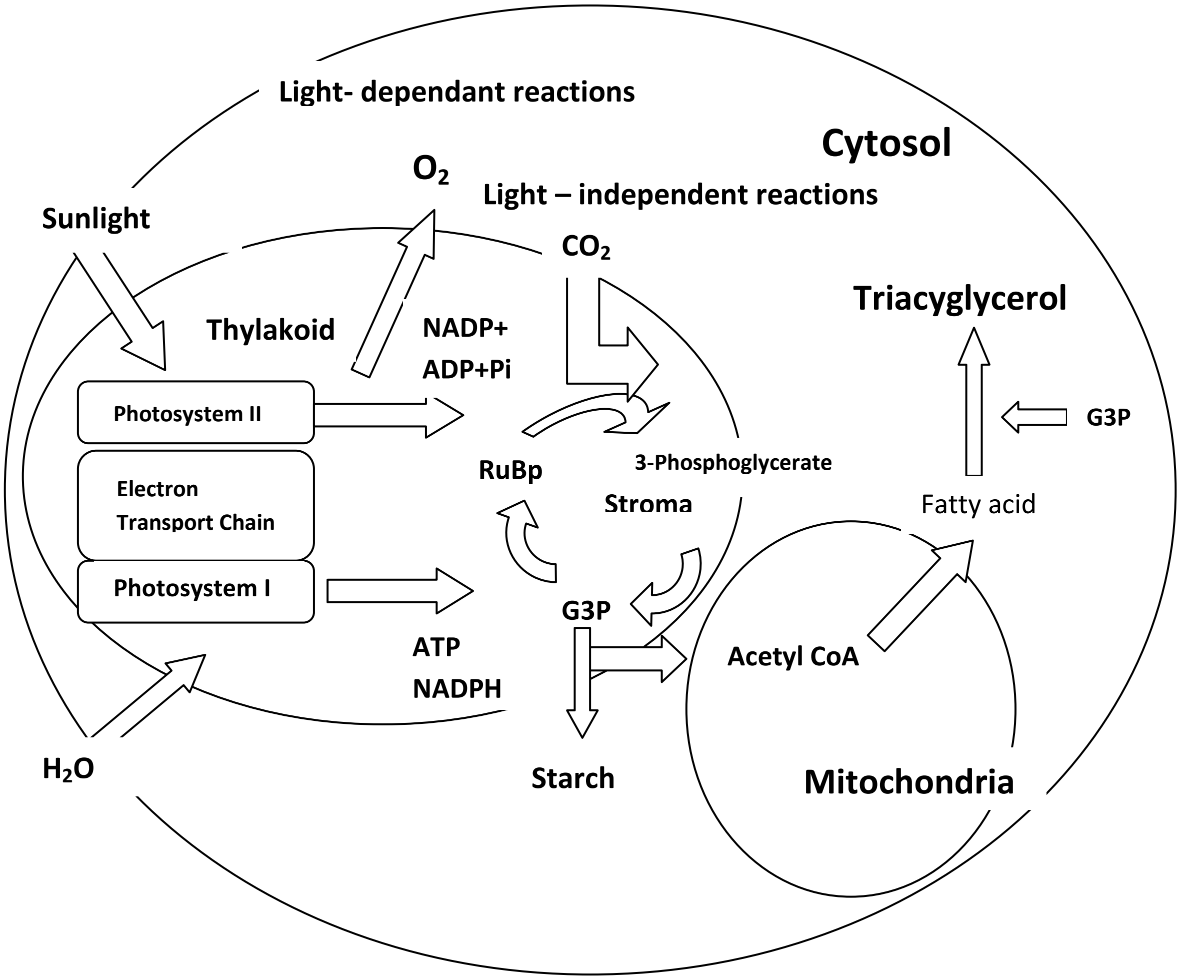


Figure 2.4: Schematics of photosynthesis, CO_2 fixation and carbon accumulation in microalgae cells

(source: Zeng et al., 2011)

Enriched carbon dioxide medium can enhance the productivity of the microalgae for great biomass production. Freshwater species that grown at high carbon dioxide concentration can provide better recovery from photoinhibition. Low level CO_2 (ambient condition) which is below critical concentration can cause limited algal growth in terms of cell size and growth rate (Yang and Gao, 2003). Fast growing species requires higher carbon concentration and they are sensitive to lower levels of CO_2 compared to slow growing species. Of three species grown under different CO_2 levels, the shape of cells is circular in *Chlamydomonas reinhardtii* and *Chlorella pyrenoidosa* at high CO_2 level but spindly in *Scenedesmus obliquus* at low CO_2 level proving that the requirement of carbon varies from species to species. Whereas, for marine phytoplankton, the enrichment of carbon dioxide that results in pH decline can increase primary production (Yang and Gao, 2003).

According to Nedbal et al, (2010), the utilization of carbon species from the medium for growth, is species dependent. Some microalgal strains uptake carbon dioxide from the medium and then release the carbonic acid while some other strains take up carbonic acid and release carbon dioxide.

2.6.2 Tolerance of Microalgae to Elevated Levels of Carbon Dioxide

Generally, both freshwater and marine microalgae may be used to grow on both flue gas and other concentrated carbon dioxide streams. Both the microalgae are of interest since freshwater species could sequester carbon dioxide from power stations located inland whereas marine species can mitigate carbon dioxide from power stations located along the sea-shore (Bilanovic et al., 2009). In the applications of microalgae for removal of carbon dioxide and energy production, the tolerance of microalgal species to the carbon dioxide places a great importance.

Several microalgae have been studied for the mitigation of carbon dioxide due to tolerance to extreme conditions. *Chlorella vulgaris* has been studied extensively due to its ability to mitigate high levels of carbon dioxide and its higher efficiency in utilizing CO_2 through photosynthesis compared to terrestrial plants. It has been proved that *Chlorella vulgaris* has the ability to grow in extreme carbon dioxide levels up to 40% (Sasi, 2009). A study showed the growth of *Spirulina* LEB18 and *Chlorella kessleri* that were exposed to controlled and non-controlled conditions by injecting different concentrations of carbon dioxide ranging from 0.03% -18% (v/v) into the cultures. The *Spirulina* LEB18 achieved maximum fixation and biomass production at 6% CO_2 (v/v) while *Chlorella kessleri* showed maximum growth rate at 18% CO_2 (v/v) (Rosa et al., 2011).

Other species like *Nannochloris oculata* can grow well between 5-15% CO_2 (Chiu et al., 2009) and *Chlorococcum littorale* showed an exceptional tolerance up to 40% (v/v) (Zeng et al., 2011). It has also been reported that *Scenedesmus obliquus* and *Spirulina* attained good capacities for the fixation of CO_2 when cultivated at 30◦C in a temperature-controlled three-stage serial tubular photobioreactor. *Spirulina* recorded maximum specific rate and maximum productivity at 6% and 12% CO_2 (Morais and Costa, 2007). Another study stated that *Botryococcus braunii*, *Chlorella vulgaris*, and *Scenedesmus* were cultivated with 10% CO_2 and flue gas for high biomass and lipid productivity. Both the strains grew well in 10% CO_2 and *Scenedesmus* produced high lipid content, showing that it is a good strain to mitigate elevated levels of carbon dioxide (Yoo et al., 2010).

2.7 BIOMASS AS A RENEWABLE ENERGY SOURCE

Energy source is the primary aspect of all living things. Generally the energy resources can be divided into renewable and non-renewable sources. It is known that fossil fuels such as petroleum, coal and natural gaseous are the form of non renewable energy resources. Fossil fuels bring about 80% of energy where it emits 98% of carbon dioxide to the atmosphere from combustion. Hence, it leads to global warming, climate change and acid rain that becomes the major problems worldwide today. According to Ong et al., (2011), it is reported that the rate of world carbon dioxide emissions has increased dramatically from 19380 million tons in 1980 to 31577 million tons in the year of 2008. Beside large amount of carbon dioxide emission, petroleum diesel is also a causative agent for the contamination of other gaseous such as nitrogen oxide (NOx), sulphur oxide (SOx), carbon monoxide (COx), particulate matters and volatile compounds.

Other than that, uneven distribution of petroleum resources also makes the energy system less sustainable. Therefore, renewable energy resources have emerged as an alternative to this depleting energy source, which also can reduce the emission of green house gases and other pollutants. Renewable energy resources are including hydro, wind, solar, geothermal, biomass and marine energy sources that supply as energy worldwide. (Demirbas and Fatih, 2011; Campbell, 2008; Hossain et al., 2008).

The present rapid and advanced growth of global economy has lead to increase in population size and living standard. This situation has created great complications and higher demand on energy resources (Zhou et al., 2011). In order to fulfill the needs, non-renewable energy resources play a vital role for sustainable energy supply for now and also for the future. Renewable energy source has overcome the non renewable sources because it is clean, environmentally friendly and reduces pollution (Demirbas and Fatih, 2011).

Recently, biomass which is also known as carbon source becomes the most promising renewable energy source that has captured the attention of people because it can be directly converted into liquid fuel (Yanli et al., 2010). According to Chen et al (2009), biomass has become the third largest energy source in the world that constitutes 14% of world yearly energy utilization. The availability of advanced technologies enhances the production of biomass that can be used for various purposes. The production of biomass requires carbon dioxide, water, sunlight and other nutrients by the process of photosynthesis. Biomass is highly potential energy source that can reduce the carbon dioxide emission in the atmosphere by the process of carbon fixation. In addition to that, it also contains less sulfur and ash but can produce more hydrogen than coal. Energy obtained from biomass can be converted through direct combustion, biochemical conversion and thermo chemical conversion to fuel (Figure 2.5) (Tsukahara and Sawayama, 2005).

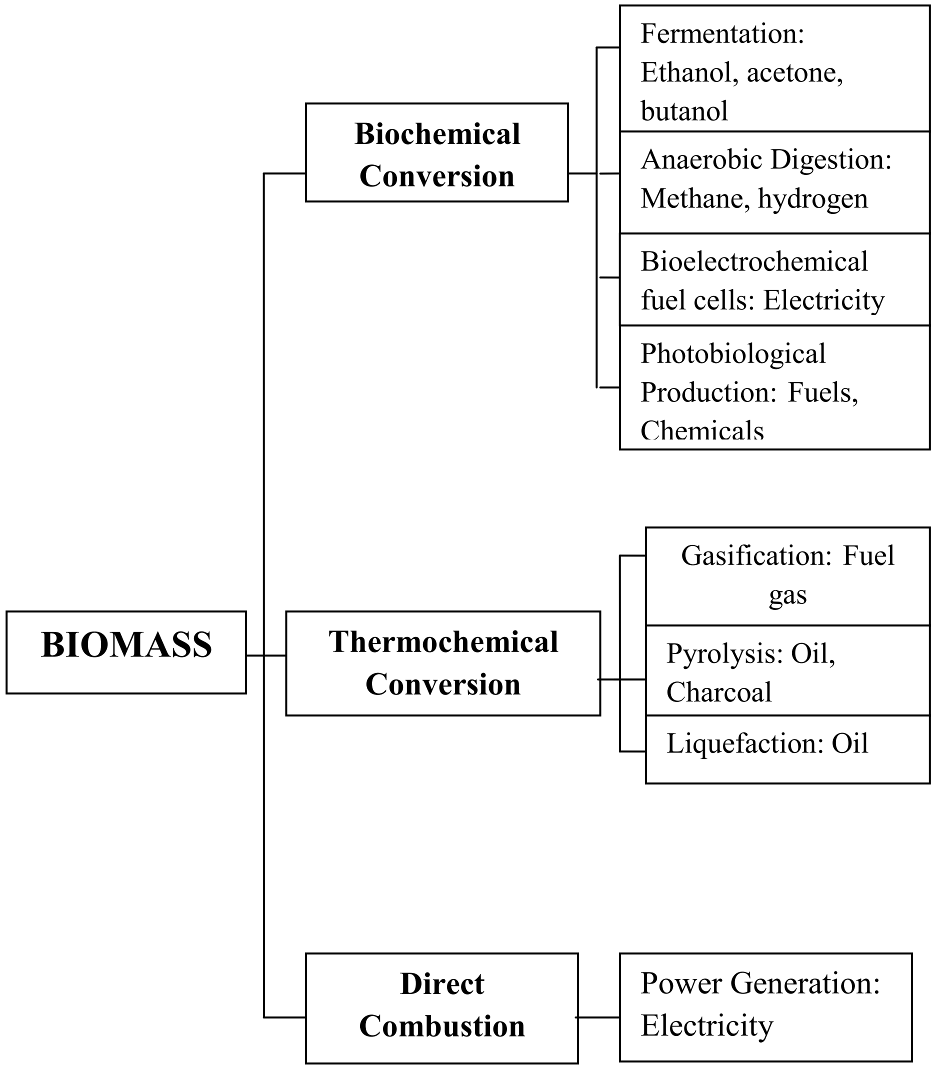


Figure 2.5: Energy Conversion processes from biomass
Source: (Tsukahara and Sawayama, 2005)

Biomass can be obtained from various sources such as agriculture waste, wood residues, organic wastes, animals manure, biomaterials and energy crops. In Malaysia, the main source of biomass is from the oil palm residues, as palm oil is the main agricultural product (Abnisa et al., 2011). The major components in biomass are cellulose, hemicelluloses, lignin, lipid, protein, sugar, starch and other compounds. The biomass energy is derived from the light energy that is accumulated in wastes, animal and plant residues. Direct combustion and thermo chemical conversion are the techniques most widely applied currently in which combustion accounts for about 97% of world energy production. Since the biomass is require in large amounts to generate energy, various techniques and developments been carried out in order to increase the production. Biomass is believed to be a great potential source of sustainable energy that can largely contribute to the global economy besides playing a vital role in the reduction of green house gaseous and other pollutants (Demirbas, 2005). It accomplishes the requirement for energy needs by providing clean and safe heat, power and transportation fuels for global energy consumption (Phukan et al., 2011).

2.8 EMERGENCE OF BIODIESEL

Biofuel is found to be the alternative energy source for the depleting non-renewable energy source that lead to an energy crisis and the demand for biofuel has risen greatly. The petroleum resource is slowly depleting leading to increase in price of the energy fuel. On the other hand, the more threatening issue is the global warming which is associated with burning of fossil fuels. In order to curb this problem, biodiesel becomes the most appropriate choice and the technology for biodiesel production has been established more than 50 years ago (Chisti, 2007). By using biodiesel as energy source, the release of sulfur content can be reduced to 30% and carbon monoxide to 10 %, in the environment. It is also known as environmentally friendly because there is no production of any chemical or aromatic substances from biodiesel (Huang et al., 2010). According to Miao and Wu (2006), biodiesel is known to be non toxic, renewable and biodegradable that gives rise to less emission of polluting green house gaseous such as carbon dioxide and sulfur dioxide to the environment than the conventional petroleum diesel.

Generally, biofuel is made of monoalkyl esters that can be obtained from organic oils, plant or animal, through the process of transesterification as shown below:

Triglyceride + 3 Methanol → Glycerine + 3 Methyl Esters (Biodiesel)

Based on the above equation, triglyceride is energy stored in plants and animals that can be esterified into glycerine and methyl esters which is then converted into biofuel. The large scale feedstock for biofuel production includes seed oils, such as soybean, rapeseed, palm, and corn oils as described in (Table 2.2) (Campbell, 2008).

The process of transesterification has the ability to reduce the viscosity of the vegetable oils or animal fats because high viscosity can cause some operational difficulty in diesel engine (Knothe, 2010). In order to extract biodiesel from plants, several techniques been applied such as direct use and blending, microemulsion, thermal cracking and transesterification (Miao and Wu, 2006).

Currently, microalgae have attained much attention as the most promising source of biodiesel as a replacement for fast depleting non-renewable resources such as fossil fuels worldwide. Even the large scale production of biodiesel from oil crops and bioethanol from sugar cane are found to be less sustaining in comparison with microalgae. The highly efficient photosynthetic microscopic algae are known to be the most efficient biological producer of oil and biomass source compared to other feedstocks as shown in Table 2.2 (Moazami et al., 2011; Phukan et al., 2011; Chisti, 2008). Microalgae are capable of producing valuable renewable energy sources such as oil, methane from anaerobic digestion of algal biomass and biohydrogen (Chisti, 2007). Besides, the biodiesel produced from microalgae is proven to be non-toxic and highly appropriate in transporting in adverse environment. It is also proven to be less polluting where no extra carbon dioxide and sulfur is emitted to the environment compared to petroleum diesel (Fulke et al., 2010).

Table 2.2: Comparison of some sources of biodiesel

Feedstock	Oil yield (kg ha^{-1} $year^{-1}$)	Conversion (%)	Biodiesel yield (kg ha^{-1} $year^{-1}$)
Soybean	375	95	356
Rapeseed	1000	95	950
Jatropha	2000	98	1960
Palm Oil	5000	94	4700
Microalgae (50 wt % oil in biomass)	75,000	80	60,000

Source: (Moazami et al., 2011)

Microalgae are good feedstocks for biodiesel as they produce high biomass production. The simpler cell structure of microalgae enables it to give rise to higher rate biomass and oil production which is about 15 to 20 times higher oil yield per hectare than conventional crops. Besides that, it does not require any food source and land space for cultivation as it can also grow in salty and waste water systems that are unsuitable for conventional food crops. Since they can grow in extreme condition, they are highly capable of synthesizing valuable co products like metabolites efficiently depending on type of species. Another advantage of microalgae is that lipid can be manipulated by the alteration of growth medium and can be harvested by batch system throughout the year. In addition to that, it can produce biofuel while involved in bioremediation and mitigate carbon dioxide from the atmosphere (Phukan et al., 2011; Chisti, 2008; Miao and Wu, 2006).

Microalgae have the ability to produce more than 50% lipid content which is the higher yield at higher density biomass than other crops (Table 2.3). This proves that microalgae are a desired source for the large scale production of oil and feedstock (Campbell, 2008).

Table 2.3: Comparison of the oil content in green algae

Microalgae	Oil content (% dry weight)
Chlorella	28-32
Botyrococcus braunii	25-75
Nitzschia	45-75
Nannochloropsis	31-68
Schizochytrium	50-77
Crypthecodinium cohnii	20
Dunaliella primolecta	23
Isochyysis	25-33
Monallanthus salina	>20
Nannocholorolis	45-47
Phaeodactylum tricornutum	20-30
Tetraselmis sueica	15-23

Source: (Chisti, 2007)

According to Demirbas and Fatih (2011), it is known that oil that extracted from microalgae has similar properties of standard biodiesel required by the American Society for Testing and Materials (ASTM). There are few techniques including pyrolysis, thermomechanical liquefaction, gasification and direct combustion that have been applied for the production of biofuel from the microalgae. To date, pyrolysis is the most promising method of to extract biofuel compounds from microalgae as it can allows the production of high aromatic hydrocarbons and octane number (Varfolomeev and Wasserman, 2011).

Other renewable biofuels that can be produced from microalgae include methane and biohydrogen (Chisti, 2007). The production of biohydrogen was first studied in *Scenedesmus obliquus* and eventually many other microalgae have been exploited for their advantages. Due to the physio chemical properties of microalgae culture, the hydrogen is converted into gas phase quickly and does not accumulate in biomass as in the terrestrial plants (Varfolomeev and Wasserman, 2011). Bioethanol from microalgae is produced through biochemical process that involves fermentation and thermochemical process by gasification. Microalgae provide carbohydrates and proteins that can be used as carbon sources for fermentation to produce ethanol under anaerobic condition (Harun et al., 2010).

Since the microalgae appear to be a reliable and continuous source biofuel and energy, much research is now directed to further develop microalgae as high quality feedstock for biodiesel production, so as to improve the production of an economically and environmentally sustainable biofuel.

2.9 MASS CULTIVATION AND TECHNOLOGY OF MICROALGAE UTILIZATION FOR BIOFUEL PRODUCTION

The production and the nutritive value of microalgae can be enhanced through the application of biotechnology. Since the cost of biofuel production from microalgal cells is high, it is important to develop the techniques in order to improve the quality of production, making it cost effective and economically viable.

The cultivation technique of microalgae is another approach to improve the productivity of the microalgae. Generally, large scale cultivation system holds the major place for efficient productivity. Open raceway ponds and closed photobioreactor are the two main systems for large cultivation. The open pond system been applied since 1950's in which this method is much cheaper and require less space (Rawat et al., 2010). Raceway pond is one of the good examples of this type of system that requires less energy input and ease of maintenance. Despite this, this method is less efficient in terms of biomass production in large scale. It is because of evaporation, limited light source, inappropriate mixing and changes in temperature and prone to contamination.

For the closed system, unlike open system, is less prone to contamination and able to cultivate single culture for a longer period of time. Since it has less risk of contamination, this approach can be used for the production of high value products. Yet the cost of maintenance is higher (Brennan and Owende, 2010). Normally, cultivation of algae in closed system is more applicable and reliable for commercial purposes that uses few types of reactors such as tubular, bubble, column and airlift photobioreactors (Celis et al., 2008).

Algae are one of the most promising organism for climate management as well as energy supply. Therefore, it is important to explore and develop the algal technology for the increasing demand of algal biomass that can be converted into biodiesel as the energy source for world consumption.

Genetic engineering of microalgae is one of the approaches that may allow enhanced productivity and yield. For an example, the oil content of *Botryococcus braunii* is about 75% (w/w DW) but it gives lower productivity due to lower growth rate. While the other species such as *Chlorella, Dunaliella, Isochrysis, Nannochloris, Nannochloropsis, Neochloris, Nitzschia, Phaeodactylum* and *Porphyridium* attained oil levels between 20% and 50% but give higher productivity. Therefore, genetic manipulation is vital for the microalgae to enhance the biofuel production with higher growth rate that can meet the global demand for bioenergy. The depletion of nitrogen coupled with RNAi suppression allows changes in the lipid and protein profiles of *Chlamydomonas reinhardtii* that increases the fatty acids by 2.4 fold within 72 hours (Amaro et al., 2011).

Besides biofuel, microalgae are genetically modified to yield high recombinant proteins such as hormones, antibodies and vaccines. Transgenic algae are suitable for containment and controlled growth in bioreactors under phototrophic and heterotrophic condition. The stable genetic transformation in the chloroplast and nucleus of *Chlamydomonas reinhardtii* has been reported (Courchesne et al., 2009; Banares et al., 2004). The recent advances in genetic manipulation tools for microalgae system promotes the manipulation of central carbon metabolism of the microalgae for improved productivity. Furthermore, the availability of genome database for microalgae enabled the characterization of fatty acid biosynthesis (Tatabaei et al., 2011).

The lipid extraction of microalgae may leave effects on human and environment in order to produce biofuel. The conventional lipid extraction method using hexane can also cause the deterioration of PUFA. Supercritical carbon dioxide extraction is a promising green technology, and an alternative to the traditional organic solvents in lipid extraction. It is because the extraction is conducted at low temperatures as it can prevent denaturation of PUFA. This method is non toxic, non flammable, cost effective and environmental friendly. This method has been studied in *Botryococcus braunni*, *Chlorella vulgaris*, *Dunaliella salina* and the cyanobacteria *Arthrospira* (Amaro et al., 2011; Cheng et al., 2011; Mendes et al., 2003).

In order to enhance the production efficiency, biorefinery is one method that reduces the cost of microalgal production. In biorefinery, all the biomass elements such as protein, carbohydrate, metabolites and others will be converted into usable forms. For example, residual biomass can be used as animal feed to produce methane by anaerobic digestion and also generate electrical power. This advanced method is proven to be able to reduce the cost of the biodiesel production.

The optimization of biodiesel production in microalgae needs to be considered as it is one of the most promising renewable sources that can substantially displace petrodiesel. The innovation to increase the productivity of algae not only fulfills the bioenergy demand but also reduces pollution and can save the environment.

CHAPTER 3

METHODOLOGY

3.1 MICROALGAE USED IN THIS STUDY

Ten strains of microalgae were obtained from the University Malaya Algae Culture Collection (UMACC): *Chlorella vulgaris* Beijerinck UMACC 001, *Scenedesmus quadricauda* UMACC 041, *Oocystis* UMACC 074, *Scenedesmus obtusus* UMACC 100, *Chlorococcum miniatum* UMACC 102, *Chlorella vulgaris* UMACC 103, *Chlorella vulgaris* UMACC 245, *Chlorella* UMACC 253, *Chlorella* UMACC 255 and *Chlorella* UMACC 275 (Phang and Chu, 1999). The *Chlorella* UMACC 275 was isolated from palm oil mill effluent (POME). The origin of the microalgae used in this study is shown in Table 3.1. The photomicrographs of the ten strains are shown in Figure 3.1.

3.2 PREPARATION OF MEDIUM

All the selected strains were grown in Bold's Basal Medium (BBM) (Table 3.2) for freshwater strains and Pro V Medium (Table 3.3) for marine strains.

3.2.1 Preparation of BBM Medium

The BBM medium was prepared in 1200ml conical flask by adding stock solution based on the composition described in Table 3.2. According to the composition, 10ml of stock solution from 1 to 6 added, followed by addition of 1ml stock solution from 7 to 10 into the conical flask. After that, distilled water was added up to 1L.

Table 3.1: Origin of the ten microalgae strains used in this study

Strains	Origin	Culture Medium
1. *Chlorella vulgaris* Beijerinck UMACC 001	IPSP Farm, University Malaya	BBM
2. *Scenedesmus quadricauda* UMACC 041	Fish tank (tilapia),IPSP Farm, University of Malaya	BBM
3. *Oocystis* UMACC 074	Painted masonry at new building, Science faculty, UM	BBM
4. *Scenedesmus obtusus* UMACC 100	Tasik Aman, Petaling Jaya, Selangor	BBM
5. *Chlorococcum miniatum* UMACC 102	Wall scraping	BBM
6. *Chlorella vulgaris* Beijerinck UMACC 103	Klang estuary, Selangor	ProV
7. *Chlorella vulgaris* UMACC 245	Global Satria Shrimp Pond, Tawau, Sabah	ProV
8. *Chlorella vulgaris* UMACC 253	Global Satria Shrimp Pond, Tawau, Sabah	ProV
9. *Chlorella* UMACC 255	Global Satria Shrimp Pond, Tawau, Sabah	ProV
10. *Chlorella* UMACC 275	Tennamaran Palm Oil Mill Effluent (POME)	BBM

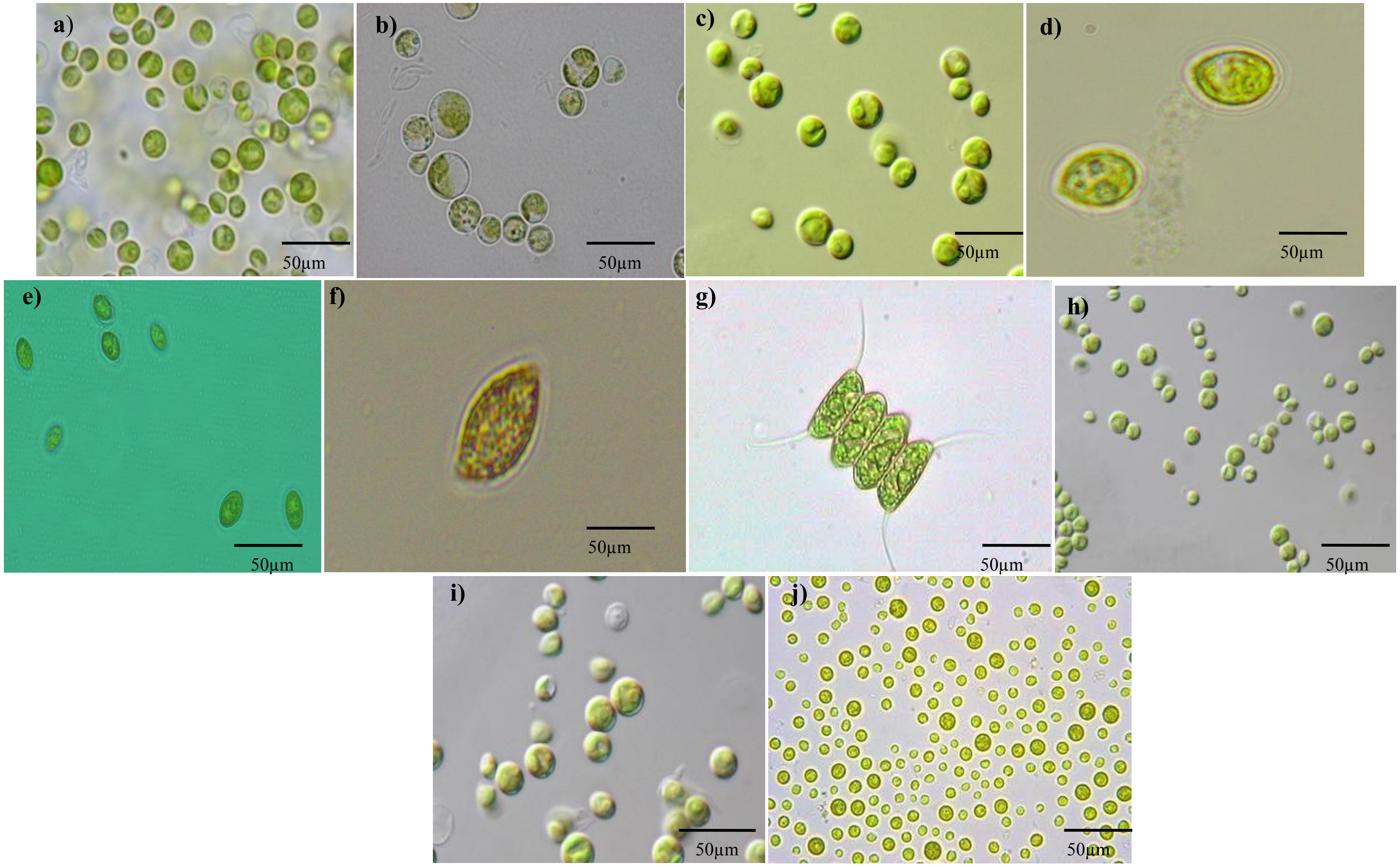


Figure 3.1: Photomicrograph of ten microalgae strains a) *Chlorella vulgaris* Beijerinck UMACC 001 b) *Chlorella* UMACC 275 c) *Chlorella vulgaris* Beijerinck UMACC 103 d) *Chlorococcum miniatum* UMACC 102 e) *Oocystis* UMACC 074 f) *Scenedesmus obtusus* UMACC 100 g) *Scenedesmus quadricauda* UMACC 041 h) *Chlorella vulgaris* UMACC 245 i) *Chlorella vulgaris* UMACC 253 j) *Chlorella* UMACC 255

Table 3.2: The Composition of Bold's Basal Medium (BBM) (Nichols and Bold, 1965)

Stocks	
	Per 400ml
1. $NaNO_3$	10.0g
2. $MgSO_4 \cdot 7H_2O$	3.0g
3. K_2HPO_4	4.0g
4. KH_2PO_4	6.0g
5. $CaCl_2$	1.0g
6. NaCl	1.0g
7. Trace element solution	Per L
$ZnSO_4$	8.82g
MoO_3	0.71g
$Co(NO_3)_2 \cdot 6H_2O$	0.49g
$MnCl_2$	1.44g
$CuSO_4 \cdot 5H_2O$	1.57g
Autoclave to dissolve	
	Per 100ml
8. H_3BO_4	1.14g
9. EDTA-KOH solution	
$EDTA \cdot Na_2$	5.0g
KOH	3.1g
	Per L
10. FeSO4·7H2O	4.98g
Concentrated HCl	1.0ml

The pH of the medium was adjusted to 6.8 by adding of hydrochloric acid or potassium hydroxide (as buffer). Then, the mouth of conical flask was tightly closed with cotton plug. The conical flask was covered completely with aluminium foil and autoclaved at 15psi, 120ºC for 20 minutes.

3.2.2 Preparation of ProV medium

The ProV medium was prepared in 1200ml conical flask by adding stock solution based on the composition described in Table 3.3. According to the composition, 1ml of $NaNO_3$ stock solution, $NaH_2PO_4 \cdot H_2O$, f/2 trace metal solution and NH_4Cl was added, followed by addition of 0.5ml of vitamin solution into the conical flask. After that, filtered seawater was added up to 1L. The pH of the medium was adjusted to 8.0 by adding of hydrochloric acid or potassium hydroxide (as buffer). Then, the mouth of conical flask was tightly closed with cotton plug. The conical flask was covered completely with aluminium foil and autoclaved at 15psi, 120ºC for 20 minutes.

Table 3.3: The Composition of ProV Medium (CCMP, 1996)

Stocks	
$NaNO_3$	75.0 g/L
$NaH_2PO_4 \cdot H_2O$	5.0g/L
f/2 trace metal solution	
$Na_2EDTA \cdot 2H_2O$	4.36g
$FeCl_3 \cdot 6H_2O$	3.15g
$CoCl_2 \cdot 6H_2O$	1.0ml (from 10.0g/L)
$ZnSO_4 \cdot 7H_2O$	1.0ml (from 22.0g/L)
$MnCl_2 \cdot 4H_2O$	1.0ml (from 180.0g/L)
$CuSO_4 \cdot 5H_2O$	1.0ml (from 9.8g/L)
$Na_2MoO_4 \cdot 2H_2O$	0.7mg
Make up to 1.0L with deionised water	
Vitamin solution	
Vitamin B12	1.0ml (from 1.0g/L)
Biotin	10.0ml (from 0.1g/L)
Thiamine HCl	100.0mg

3.3 STOCK CULTURE MAINTENANCE

All the ten strains were grown in 250ml conical flasks containing 90ml medium added with 10ml (10%) of inoculum. All the cultures were maintained in an incubator shaker set at 100rpm at 25°C±1. The cultures were illuminated with cool fluorescent lamps with the irradiance of 42µmolm^{-2}s^{-1} on 12:12 hour light dark-cycle. The stock cultures were maintained by subculturing into new medium every two months.

3.4 PREPARATION OF INOCULUM

The inoculum was prepared from the maintained stock culture for further cultivation. The inoculum was obtained from exponential phase cultures standardised at an optical density at 620nm (OD_{620nm}) of 0.2. The OD_{620nm} was measured using Shimadzu UV-vis Spectrophotometer. (Teoh et al., 2004).

3.5 ANALYTICAL METHODS

The following parameters were determined for all the ten microalgae strains: OD_{620nm}, cell count, chlorophyll-a content, carotenoid content, biomass and biochemical composition such as protein content, carbohydrate content, lipid content and the distribution of fatty acids.

3.5.1 Optical Density

The optical density of the culture was determined by taking about 2ml of the sample from each replicate in triplicates and placed in a plastic cuvette. Then, the optical density of the samples was measured at 620nm (OD_{620nm}). The optical density for each sample was measured using Shimadzu UV-vis Spectrophotometer. The optical density measured for every two days throughout the growth period (Teoh et al., 2004).

3.5.2 Cell count

The growth of the culture was also monitored based on the cell number. About 1ml of the sample was taken in an Eppendorf tube from each sample in triplicates. Then, one to two drops of formalin added into the sample to preserve the cells in the sample. One drop of the sample was placed in an improved Double- Neubauer Haemacytometer. The cell numbers on improved Double- Neubauer Haemacytometer were counted using light microscope. The cell count measured for every two days throughout the cycle.

3.5.3 Chlorophyll-a content

The chorophyll-a content of the sample was determined using colorimetric method by (Strickland and Parson, 1968). From the culture, 10ml of sample was taken from each replicate in triplicates and filtered in glass fiber filter paper (Whatman GF/C, 0.45µm). Then, the filter paper was mashed in 15ml centrifuge tube by using glass rod. After mashing, 10ml of 100% acetone was added into the centrifuge tube. The centrifuge tube was covered with aluminium foil and kept in fridge at 4°C for overnight. On the next day, the samples were taken out and centrifuged at 3000rpm for 10 minutes. After that, the supernatant was taken and measured at OD_{665nm}, OD_{645nm} and OD_{630nm} for chlorophyll –a content. Based on this, the chlorophyll-a content was determined by using the following formula (Strickland and Parsons, 1968). The chlorophyll-a content was determined for every two days throughout the cycle using the following formula:

Determination of Chlorophyll:

$$A = 11.6\,(OD_{665nm}) - 1.31(OD_{645nm}) - 0.14(OD_{630nm})$$

$$\text{Chlorophyll-a content} = \frac{\text{A X Volume of acetone (ml)}}{\text{Volume of sample (L)}}$$

$$= \text{mg/ m}^3/1000$$

$$= \text{mg/L}$$

3.5.4 Carotenoid Content

The carotenoid content of the sample was determined using colorimetric method by (Strickland and Parson, 1968). From the culture, 10ml of sample was taken and filtered in glass fiber filter paper (Whatman GF/C, 0.45µm). Then, the filter paper was mashed in 15ml centrifuge tube by using glass rod. After mashing, 10ml of 100% acetone was added into the centrifuge tube. The centrifuge tube was covered with aluminium foil and kept in fridge at 4°C for overnight. On the next day, the samples were taken out and centrifuged at 3000rpm for 10 minutes. After that, the supernatant were taken and measured at OD_{452nm} for carotenoid content. Based on this, the carotenoid content was determined by using the following formula (Strickland and Parsons, 1968). The chlorophyll-a content measured for every two days throughout the cycle.

Determination of Carotenoid:

$$\text{Carotenoid content } (\mu g/ml) = OD_{452nm} \times 3.86 \times \frac{\text{Volume of Acetone (ml)}}{\text{Volume of Sample (ml)}}$$

3.5.5 Specific growth rate

Based on the chlorophyll-a determination, the specific growth rate (µ, day $^{-1)}$ of each strain was determined by using the following formula:

$$\mu, \text{day}^{-1} = \frac{\ln X_2 - \ln X_1}{t_2 - t_1}$$

X_2 and X_1 denotes for the chlorophyll content at times whereas t_1 and t_2 are times within the exponential phase.

3.5.6 Dry Weight of Sample

The samples were harvested in triplicates by filtration to determine the dry weight (DW) of the samples. The blank Whatman filter papers (GF/C 0.45µm) were dried in oven at 100°C overnight and kept in dessicator for at least 8 hours before measuring the pre weight. The volume of 50ml of culture was filtered on pre weighed filter papers. Then, the filter papers were dried overnight at 100°C and were kept in dessicator for 8 hours before measuring the post weight. The DW of the samples was obtained by using the following formula:

$$\text{Dry weight of sample} = \frac{\text{Post weight of filter paper (g)- pre weight of filter paper(g)}}{\text{Volume of culture (L)}}$$

This dry weight (DW) was used as a measure of biomass DW.

3.5.7 Protein Extraction of Microalgae

Firstly, 50ml of algal sample was filtered using Whatman filter paper (GF/C 0.45µm). Then, 5ml of 0.5M sodium hydroxide, NaOH was added little by little and mashed using hand homogenizer. The sample was then transferred into 15ml plastic centrifuge tube. 5ml of 0.5 NaOH was added into centrifuge tube to make up 10ml. It incubated in water bath for 20 minutes at 80ºC.

After incubation, the sample was centrifuged at 3000 rpm for 10 minutes at 4ºC. Then, 100µL aliquots were taken out and added with 3ml of protein reagent. It incubated for

30 minutes and then an absorbance was read at 595nm (OD_{595nm}). The protein content was determined based on the protein standard curve (Bradford, 1976).

3.5.8 Carbohydrate Extraction of microalgae

The carbohydrate content was analyzed based on the spectrophotometric method. Initially, 50ml of algal sample was filtered using Whatman filter paper (GF/C 0.45µm). Then, 6ml of 2M Hydrochloric acid, HCl was added little by little and mashed using hand homogenizer. The sample was transferred into 15ml plastic centrifuge tube. Again, 6ml of 2M HCl was added to make up 12ml. It then incubated in water bath for one hour at 80ºC and mixed regularly.

After one hour, the sample was taken and centrifuged at 3000 rpm for 10 minutes. Then, 500µL aliquots were taken out and added with 1.5ml of distilled water into glass tube. Subsequently, 100µL of 100% aqueous phenol was added followed by 5ml of concentrated sulphuric acid, H_2SO_4 added slowly into the tube. The sample was incubated for 30 minutes and then the tubes were cooled at room temperature. The absorbance was read at 485nm (OD_{485nm}). The carbohydrate content was determined based on the carbohydrate standard curve (Dubois et al., 1956).

3.5.9 Lipid Extraction of Microalgae

The total lipid content in microalgae was determined gravimetrically with chloroform-methanol (2:1 v/v) extraction (Bligh and Dyer, 1959). First, 50ml of algal sample was filtered on Whatman glass microfiber filter paper (0.45µm). After filtration, 5ml of methanol- chloroform (2:1 v/v) was added and mashed using hand homogenizer or tissue grinder. Then, the extract was transferred into a 15ml centrifuge tube and centrifuged at 3000 rpm for 10 minutes. The supernatant was removed and transferred into a new centrifuge tube using a long Pasteur pipette. Then, 2ml of chloroform followed by 2ml of distilled water were added. After that, the mixture was thoroughly shaken using vortex before being centrifuged again at 3000 rpm for 10 minutes to separate into two phases. The lower phase (green in colour) was removed with a specially drawn Pasteur pipette and transferred into a new screw capped tube. The extract was then evaporated with a gentle stream of nitrogen gas.

Then, the dried extract was redissolved with in a small volume (~1ml) of chloroform before transferred into pre weighed glass vials. The solvent again was evaporated gentle stream of nitrogen gas and dried extract was kept in dessicator for at least 24 hours. On the next day, the dried extract were weighed and kept for fatty acid transesterification. The total lipid content was determined using the following formula:

$$\text{Lipid extract(\% DW)} = \frac{\text{(weight of vial+lipid)-(Weight of empty vial)}}{\text{DW}} \times 100\%$$

3.5.10 Fatty Acid Transesterification

The dried lipid extract was dissolved in 1ml of 1% sulphuric acid- methanol (H_2SO_4 – MeOH). Then, the solvent was transferred into screw capped test tube using a long Pasteur pipette and the cap sealed with parafilm. The tubes were then heated to 90°C in a test tube heater (Brad) for 1 hour. The test tubes were shaken regularly.

After that, the test tubes were allowed to be cool at room temperature for about 10 to 15 minutes before adding hexane. Then, 1ml of hexane was added and the mixture was vortexed vigorously and was left to separate into two layers. The upper layer (yellowish colour) that consists of fatty acid methyl esters (FAME) was removed and transferred into a new screw capped test tube. The remaining lower layer was added with 1ml of hexane and vortexed again vigorously to form two layers. The upper layer was removed and the two layers were pooled together.

The pooled extracts were dried with gentle stream of nitrogen gas. The residue was then redissolved in 0.1ml of hexane and transferred into a clean glass vial. Then, the glass vial was sealed with parafilm and stored in freezer for further analysis by gas chromatography for the determination of fatty acid composition (Chu et al., 1994).

3.6 EXPERIMENT I: GROWTH AND BIOCHEMICAL CHARACTERIZATION OF MICROALGAE CULTURED IN BBM AND PROV MEDIUM FOR 12 DAYS

For preliminary screening, the microalgae cultures were grown in 1500ml of conical flask containing 1080ml of medium (BBM medium for freshwater strains and Pro V medium for marine strains) and 120ml (10%) of inoculum. The 10% inoculum was from the exponential phase of culture with the optical density at OD_{620nm} 0.2. All the cultures were grown under illuminated shelves. Each strain was grown in triplicate cultures in batch culture system. The cultures were grown at room temperature, 25°C±1ºC and illumination was provided with cool fluorescent lamps in 12:12 light-dark cycles with the irradiance of $42\mu molm^2s^{-1}$. All the cultures were supplied with continuous aeration using standard aquarium air pumps and plastic tubing stuffed with glass wool to provide for some filtration of air for 12 days.

The pH of the cultures was measured on the day 0 and day 12. The growth of all strains was monitored from optical density at OD_{620nm}, cell count, chlorophyll-a content and carotenoid content on day 0, day 2, day 4, day 8 and day 12. The dry weight of samples was measured for every four days on day 0, day 4, day 8 and day 12. The biochemical composition (protein, carbohydrate and lipid content) was determined on day 0, day 4, day 8 and day 12 as described in section 3.5. The specific growth rate of each strain was calculated based on chlorophyll a content with particular strains based on the semilogarithmic graph plotted at exponential growth phase as explained in section 3.5.

3.7 EXPERIMENT II: GROWTH AND BIOCHEMICAL COMPOSITION OF SELECTED MICROALGAE AT ELEVATED LEVELS OF CARBON DIOXIDE

Based on the results obtained from Experiment I, four strains from UMACC, namely *Chlorella vulgaris* Beijerinck UMACC 001, *Scenedesmus quadricauda* UMACC 041, *Oocystis* UMACC 074 and *Chlorella* UMACC 275 were selected for further studies. All the four strains were selected based on their biomass production, lipid production and specific growth rates. All these four strains were cultured at 0.04%, 5% and 10% to determine their growth rate and biochemical composition at the elevated CO_2 levels.

3.7.1 Determination of the Flow Rate of CO_2

Initially, 400ml of BBM without inoculum was prepared. Then, carbon dioxide from the tank (MOX Linde Gas) was supplied to the medium at a flow rate of 10ml/min for one hour and the pH was measured for each 10 minutes. This process was repeated with different flow rate at 20ml CO_2/min, 30ml CO_2/min, 40ml CO_2/min and 50ml CO_2/min. Based on this, a calibration graph was plotted and 40ml CO_2/min was determined as the flow rate to grow microalgae at 5% and 10% of CO_2 and the amount of dissolved carbon dioxide was determined as described in section 3.7.3.

3.7.2 Growth Study of Microalgae at Different Levels of Carbon Dioxide

The cultures were grown in 1000ml of conical flask containing 360ml of BBM medium and 40ml (10%) of inoculum. The 10% inoculum was obtained from the stock culture at the exponential phase with the OD_{620nm} of 0.2. Each strain was grown in triplicate in batch culture system. The cultures were grown at room temperature, 25°C±1ºC and illumination was provided with cool fluorescent lamps with the irradiance of

42μmolm^2s^{-1} in 12:12 hour light- dark cycles. All the cultures were supplied with continuous aeration using standard aquarium air pump stuffed with glass wool to provide for some filtration of air for 10 days. The source of carbon dioxide was obtained in tank with the local blend gas of carbon dioxide (5% and 10%) and compressed air (MOX Linde Gas). A tube made from polyethylene was connected from the tank to the conical flask to supply the carbon dioxide mixed with compressed air with adjusted flow rate of 40ml/min based on calibration curve. All the cultures were supplied with carbon dioxide for every 12 hours from 7am to 7pm. The growth was monitored based on three parameters including optical density at OD_{620nm}, cell count and chlorophyll-a concentration on day 0, day2, day 4, day 8 and day 10. The carotenoid content was also measured on day 0, day2, day 4, day 8 and day 10. Based on the chlorophyll-a content, the specific growth rate (μ, day $^{-1)}$ of each strain was calculated as described in section 3.5.

The dry weight of the strains was calculated on day 0 day 10. The biochemical composition including protein content, carbohydrate content, lipid content and fatty acid distribution was determined at on day 0 and day 10 as in section 3.5.

3.7.3 Determination of Dissolved Carbon Dioxide

The amount of dissolved carbon dioxide was measured using the following correlation (Papazi et al., 2008).

C_{CO2} (dissolved in water) = (K_b $C_{C02\ (initial)}$ + K_W) – $10^{-\ pH}$

$K_b = 10^{-6.35}$

$K_W = 10^{-14}$

This correlation enables the determination of dissolved CO_2 at each incubation time by measuring only the corresponding pH value. The pH value was measured using calibrated pH meter for every 12 hours, before and after the CO_2 was supplied.

3.8 DATA ANALYSIS

All data were expressed in terms of mean and standard deviation (S.D). The data were analysed by one-way analysis of variance (ANOVA) followed by a Tukey's HSD post hoc comparison to determine significant differences between treatment means (P being set at 0.05). (Renaud et al., 2002).

CHAPTER 4

RESULTS

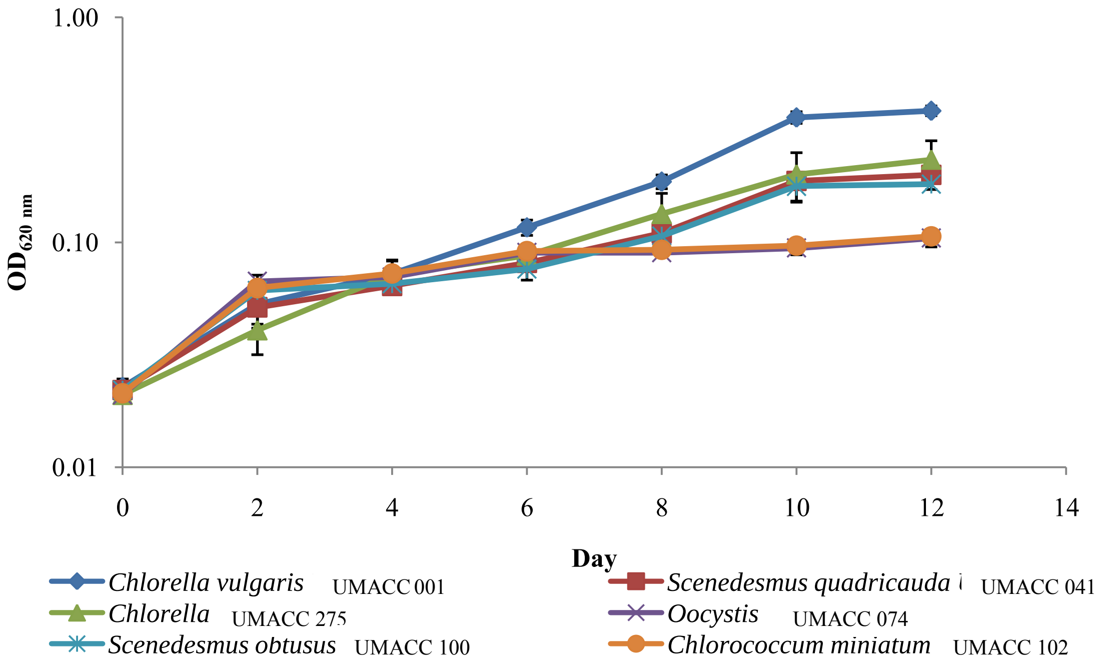
4.1 EXPERIMENT I: GROWTH AND BIOCHEMICAL CHARACTERIZATION OF MICROALGAE

4.1.1 Growth Trends (Appendix A)

4.1.1.1 Growth Trends Based on OD_{620nm}

Based on the OD_{620nm} in the graph below (Figure 4.1), *Chlorella vulgaris* UMACC 001 attained highest biomass concentration followed by *Chlorella* UMACC 275 and *Chlorella vulgaris* UMACC 103. The lowest OD_{620nm} was observed in *Oocystis* UMACC 074, *Chlorococcum miniatum* UMACC 102 and *Chlorella vulgaris* UMACC 245. The OD_{620nm} was found to be almost the same for three strains. The other strains namely *Scenedesmus quadricauda* UMACC 041, *Scenedesmus obtusus* UMACC 100, *Chlorella vulgaris* UMACC 253 and *Chlorella* UMACC 255 showed average growth among the strains. Almost all the strains have achieved stationary phase or late exponential phase on day 10.

a) Freshwater strains



b) Marine strains

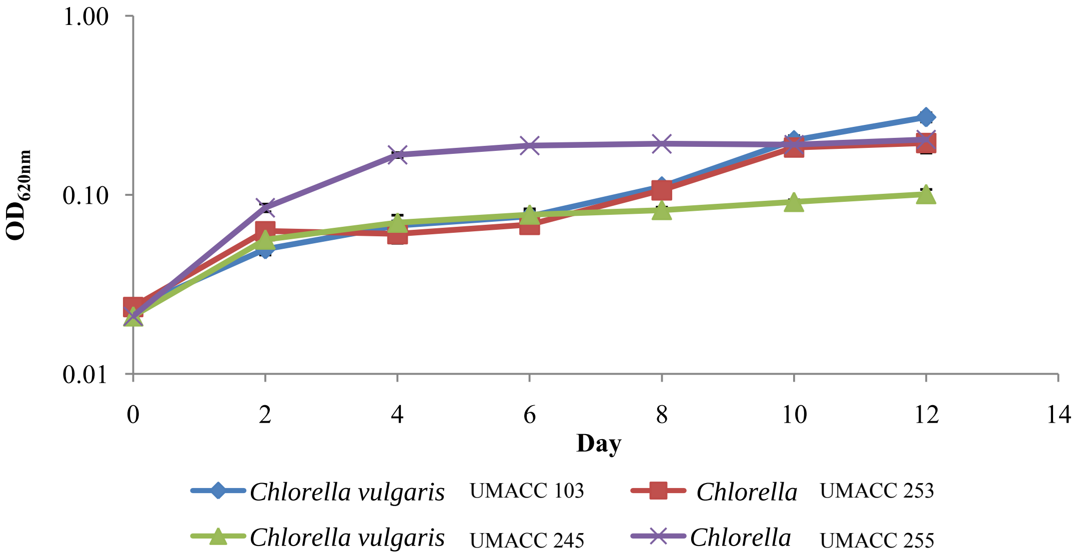
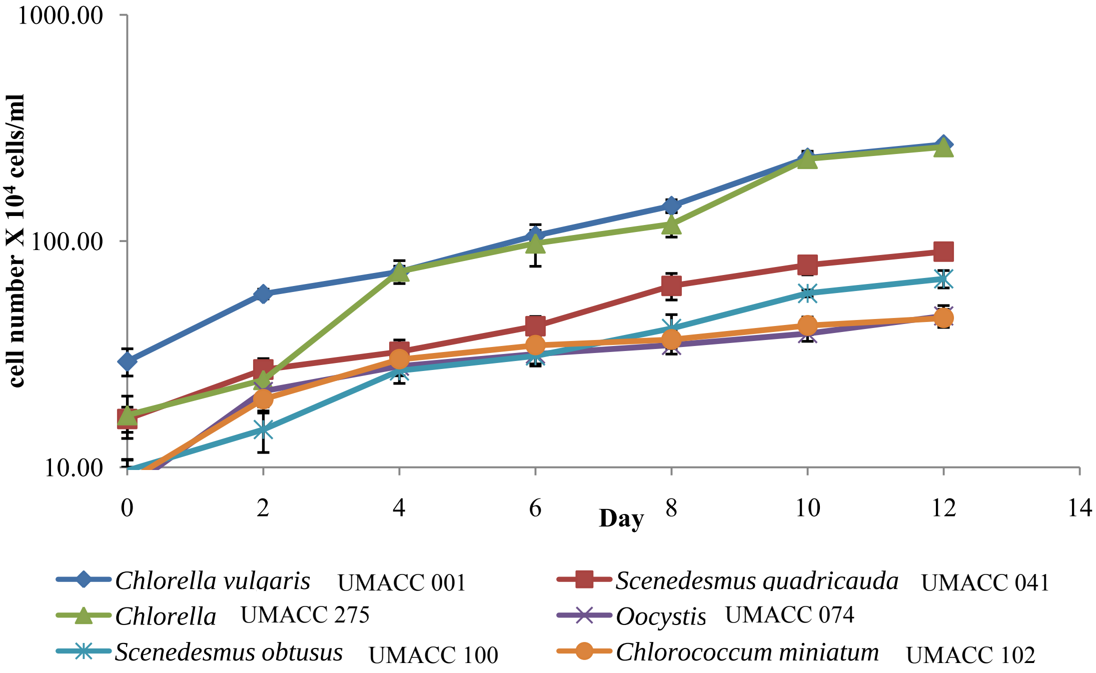


Figure 4.1: Semilogarithmic growth curve of a) Freshwater strains b) Marine strains based on OD_{620nm}. Data represents the means ±S.D.

4.1.1.2 Growth Trends Based on Cell Number

Based on the Figure 4.2, the highest cell count was observed in *Chlorella vulgaris* UMACC 001 followed by *Chlorella* UMACC 275, *Chlorella vulgaris* UMACC 103 and *Scenedesmus quadricauda* UMACC 041. The lowest cell count was observed in *Oocystis* UMACC 074 and *Chlorococcum miniatum* UMACC 102.

a) <u>Freshwater strains</u>



b) Marine Strains

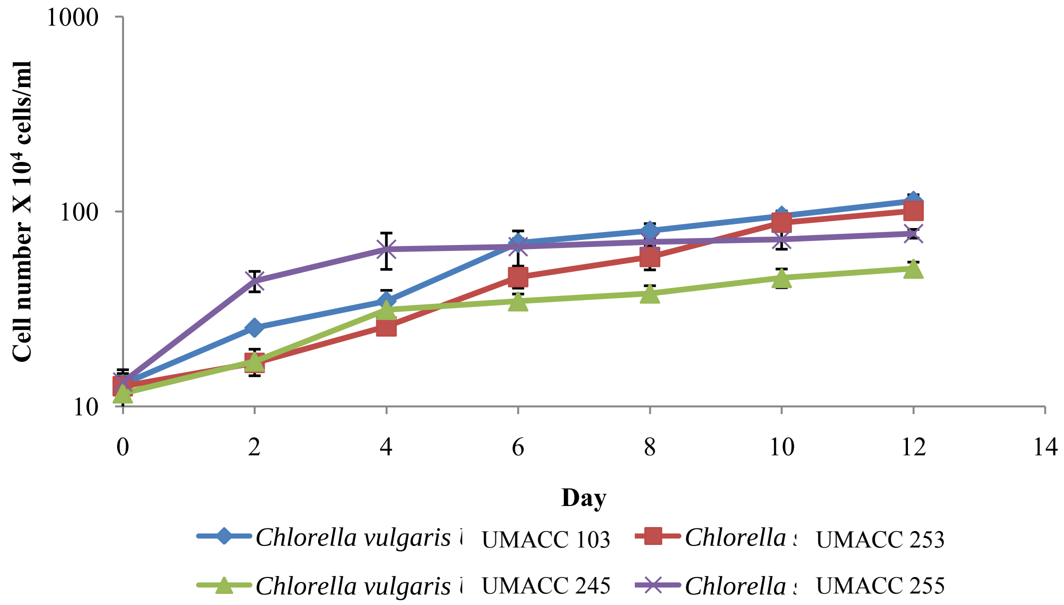
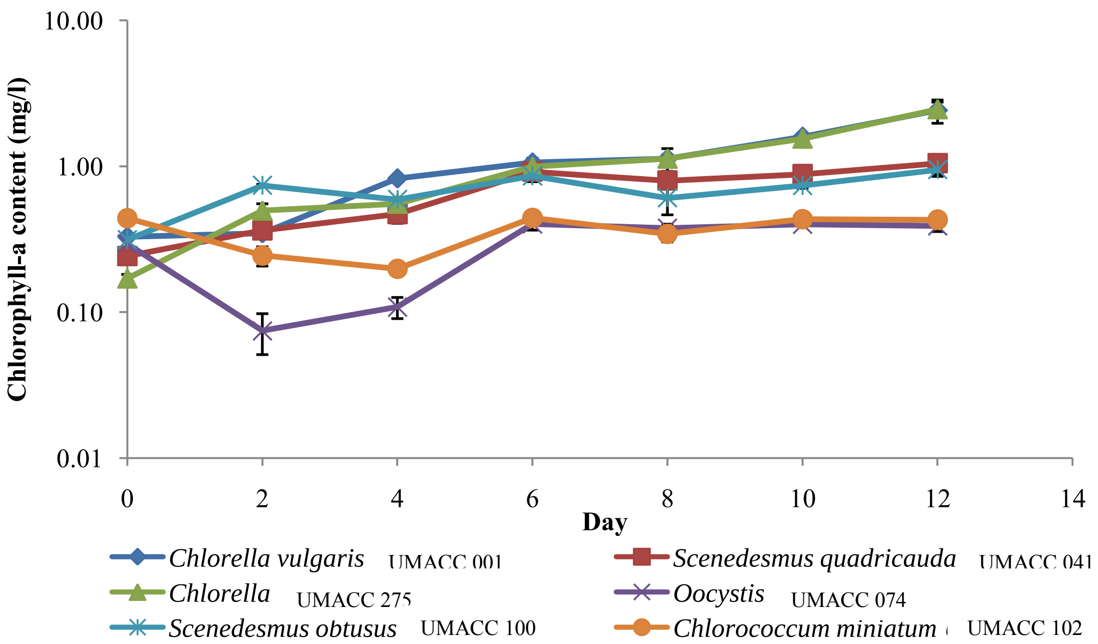


Figure 4.2: Semi logarithmic growth curves of a) Freshwater strains b) Marine strains based on cell count. Data represents the means ±S.D.

4.1.1.3 Growth Trends Based on Chlorophyll-a content

Besides that, chlorophyll a content (Figure 4.3), another parameter of growth attained higher production in *Chlorella* UMACC 275 about 2.461±0.298 mg/L on day 12. It was followed by *Chlorella vulgaris* UMACC 001 about 2.414±0.438 mg/L. The lowest content produced in *Oocystis* UMACC 074, *Chlorococcum miniatum* UMACC 102 about 0.391 ±0.033 mg/L and 0.429±0.039mg/L respectively. The other strains showed almost the constant range within day 9 and day10.

a) Freshwater strains



b) Marine Strains

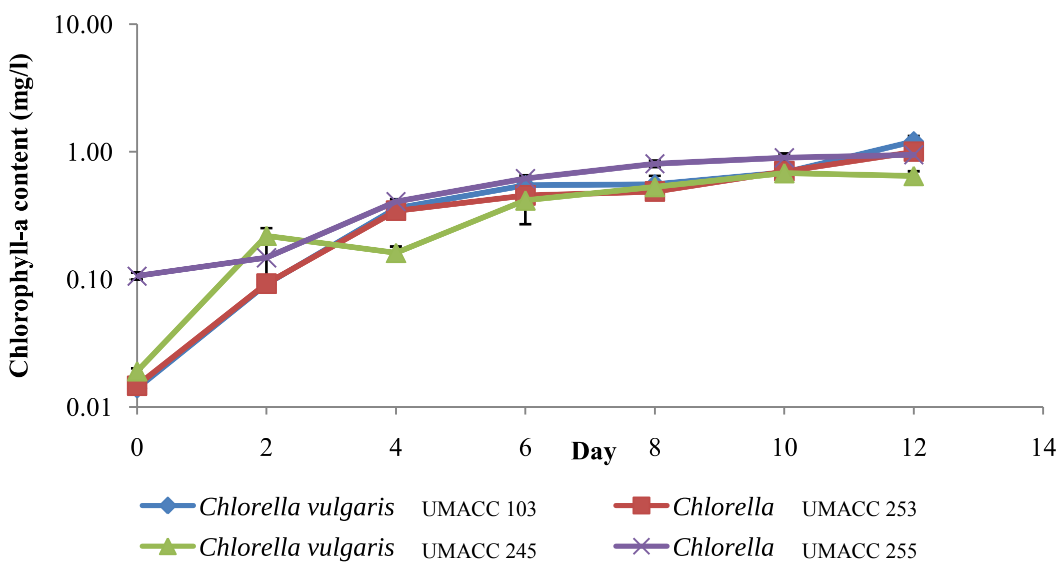


Figure 4.3: Semi logarithmic growth curves of a) Freshwater strains b) Marine strains based on chlorophyll a concentration. Data represents the means ±S.D.

4.1.1.4 Specific Growth Rate Based on Chlorophyll-a content

The entire specific growth rate was calculated based on the chlorophyll a content. Based on the Table 4.1, *Chlorella vulgaris* UMACC 001 performed highest specific growth rate (μ), 0.2433 ± 0.0168 day^{-1} among all the strains followed by *Chlorella* UMACC 275 0.2202 ± 0.0108 about day^{-1}. *Chlorococcum miniatum* UMACC 102 attained the lowest growth rate about 0.1217 ± 0.0061day^{-1}. The specific growth rate was calculated during the exponential phase of the culture between day 2 to day 6 as all the strains achieved their highest growth rate during this period of culture. For *Scenedesmus quadricauda* UMACC 041 attained (μ) 0.2140 ± 0.0110day^{-1}. All the marine strains achieved average growth rate during the exponential period of culture.

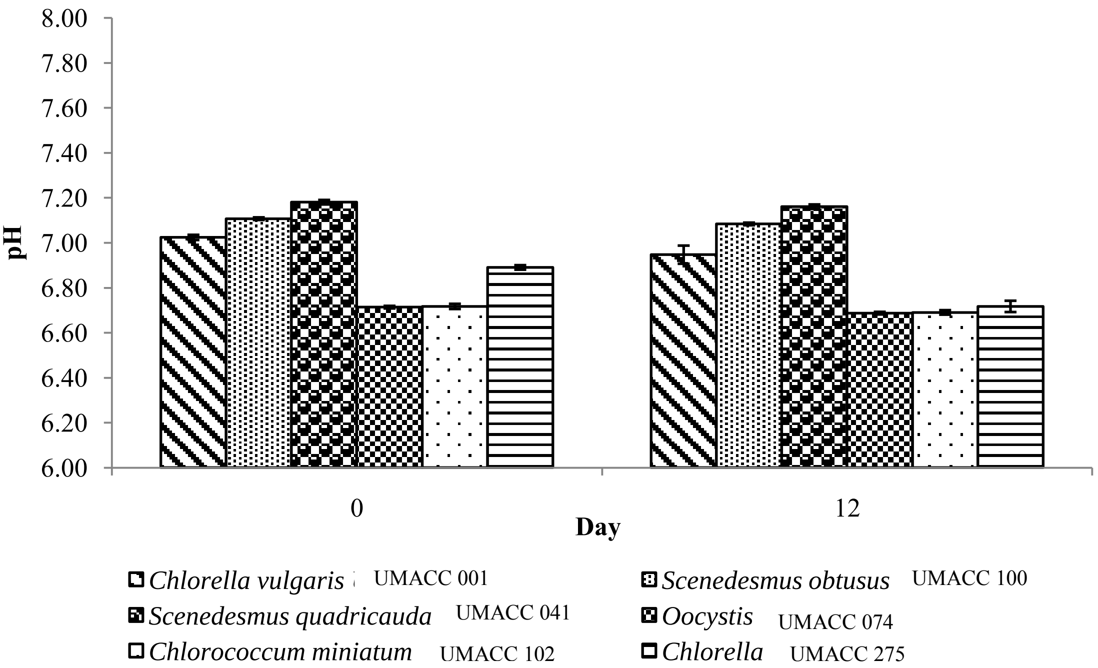
Table 4.1: Specific Growth Rates of Ten Microalgae Strains Based on Chlorophyll a content

Strains	specific growth rate (μ,day^{-1})	Exponential phase
Chlorella vulgaris UMACC 001	0.2433 ± 0.0168	day 4 -day 2
Scenedesmus obtusus UMACC 100	0.1327 ± 0.0361	day 6 -day 4
Chlorella UMACC 275	0.2202 ± 0.0108	day 6 -day 4
Scenedesmus quadricauda UMACC 041	0.2140 ± 0.0110	day 6 -day 2
Oocystis UMACC 074	0.1470 ± 0.0135	Day4 -day2
Chlorococcum miniatum UMACC 102	0.1217 ± 0.0061	day6 –day4
Chlorella vulgaris UMACC 103	0.1338 ± 0.0059	day4 –day2
Chlorella vulgaris UMACC 245	0.1272 ± 0.0065	day 6 -day 4
Chlorella UMACC 253	0.1260 ± 0.0155	Day4 -day 2
Chlorella UMACC 255	0.1298 ± 0.0209	day 4 -day2

4.1.1.5 pH of culture medium

As shown in the Figure 4.4, the pH of all the ten strains was obtained between 6.8 to 8.4. Slight change in pH was observed in all the strains. The cultures were not buffered.

a) <u>Freshwater strains</u>



b) <u>Marine strains</u>

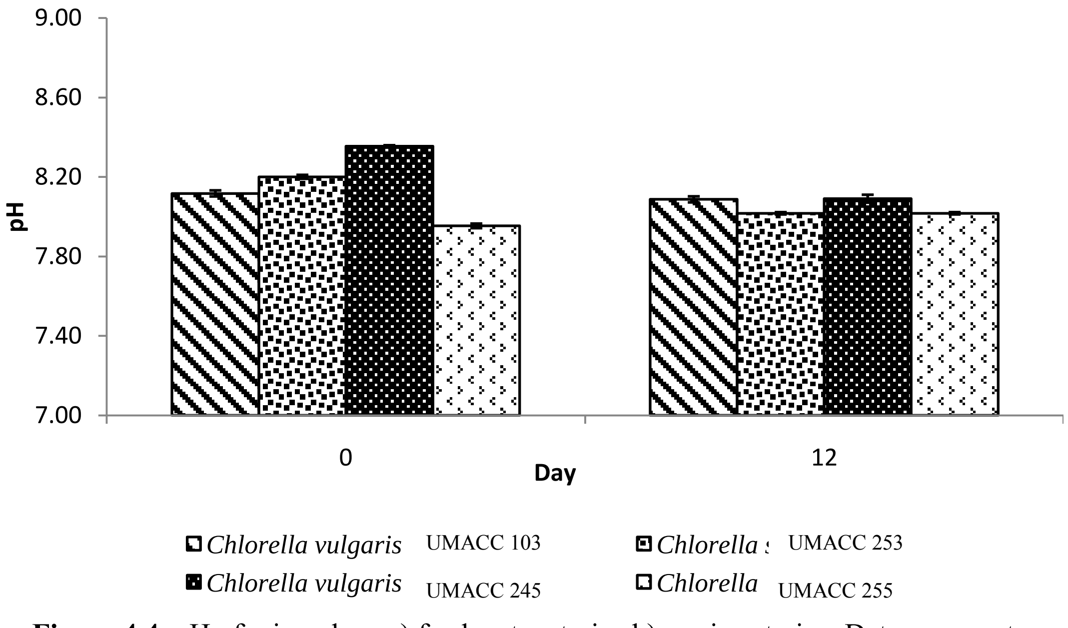
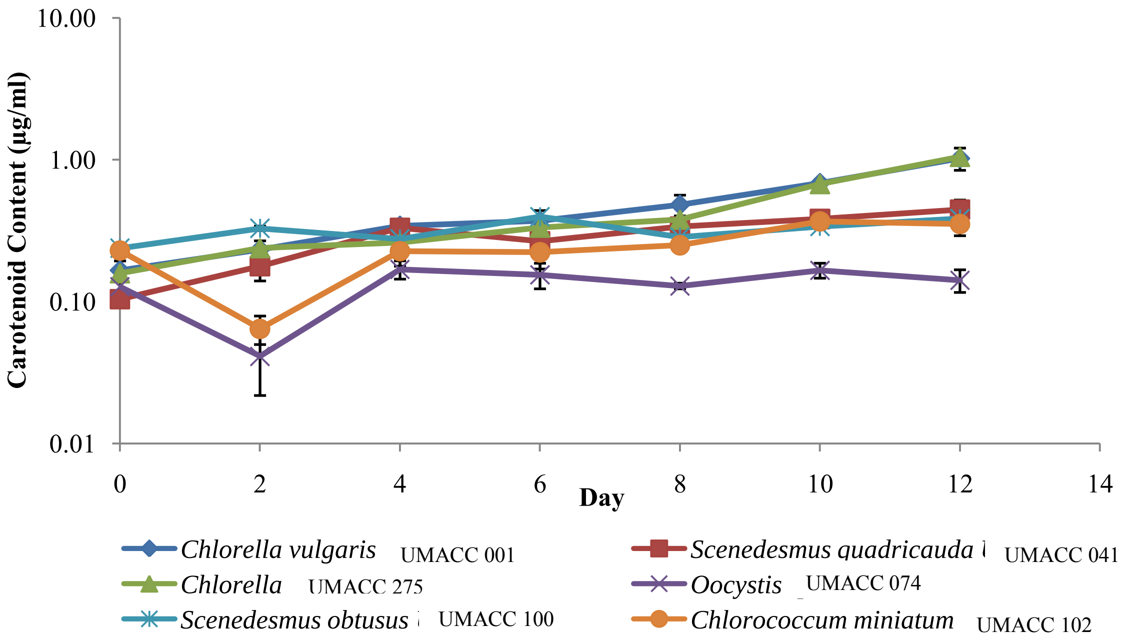


Figure 4.4: pH of microalgae a) freshwater strains b) marine strains. Data represents the means ±S.D.

4.1.2 Carotenoid Content (Appendix B)

Based on the carotenoid content shown in the Figure 4.5, *Chlorella* UMACC 275 produced highest yield about 1.049±0.126 µg/ml compared with other strains. Besides, *Chlorella vulgaris* UMACC 001 also produced higher carotenoid content of 1.023±0.183µg/ml respectively. The lowest content was observed in *Oocystis* UMACC 074 which is 0.142±0.026µg/ml. The carotenoid content of *Chlorella* UMACC 275 was 7 times higher than the lowest producer *Oocystis* UMACC 074. All the marine water strains were produced comparably lower carotenoid content with the fresh water strains.

a) Freshwater strains



b) Marine strains

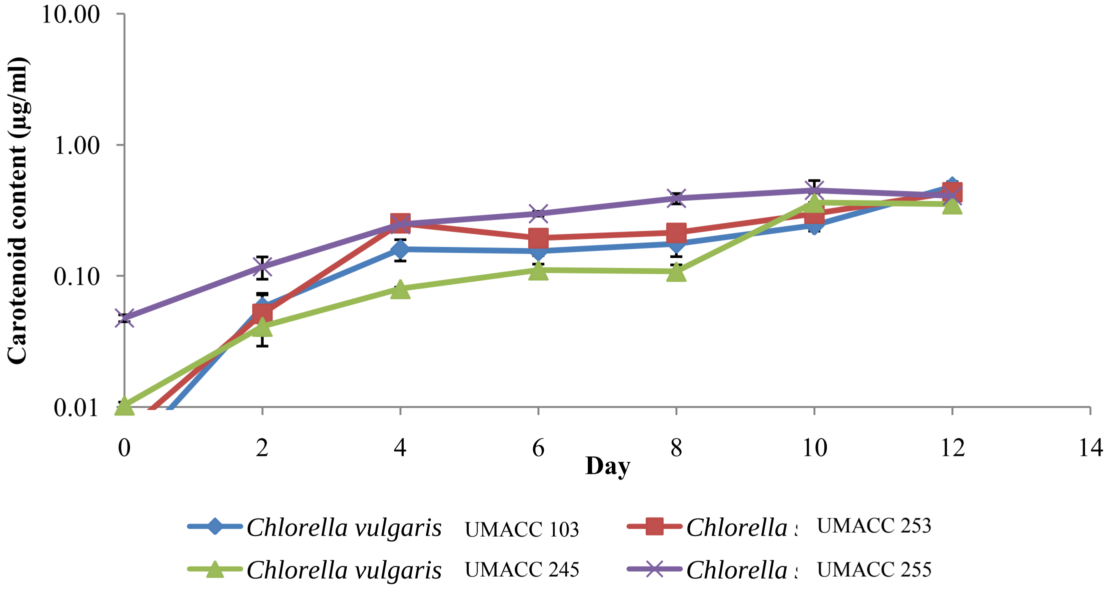
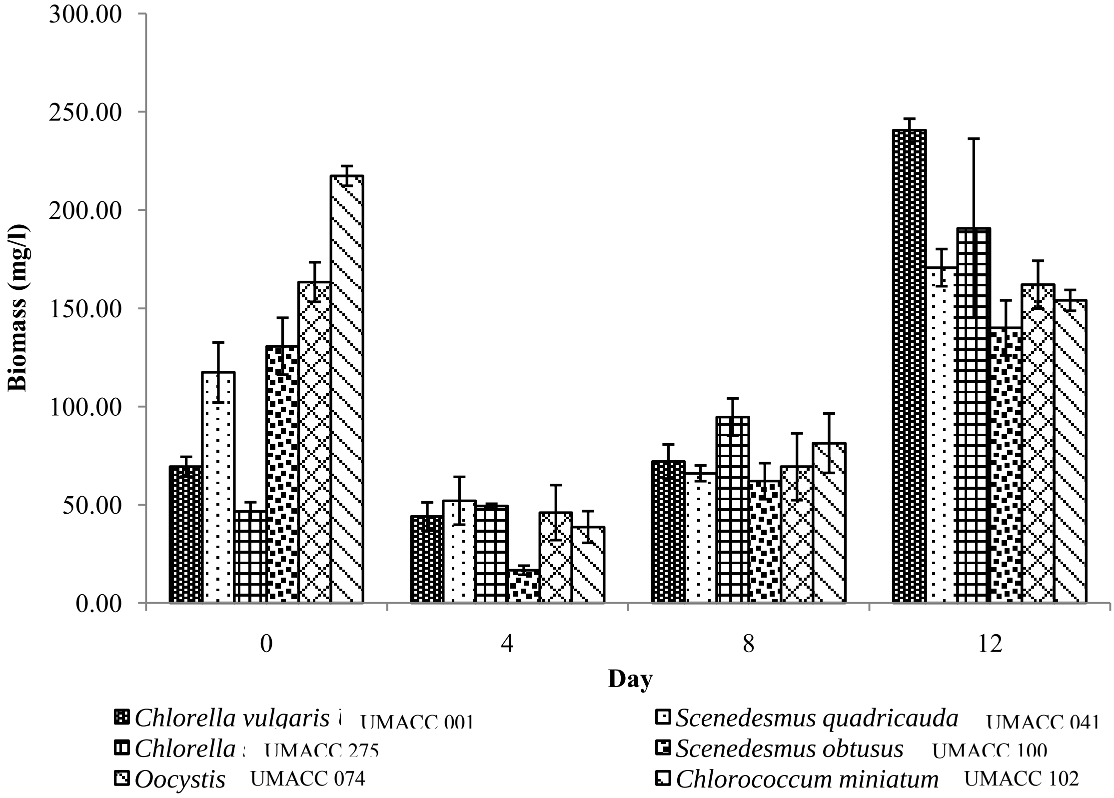


Figure 4.5: Semi logarithmic carotenoid content of ten microalgae a) freshwater strains b) marine strains. Data represents the means ±S.D.

4.1.3 Biomass Based on DW (Appendix C)

Of the microalgae screened, *Chlorella vulgaris* UMACC 001 attained the highest biomass on day 12 was 240.7±5.774mg/l DW (Figure 4.6). The biomass of *Chlorella* UMACC 275 was 190.67±45.622mg/L DW on day 12. The lowest biomass was observed in *Chlorella vulgaris* UMACC 245 108±2.00mg/L DW on day 12. All the strains attained highest biomass on the last day of the culture period (12 days). However, the biomass of *Oocystis* UMACC 074 on day 12 was almost the same as day 0. There was a great decrease in biomass from day 0 to day 4, after which growth became resumed and biomass increased until day 12. The same trend was observed for *Scenedesmus obtusus* UMACC 100 and *Chlorococcum miniatum* UMACC 102.

a) <u>Freshwater strains</u>



b) <u>Marine strains</u>

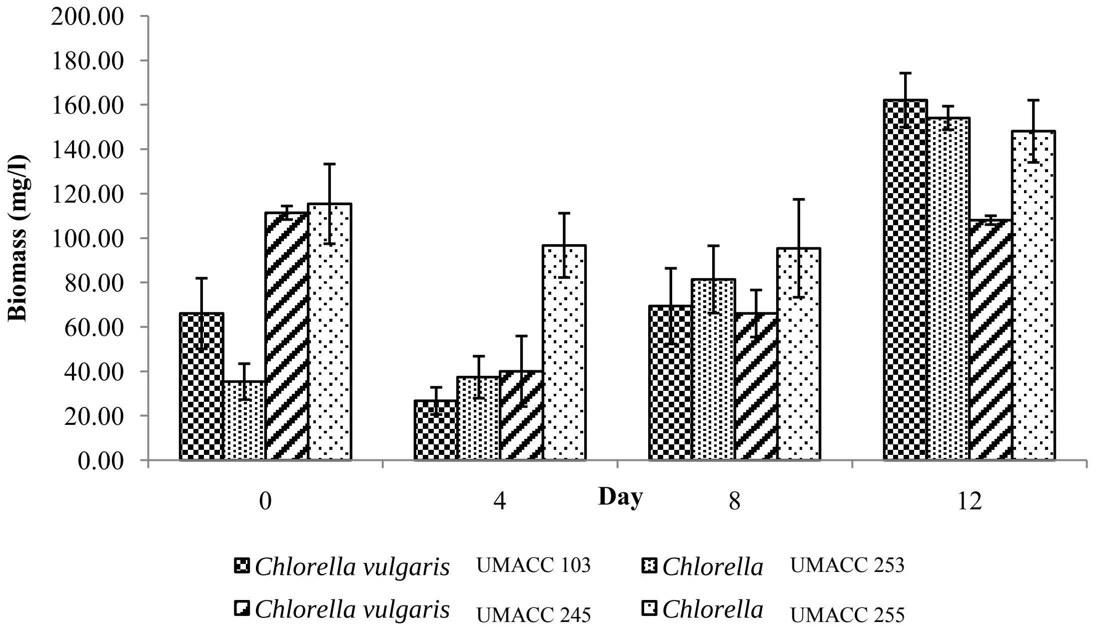


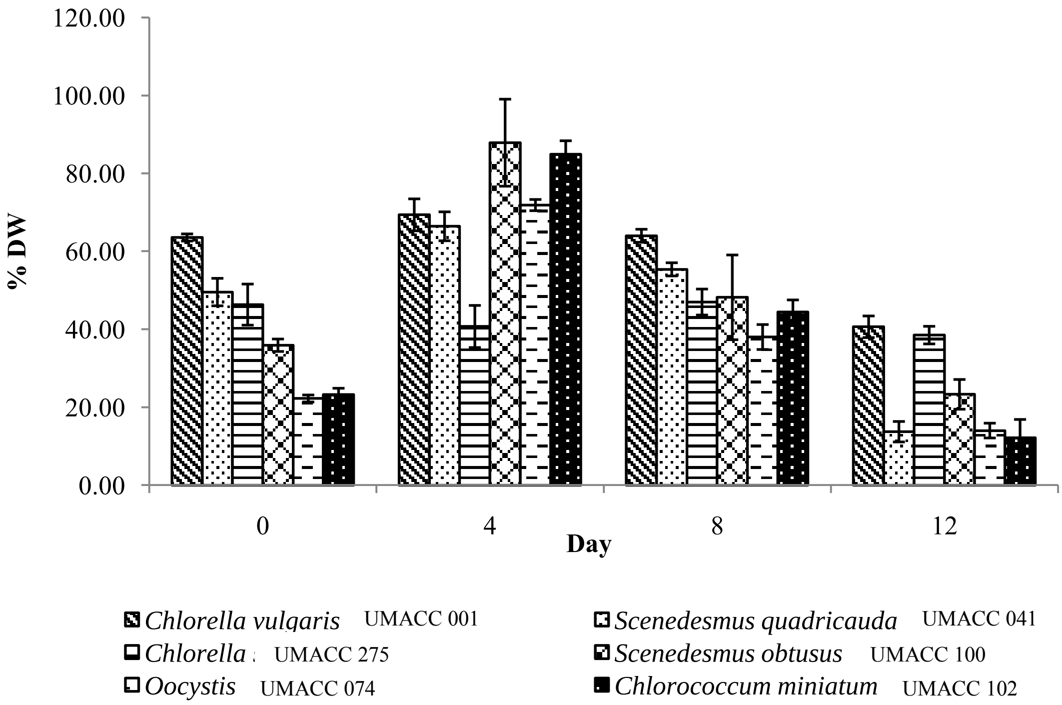
Figure 4.6: Biomass of ten microalgae a) Freshwater strains b) Marine strains based on DW. Data represents the means ±S.D.

4.1.4 Biochemical Composition (Appendix D)

4.1.4.1 Proteins

In general, the microalgae produced highest amounts of proteins on day 4, ranging from 43 to 87% of the DW (Figure 4.7). Of the strains screened, *Scenedesmus obtusus* UMACC 100 and *Chlorococcum miniatum* UMACC 102 attained the highest protein contents of 87.9 ±11.161% and 85.0 ± 3.418% DW respectively. The lowest content on day 4 was produced by *Chlorella* UMACC 275 about 40.72% DW. However, the protein content increased to 46.99± 3.332% DW on day 8. *Chlorella vulgaris* UMACC 245 also produced higher protein content on day 8, 72.53 ±1.273% DW. For *Chlorella vulgaris* UMACC 001, the protein content averaged about 69.36±4.103% of DW which was the highest protein content achieved by it on day 4.

a) Freshwater strains



b) Marine strains

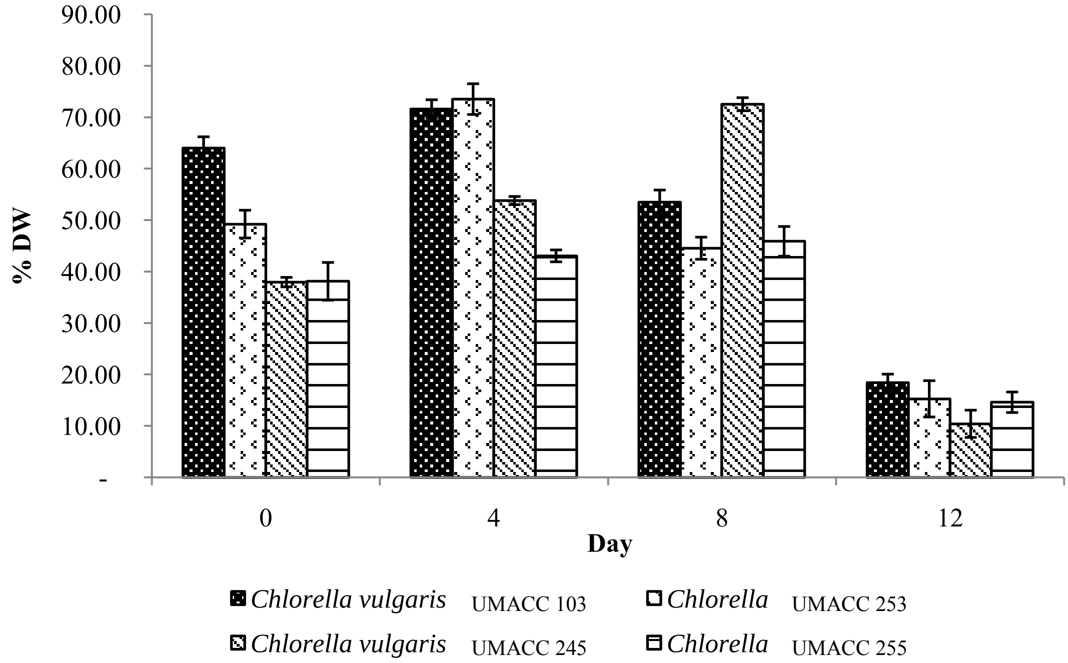
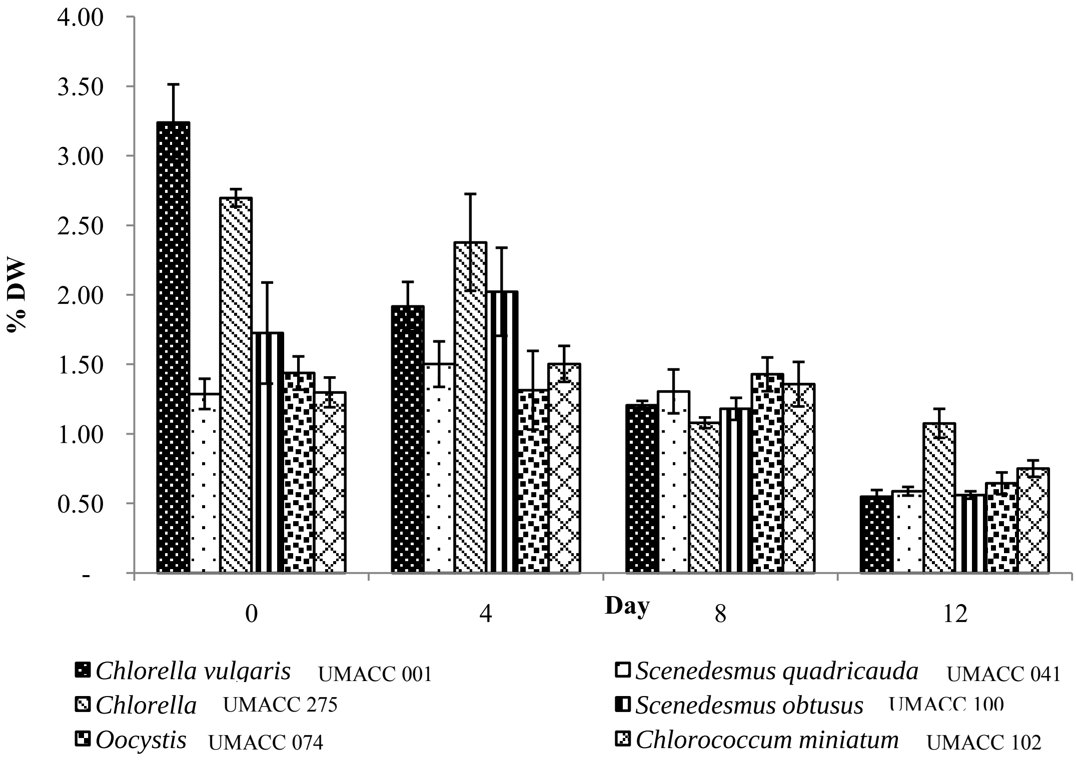


Figure 4.7: Protein content of ten microalgae a) Freshwater strains b) Marine based on percentage of DW. Data represents the means ±S.D.

4.1.4.2 Carbohydrates

Generally, the carbohydrate content attained by all the species was relatively low that is below 3% DW (Figure 4.8). The carbohydrate content decreased with the culture period.

a) Freshwater strains



b) Marine strains

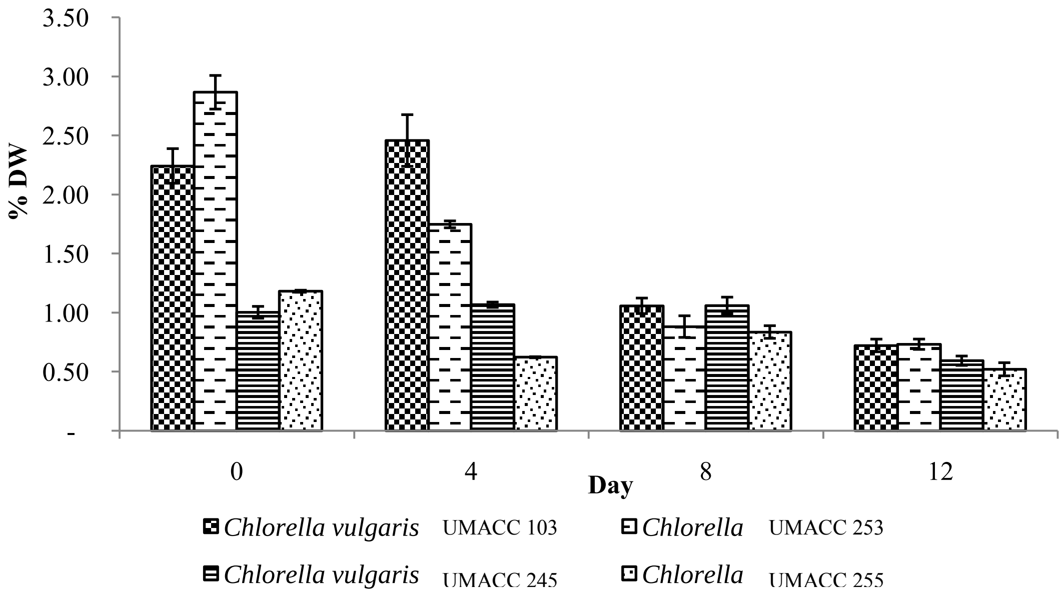
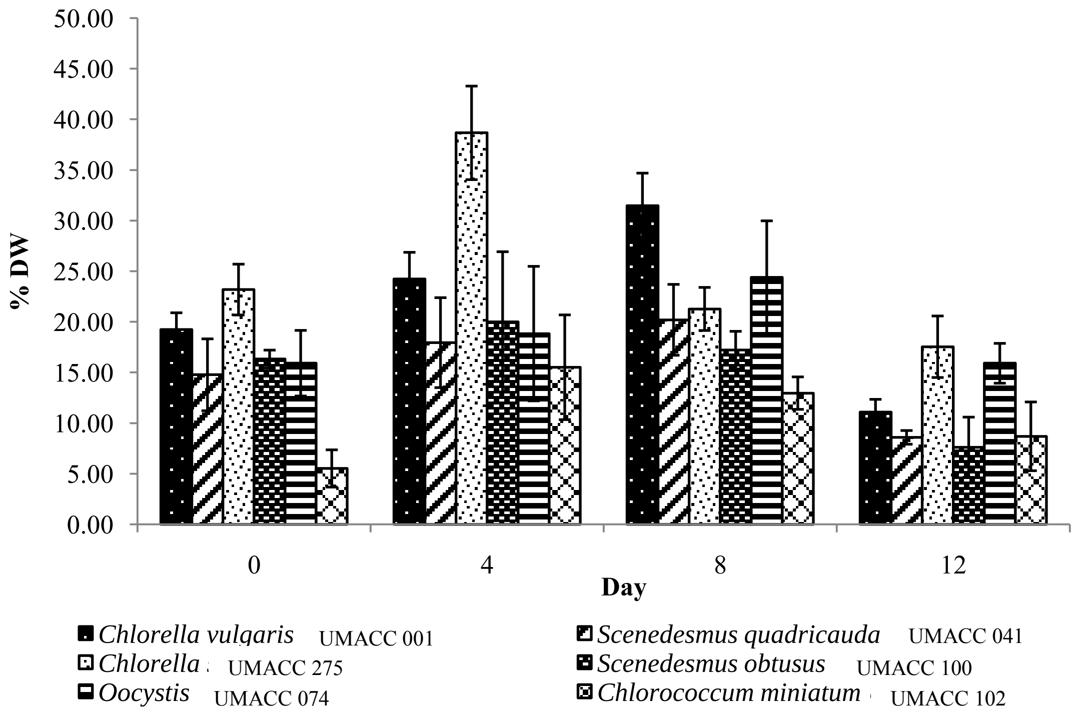


Figure 4.8: Carbohydrate content of ten microalgae a) Freshwater strains b) Marine strains based on DW. Data represents the means ±S.D.

4.1.4.3 Lipids

Chlorella UMACC 275 produced the highest amount of lipid 38.7±4.619 % DW on day 4 but decreased to 21.28 ±2.128% on day 8 (Figure 4.9). In comparison, lipid content of *Chlorella vulgaris* UMACC 001 on day 4 was only about 24.2±2.624% DW but it increased on day 8 about 31.5±3.208% DW. Both the *Scenedesmus quadricauda* UMACC 041, *Scenedesmus obtusus* UMACC 100 and *Oocystis* UMACC 074 are only produced average amount of lipid that within the range 17-25% DW on day 4 and day 8. Another strain *Chlorella vulgaris* UMACC 245 also attained its higher lipid content on day 4 25% of DW. The lipid content for the other strains was within the range of 10-25% DW. The lipid content decreased towards the end of the culture period, day 12.

a) <u>Freshwater strains</u>



b) Marine strains

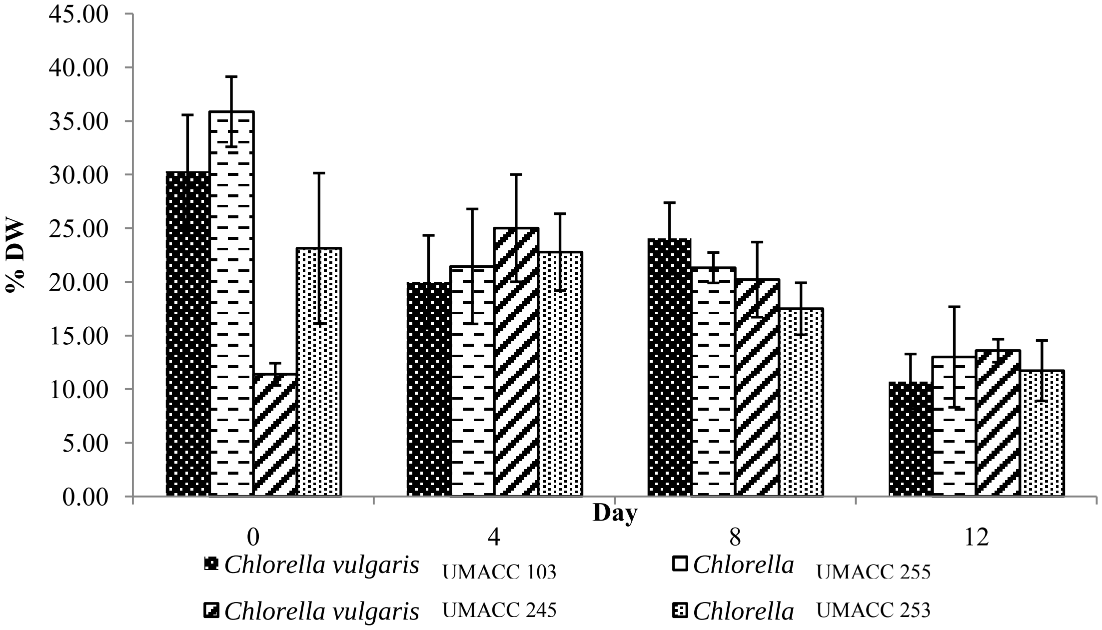


Figure 4.9: Lipid content of ten microalgae a) Freshwater strains b) Marine strains based on percentage of DW. Data represents the means ±S.D.

4.1.5 Fatty acid composition (Appendix E)

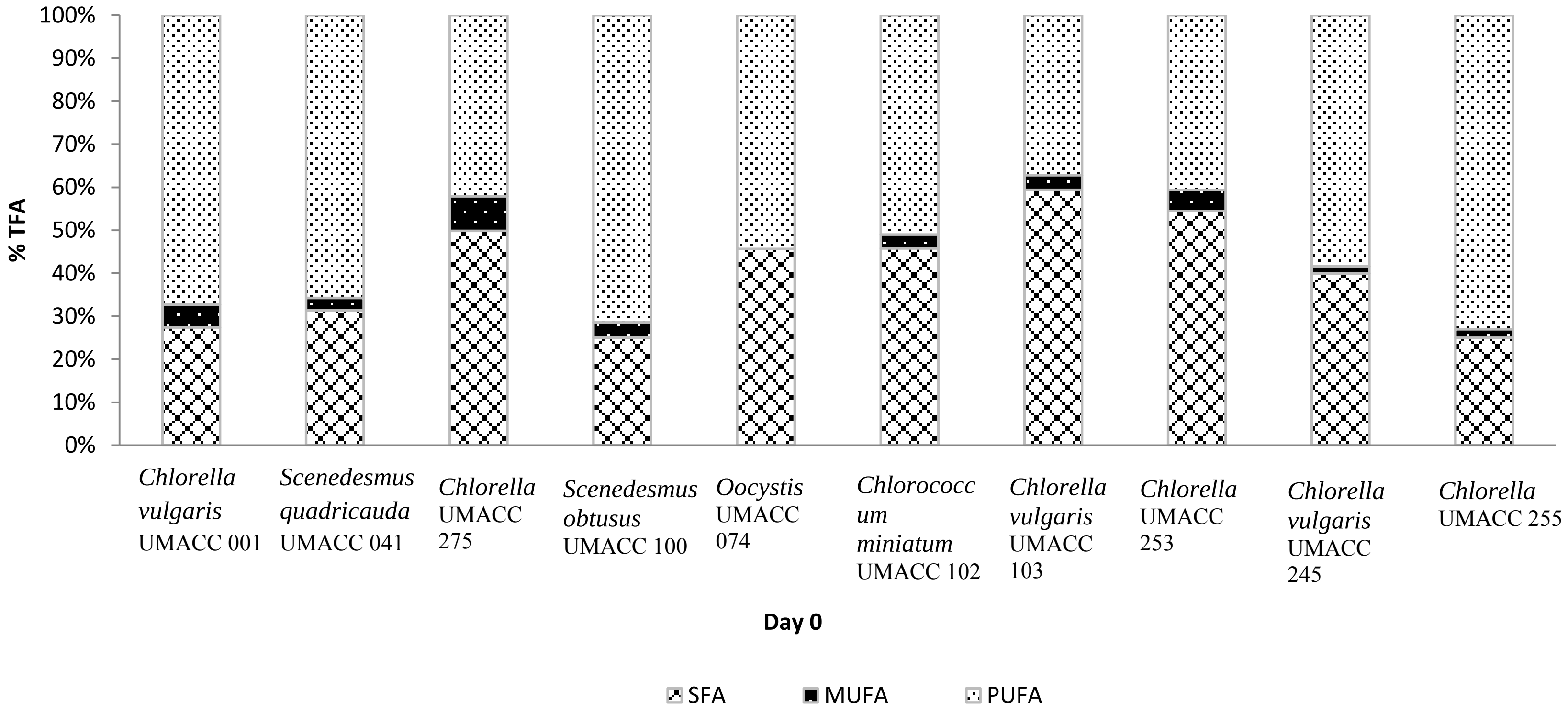
For the fatty acid composition, three types of fatty acids were determined namely saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). Based on Figure 4.10, the percentage of PUFA content was higher in all strains. *Scenedesmus obtusus* UMACC 100 produced the highest PUFA content, 71.4% TFA. From the Table 4.2, the higher PUFA content in *Scenedesmus obtusus* UMACC 100 18: 3, 50.5% TFA. The percentage of MUFA is comparably lower within the range of 2-8% TFA on day 0. In day 0, no MUFA content produced in *Oocystis* UMACC 074. Whereas, the higher SFA content was observed in *Chlorella vulgaris* UMACC 103 and *Chlorella* UMACC 253, 59.5% TFA and 59.9% TFA respectively. From this, 16:0 produced high amount of SFA for these two strains as well as for the other strains (Table 4.2).

On day 4, the SFA content was increased while the percentage PUFA was decreased in all the strains (Figure 4.10). The SFA content was produced within the range of 45-70% TFA in all the species in which high amount of SFA was observed in *Oocystis* UMACC 074 about 70.4% TFA. Besides, all the other strains were also possessed high SFA in 16:0. In addition, high percentage of MUFA content was observed in *Chlorella* UMACC 275, 12% TFA compared with other strains on day 4.

The SFA content was decreased as the PUFA content increased again on day 8 in all the ten strains (Figure 4.10). The range of SFA was about 30-50% TFA in which 16:0 attained high amount (Table 4.4), while the range of PUFA was between 40-64% TFA with 18:2 and 18:3 were observed in high amount. The content of MUFA was lower compared to SFA. *Chlorella* UMACC 255 was attained highest MUFA content of 23% TFA.

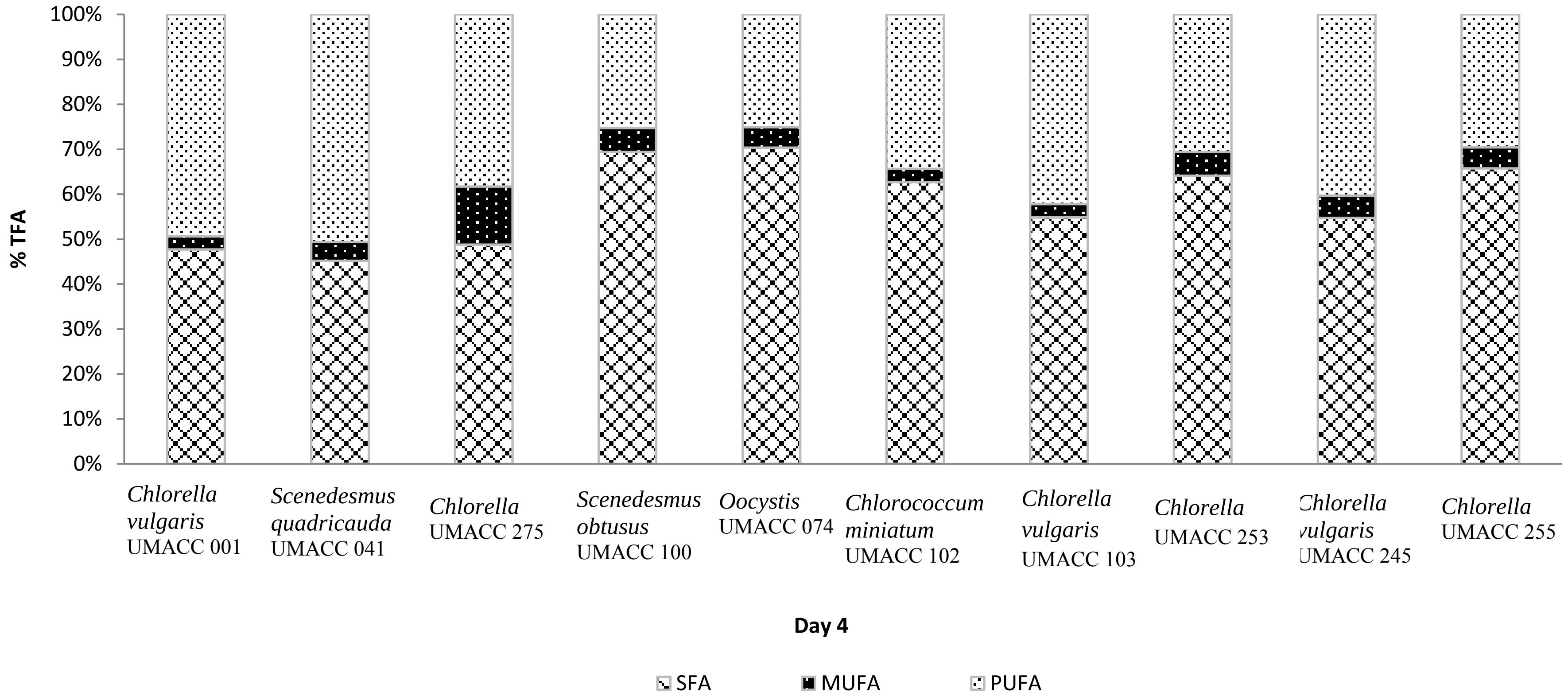
On day 12, the accumulation of PUFAwas increased as the SFA and MUFA were decreased in all the strains. The highest PUFA was observed in *Chlorella vulgaris* UMACC 245 about 84.2% TFA. From the Table 4.5, the higher PUFA content of *Chlorella vulgaris* UMACC 245 was about 23.9% TFA (18:2) and 33.5% TFA (18:3) respectively. The higher SFA content was observed in *Chlorococcum miniatum* UMACC 102, 55.1% TFA.

a) Day 0



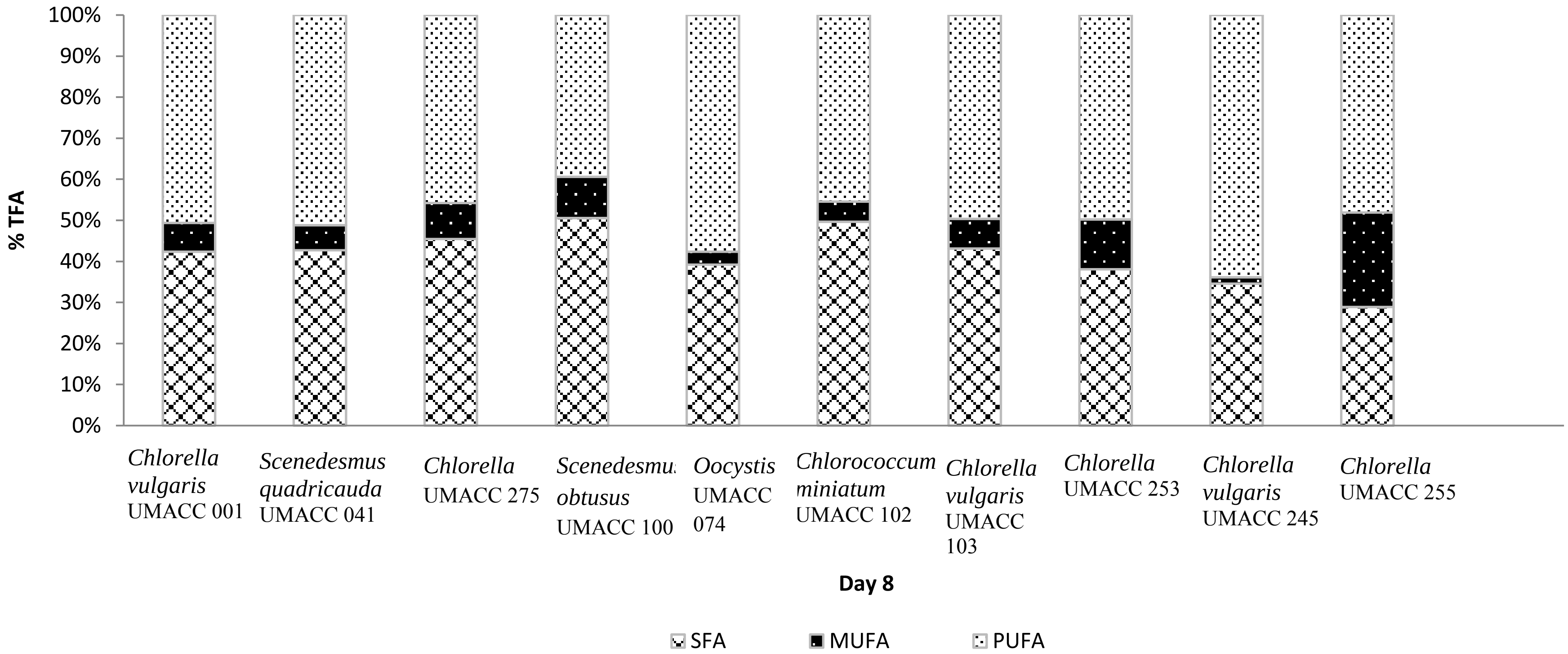
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% TFA
Chlorella vulgaris UMACC 001
Scenedesmus quadricauda UMACC 041
Chlorella UMACC 275
Scenedesmus obtusus UMACC 100
Oocystis UMACC 074
Chlorococcum miniatum UMACC 102
Chlorella vulgaris UMACC 103
Chlorella UMACC 253
Chlorella vulgaris UMACC 245
Chlorella UMACC 255
Day 0
SFA
MUFA
PUFA

b) Day 4



100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% TFA
Chlorella vulgaris UMACC 001
Scenedesmus quadricauda UMACC 041
Chlorella UMACC 275
Scenedesmus obtusus UMACC 100
Oocystis UMACC 074
Chlorococcum miniatum UMACC 102
Chlorella vulgaris UMACC 103
Chlorella UMACC 253
Chlorella vulgaris UMACC 245
Chlorella UMACC 255
Day 4
SFA
MUFA
PUFA

c) Day 8



100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% TFA
Chlorella vulgaris UMACC 001
Scenedesmus quadricauda UMACC 041
Chlorella UMACC 275
Scenedesmus obtusus UMACC 100
Oocystis UMACC 074
Chlorococcum miniatum UMACC 102
Chlorella vulgaris UMACC 103
Chlorella UMACC 253
Chlorella vulgaris UMACC 245
Chlorella UMACC 255
Day 8
SFA
MUFA
PUFA

d) Day 12

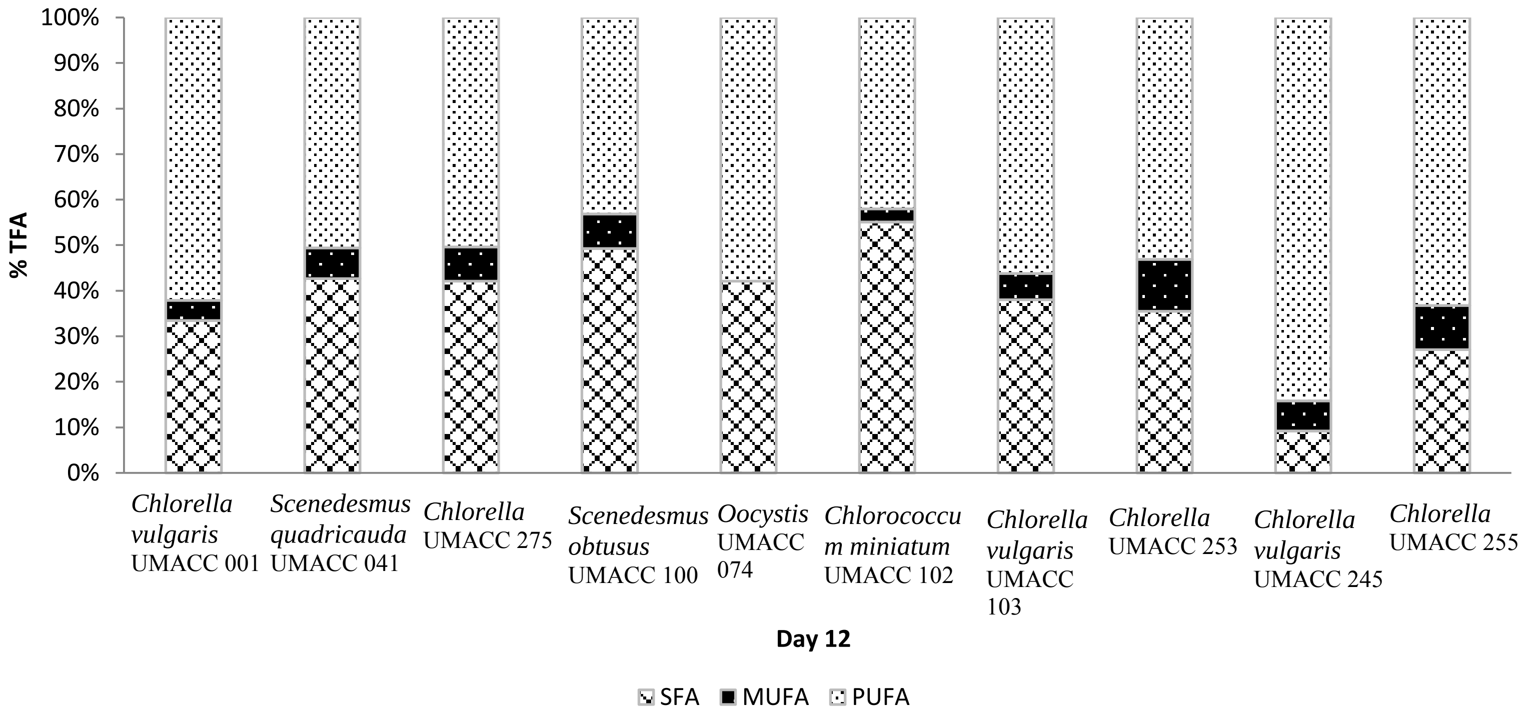


Figure 4.10: Distribution of saturated, monounsaturated and polyunsaturated fatty acids of ten microalgae strains on a) day 0 b) day 4 c) day 8 d) day 12

Table 4.2: Fatty acid distribution of ten microalgae strains on day 0

Fatty acid	*Chlorella vulgaris* UMACC 001	*Scenedesmus quadricauda* UMACC 041	*Chlorella* UMACC 275	*Scenedesmus obtusus* UMACC 100	*Oocystis* UMACC 074	*Chlorococcum miniatum* UMACC 102	*Chlorella vulgaris* UMACC 103	*Chlorella* UMACC 253	*Chlorella vulgaris* UMACC 245	*Chlorella* UMACC 255
Saturated										
14:0	1.7	0.8	3.4	1.5	2.3	1.1	3.2	3.3	1.3	1.6
16:0	22.6	22.8	42.6	19.7	33.2	26.7	45.9	45.3	26.1	22.7
18:0	3.1	7.8	4.0	3.9	10.2	18.0	10.3	11.3	22.5	0.6
Sum	**27.4**	**31.4**	**49.9**	**25.1**	**45.7**	**45.8**	**59.5**	**59.9**	**50.0**	**25.0**
Monounsaturated										
16:1	3.2	2.9	6.5	2.6	0.0	3.0	3.4	4.6	4.6	2.0
18:1	2.2	0.0	1.5	1.0	0.0	0.2	0.0	0.0	0.7	0.0
Sum	**5.3**	**2.9**	**8.0**	**3.5**	**0.0**	**3.2**	**3.4**	**4.6**	**5.3**	**2.0**
Polyunsaturated										
16:2	5.1	0.6	0.0	0.4	2.3	0.2	0.0	0.0	0.0	13.2
16:3	10.1	16.3	5.3	14.6	7.9	11.5	7.5	9.5	4.8	13.9
18:2	11.5	11.0	15.3	5.8	16.2	39.3	5.5	7.2	25.2	18.2
18:3	40.6	37.7	21.4	50.5	27.9	0.0	24.1	18.8	14.7	27.6
Sum	**67.3**	**65.7**	**42.1**	**71.4**	**54.3**	**51.0**	**37.2**	**35.6**	**44.7**	**73.0**

Table 4.3: Fatty acid distribution of ten microalgae strains on day 4

Fatty acid	*Chlorella vulgaris* UMACC 001	*Scenedesmus quadricauda* UMACC 041	*Chlorella* UMACC 275	*Scenedesmus obtusus* UMACC 100	*Oocystis* UMACC 074	*Chlorococcum miniatum* UMACC 102	*Chlorella vulgaris* UMACC 103	*Chlorella* UMACC 253	*Chlorella vulgaris* UMACC 245	*Chlorella* UMACC 255
Saturated										
14:0	2.7	2.8	3.3	4.5	3.5	3.2	3.1	4.5	2.1	5.0
16:0	39.3	34.2	40.8	53.2	50.3	46.7	43.1	50.3	33.3	59.1
18:0	5.8	8.2	4.8	11.8	16.5	12.8	8.6	9.4	19.3	1.7
Sum	**47.7**	**45.2**	**48.8**	**69.4**	**70.4**	**62.7**	**54.8**	**64.1**	**54.7**	**65.8**
Monounsaturated										
16:1	2.9	2.6	11.1	3.9	4.6	2.9	3.0	5.3	4.1	4.7
18:1	0.0	1.6	1.8	1.4	0.0	0.0	0.0	0.0	0.8	0.0
Sum	**2.9**	**4.2**	**12.9**	**5.3**	**4.6**	**2.9**	**3.0**	**5.3**	**5.0**	**4.7**
Polyunsaturated										
16:2	1.5	1.8	0.0	0.0	0.0	0.0	1.9	0.0	0.0	0.0
16:3	8.6	9.9	6.0	16.3	5.2	10.1	7.8	4.3	4.7	5.2
18:2	13.2	12.9	23.8	7.1	19.9	24.3	12.7	13.7	23.7	5.2
18:3	26.2	26.0	8.6	1.8	0.0	0.0	19.8	12.6	11.9	19.2
Sum	**49.4**	**50.7**	**38.3**	**25.3**	**25.1**	**34.4**	**42.2**	**30.6**	**40.3**	**29.6**

Table 4.4: Fatty acid distribution of ten microalgae strains on day 8

Fatty acid	*Chlorella vulgaris* UMACC 001	*Scenedesmus quadricauda* UMACC 041	*Chlorella* UMACC 275	*Scenedesmus obtusus* UMACC 100	*Oocystis* UMACC 074	*Chlorococcum miniatum* UMACC 102	*Chlorella vulgaris* UMACC 103	*Chlorella* UMACC 253	*Chlorella vulgaris* UMACC 245	*Chlorella* UMACC 255
Saturated										
14:0	2.7	2.7	2.4	3.3	1.7	1.7	1.4	2.6	0.9	1.1
16:0	34.1	33.5	38.5	39.2	28.4	31.3	32.8	24.1	22.6	22.6
18:0	5.6	6.6	4.5	8.1	9.1	16.8	8.9	11.4	11.1	5.2
Sum	**42.4**	**42.7**	**45.4**	**50.6**	**39.2**	**49.7**	**43.1**	**38.1**	**34.6**	**28.9**
Monounsaturated										
16:1	6.0	3.5	7.5	7.2	3.2	4.9	6.4	11.7	1.5	1.7
18:1	1.1	2.6	1.3	2.8	0.0	0.0	0.8	0.5	0.0	21.3
Sum	**7.0**	**6.1**	**8.8**	**10.0**	**3.2**	**4.9**	**7.2**	**12.2**	**1.5**	**23.0**
Polyunsaturated										
16:2	4.8	3.6	0.0	0.7	0.0	0.0	0.0	0.0	8.2	6.1
16:3	7.4	11.9	6.9	11.2	8.1	9.1	12.1	8.3	8.0	7.3
18:2	14.9	14.5	22.2	10.3	20.0	22.3	14.8	25.0	24.5	13.3
18:3	23.6	21.2	16.6	17.3	29.5	14.0	22.7	16.5	23.1	21.5
Sum	**50.6**	**51.2**	**45.7**	**39.4**	**57.6**	**45.4**	**49.6**	**49.8**	**63.9**	**48.1**

Table 4.5: Fatty acid distribution of ten microalgae strains on day 12

Fatty acid	*Chlorella vulgaris* UMACC 001	*Scenedesmus quadricauda* UMACC 041	*Chlorella* UMACC 275	*Scenedesmus obtusus* UMACC 100	*Oocystis* UMACC 074	*Chlorococcum miniatum* UMACC 102	*Chlorella vulgaris* UMACC 103	*Chlorella* UMACC 253	*Chlorella vulgaris* UMACC 245	*Chlorella* UMACC 255
Saturated										
14:0	1.9	1.7	2.7	2.3	2.2	2.9	1.1	1.0	1.6	1.3
16:0	26.0	32.5	35.5	41.2	30.4	40.5	31.0	29.1	2.4	21.6
18:0	5.5	8.5	3.8	5.7	9.5	11.7	5.9	5.3	5.2	4.2
Sum	**33.5**	**42.7**	**42.1**	**49.3**	**42.1**	**55.1**	**38.0**	**35.5**	**9.2**	**27.1**
Monounsaturated										
16:1	3.6	4.4	6.4	4.0	0.0	2.9	5.5	5.4	0.0	3.5
18:1	0.7	2.3	1.1	3.7	0.0	0.0	0.4	6.0	6.6	6.2
Sum	**4.4**	**6.7**	**7.5**	**7.6**	**0.0**	**2.9**	**5.8**	**11.4**	**6.6**	**9.7**
Polyunsaturated										
16:2	4.3	0.0	0.0	0.8	3.0	2.2	2.2	7.2	13.4	8.0
16:3	11.0	14.6	5.9	14.6	2.7	10.9	12.8	6.3	13.3	8.7
18:2	18.2	25.3	23.8	14.2	19.1	21.7	15.7	24.5	23.9	16.9
18:3	28.6	10.8	20.7	13.5	33.0	7.2	25.5	15.1	33.5	29.5
Sum	**62.2**	**50.6**	**50.4**	**43.1**	**57.9**	**42.0**	**56.2**	**53.1**	**84.2**	**63.2**

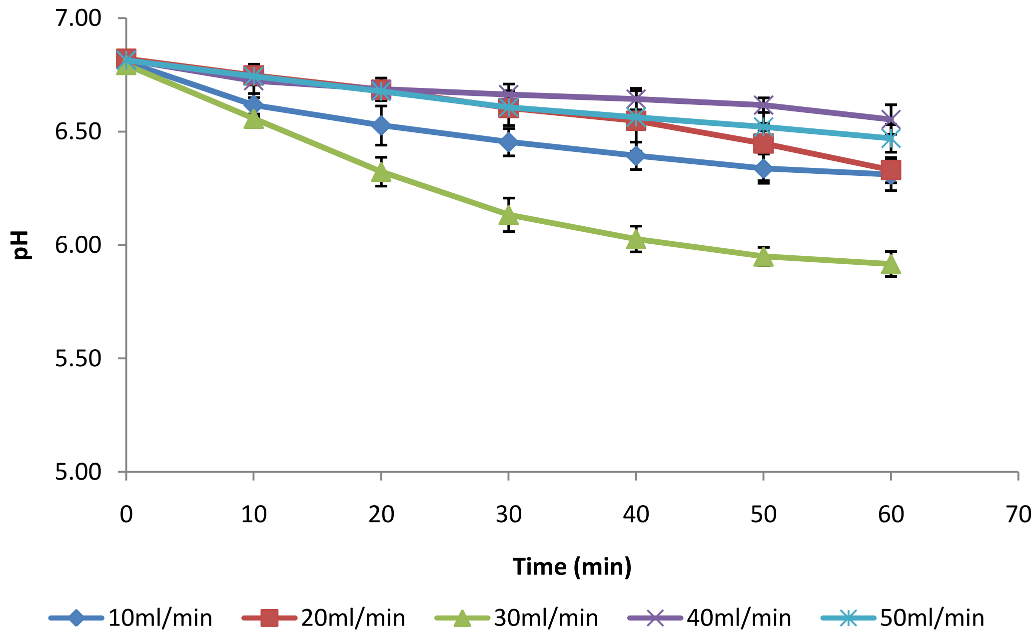
4.2 EXPERIMENT II: GROWTH AND BIOCHEMICAL COMPOSITION OF SELECTED MICROALGAE AT DIFFERENT LEVELS OF CARBON DIOXIDE

4.2.1 Calibration of CO_2 Levels (Appendix F)

4.2.1.1 pH of BBM at different flow rates

Based on Figure 4.11, the higher pH was obtained at the flow rate of 40ml CO_2/min in both 5% CO_2 and 10% CO_2. The lowest pH was observed at flow rate of 30ml CO_2/min in BBM medium.

a) Flow rates at 5% CO_2



b) Flow rates at 10% CO_2

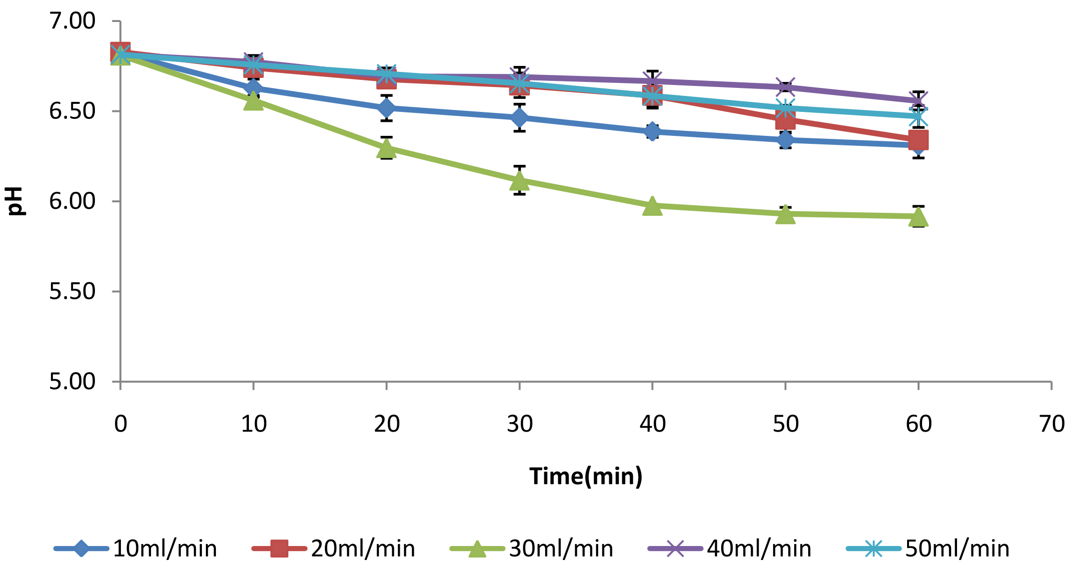
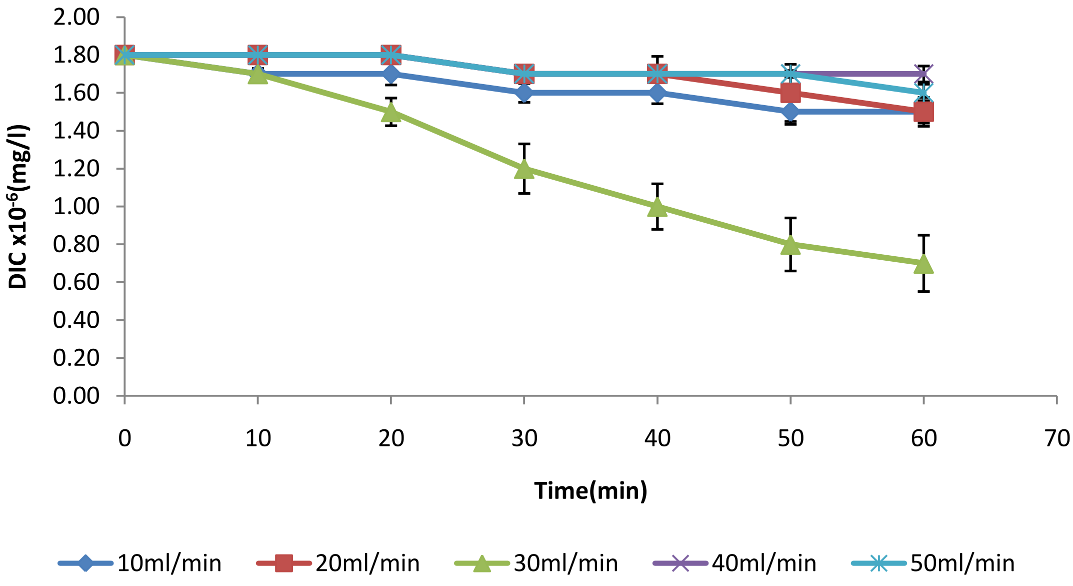


Figure 4.11: pH of BBM at flow rates of a) 5% CO_2 b) 10% CO_2. Data represents the means ±S.D.

4.2.1.2 Dissolved Inorganic Carbon (DIC) at different flow rates

Based on the pH (Figure 4.11), the amount of DIC was calculated (section 3.7.3) and plotted shown in Figure 4.12. Based on this figure, it was shown that high amount of DIC was observed at 40ml CO_2/min. Based on this calibration graph, 40ml CO_2/min was determined as the flow rate to grow the selected microalgae at 5% and 10% of CO_2.

a) 5% CO_2



b) 10% CO_2

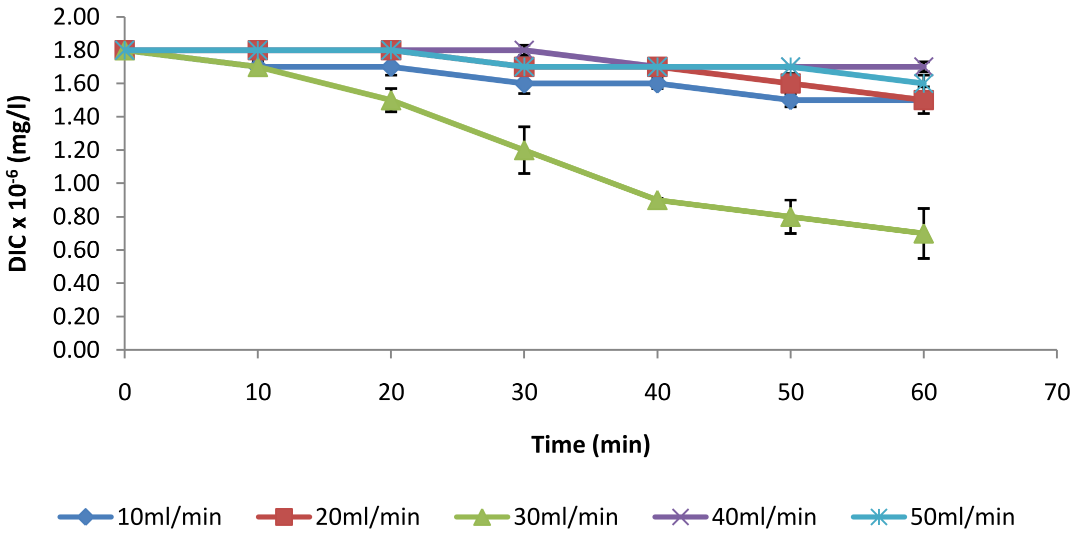


Figure 4.12: DIC at different flow rates of a) 5% CO_2 b) 10% CO_2. Data represents the means ±S.D.

4.2.2 Growth Trends (Appendix G)

The cultures were grown under conditions:

i) Control where no aeration was supplied

ii) Aeration with aquarium pump, supply 0.04% CO_2 (ambient condition)

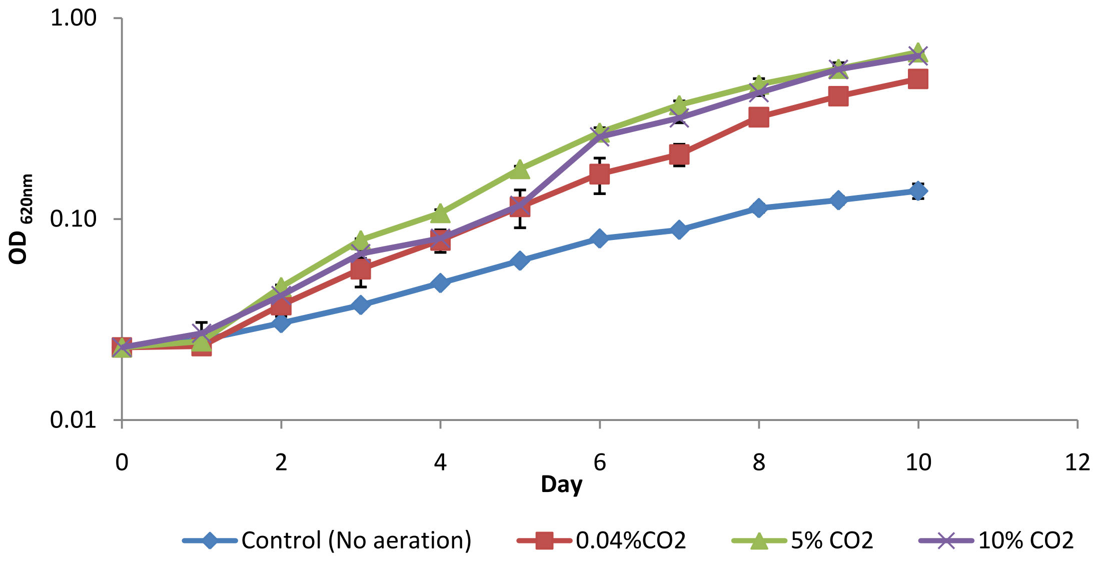
iii) Aeration with 5% CO_2 mixed with compressed air (MOX Linde Gas)

iv) Aeration with 10% CO_2 mixed with compressed air (MOX Linde Gas)

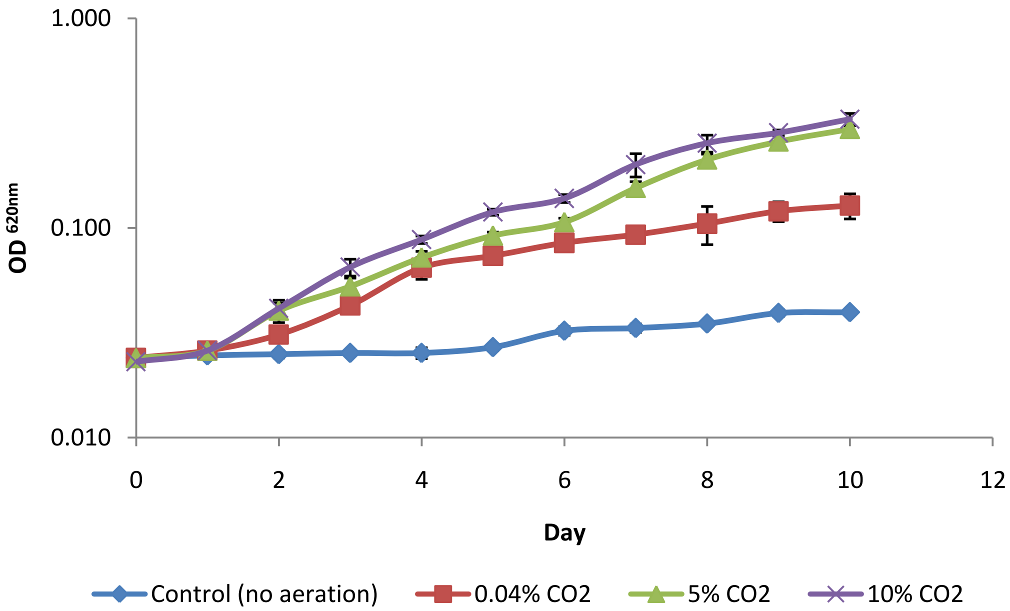
4.2.2.1 Growth Trends based on OD_{620nm}

The higher growth was attained at 5% CO_2 in *Chlorella vulgaris* UMACC 001, *Scenedesmus quadricauda* UMACC 041 and *Oocystis* UMACC 074 whereas *Chlorella* UMACC 275 attained highest growth rate at 10% CO_2 (Figure 4.13). The lowest growth was observed in control in all the four strains. At 5% the highest growth trend was observed in *Chlorella vulgaris* UMACC 001 followed by *Scenedesmus quadricauda* UMACC 041 and *Chlorella* UMACC 275 respectively. *Chlorella vulgaris* UMACC 001 grew better than other strains in all CO_2 levels.

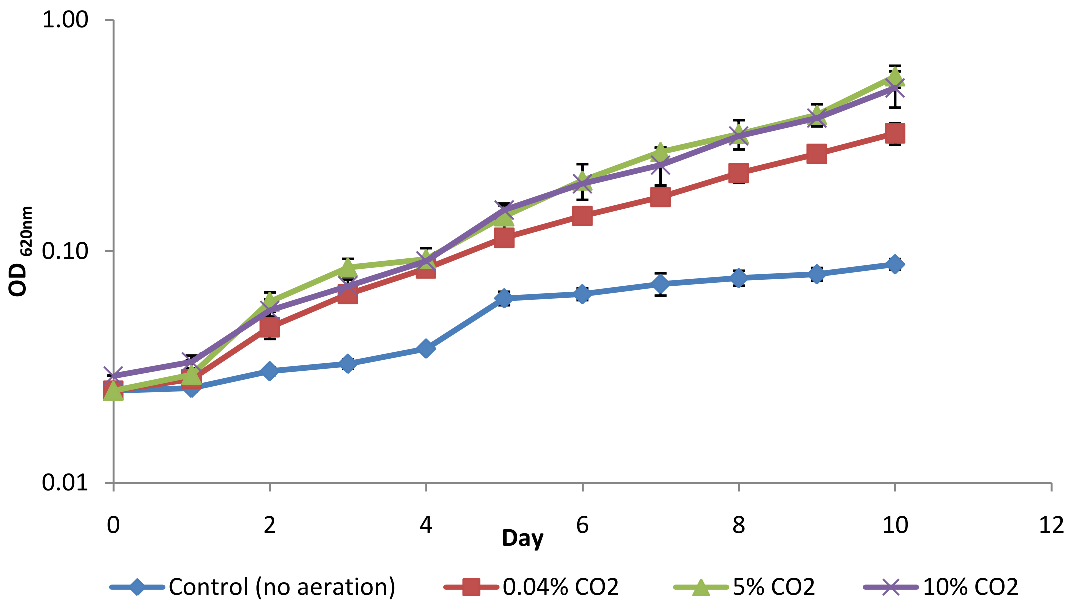
a) *Chlorella vulgaris* UMACC 001



b) *Chlorella* UMACC 275



c) *Scenedesmus quadricauda* UMACC 041



d) *Oocystis* UMACC 074

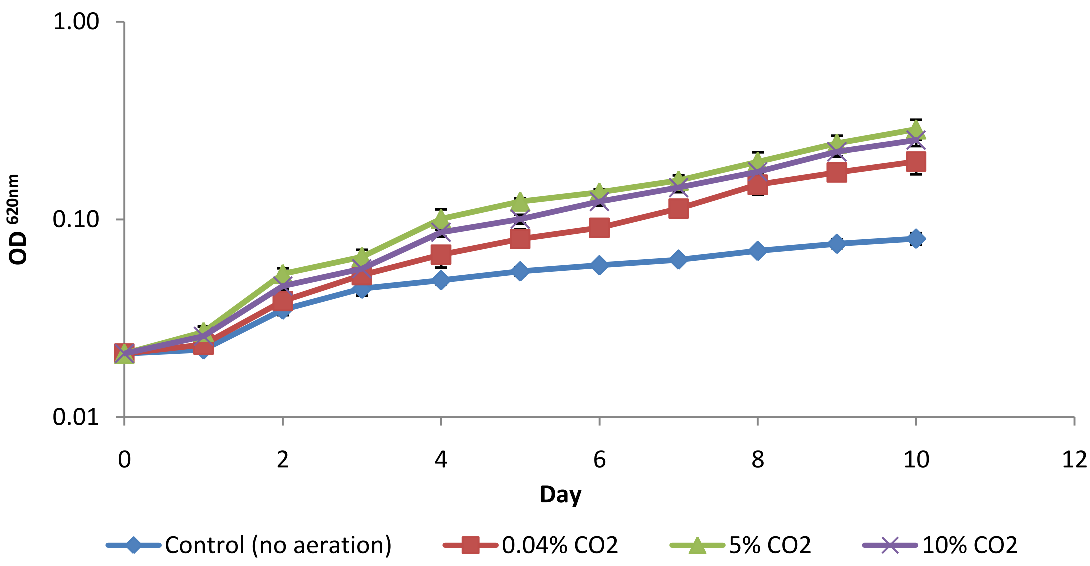
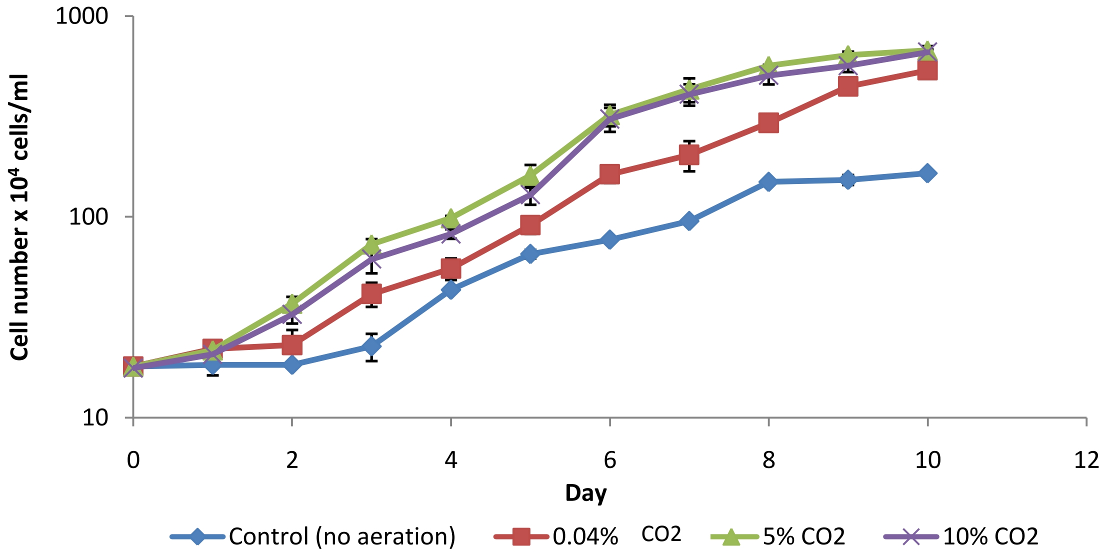


Figure 4.13: Semi logarithmic growth curves of a) *Chlorella vulgaris* UMACC 001 b) *Chlorella* UMACC 275 c) *Scenedesmus quadricauda* UMACC 041 and d) *Oocystis* UMACC 074 based on OD_{620nm}. Data represents the means ±S.D.

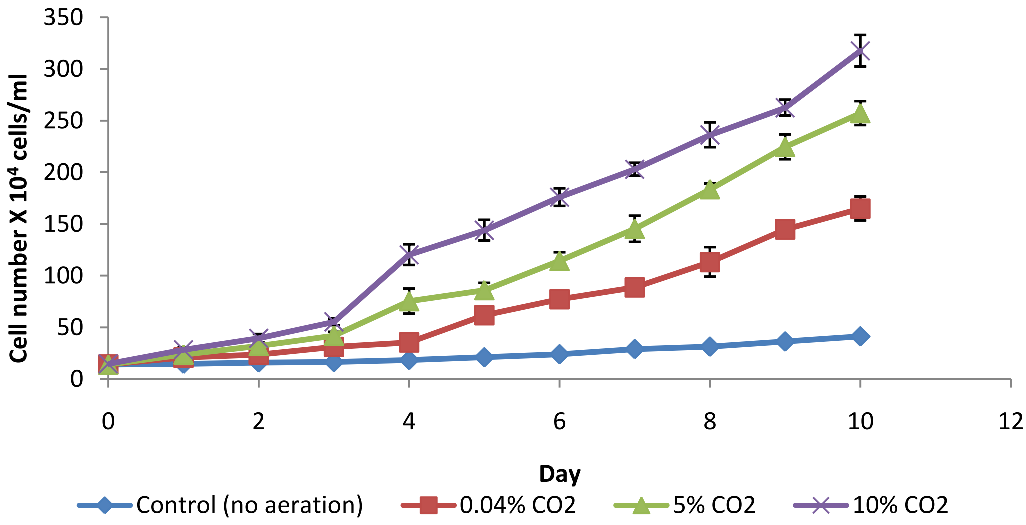
4.2.2.2 Growth trends based on cell number

Similar growth trend based on OD_{620nm} was observed in all the strains. The highest cell count was produced in *Chlorella vulgaris* UMACC 001 at 5% CO_2 followed by *Chlorella* UMACC 275 and *Scenedesmus quadricauda* UMACC 041 (Figure 4.14). The lowest growth was observed in *Oocystis* UMACC 074. For *Chlorella* UMACC 275, higher growth was produced at 10% CO_2 compared with other strains.

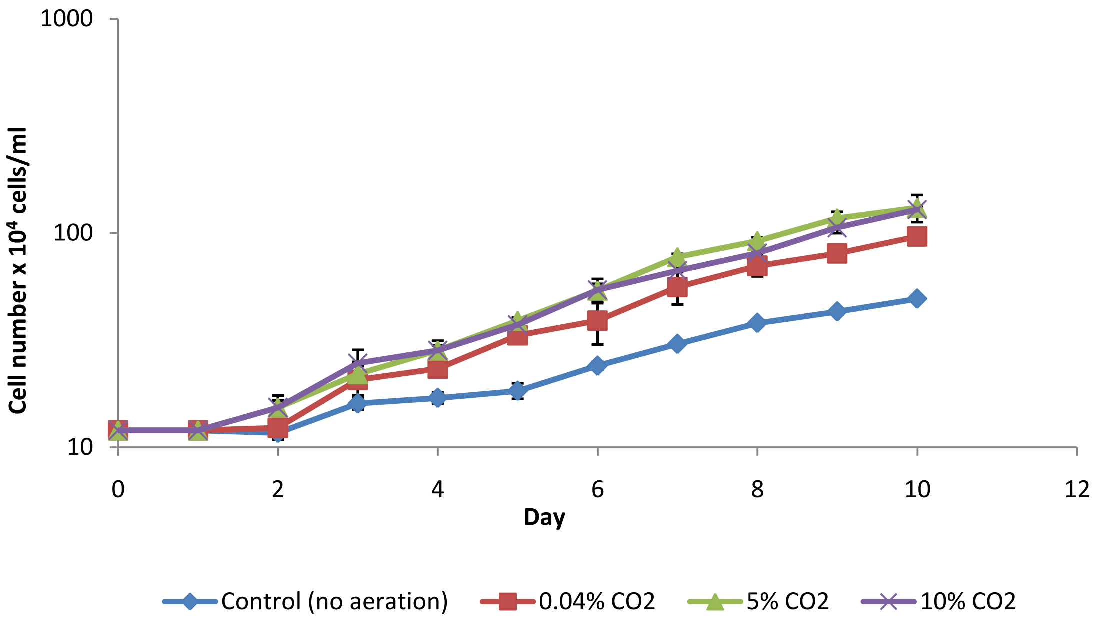
a) *Chlorella vulgaris* UMACC 001



b) *Chlorella* UMACC 275



c) *Scenedesmus quadricauda* UMACC 041



d) *Oocystis* UMACC 074

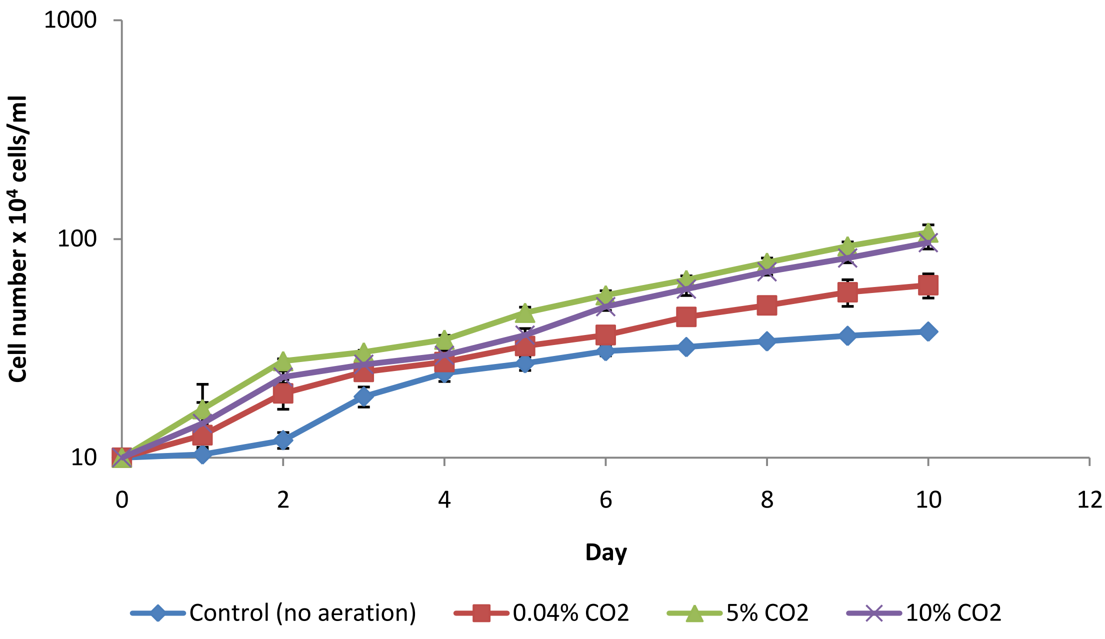
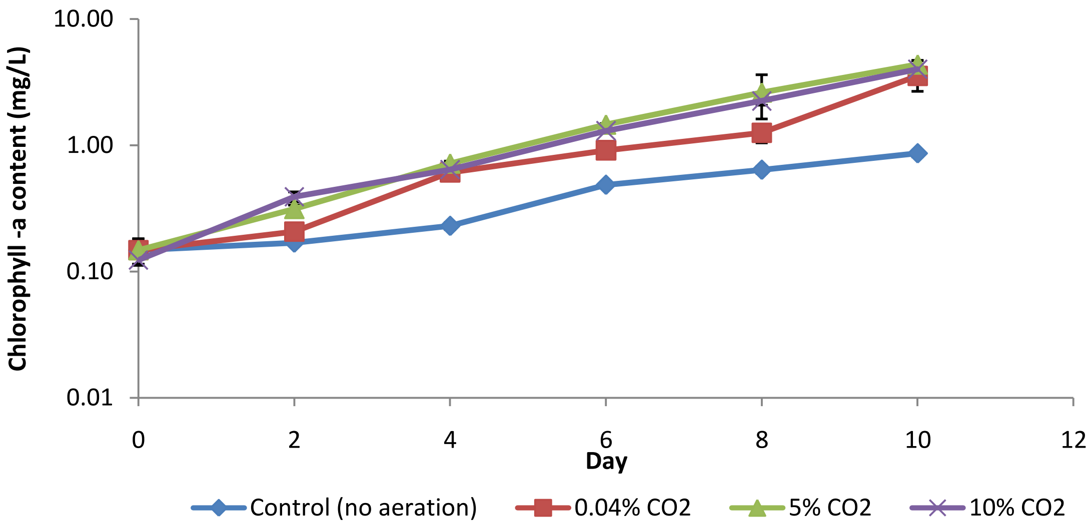


Figure 4.14: Semi logarithmic growth curves of a) *Chlorella vulgaris* UMACC 001 b) *Chlorella* UMACC 275 c) *Scenedesmus quadricauda* UMACC 041 and d) *Oocystis* UMACC 074 based on cell count. Data represents the means ±S.D.

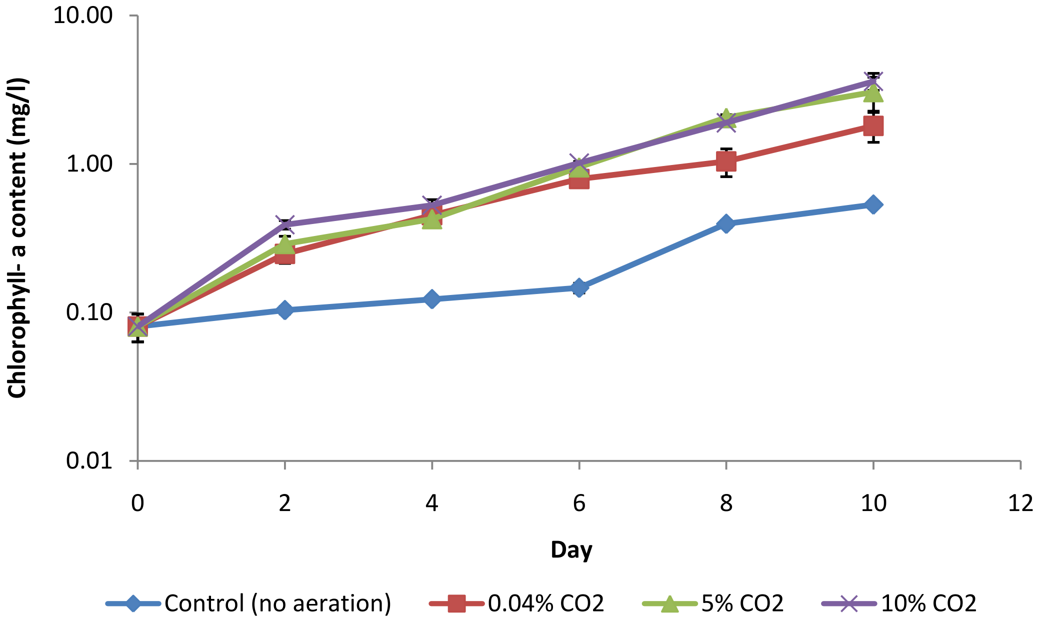
4.2.2.3 Growth trends based on chlorophyll-a content

Based on the Figure 4.15, chlorophyll-a content possessed similar trend as OD_{620nm} and cell count. The highest chlorophyll a content was obtained on day 10 in *Chlorella vulgaris* UMACC 001 about 4.35±0.325mg/l at 5% CO_2. While in *Chlorella* UMACC 275, the highest chlorophyll a content was 3.60±0.473mg/L at 10% CO_2. For *Scenedesmus quadricauda* UMACC 041, the higher chlorophyll a content was 2.933±0.451 mg/l at 5% CO_2 and *Oocystis* UMACC 074 attained higher chlorophyll-a content 1.88±0.083mg/l at 10% CO_2. From all the four strains, the lowest cell concentration was observed at control while the in the ambient condition the cell growth relatively lower than the cultures aerated with 5% CO_2 and 10% CO_2.

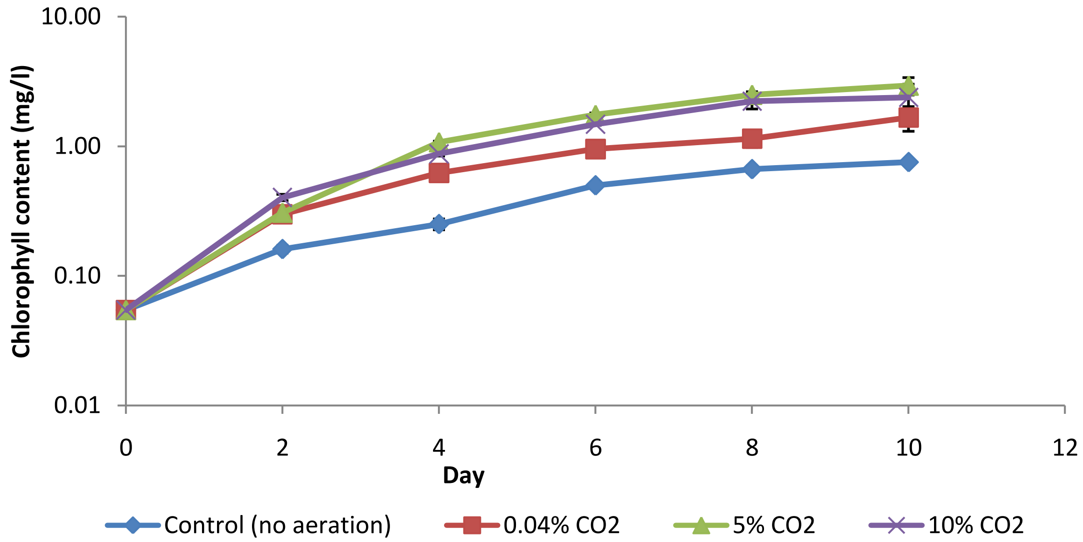
a) *Chlorella vulgaris* UMACC 001



b) *Chlorella* UMACC 275



c) *Scenedesmus quadricauda* UMACC 041



d) *Oocystis* UMACC 074

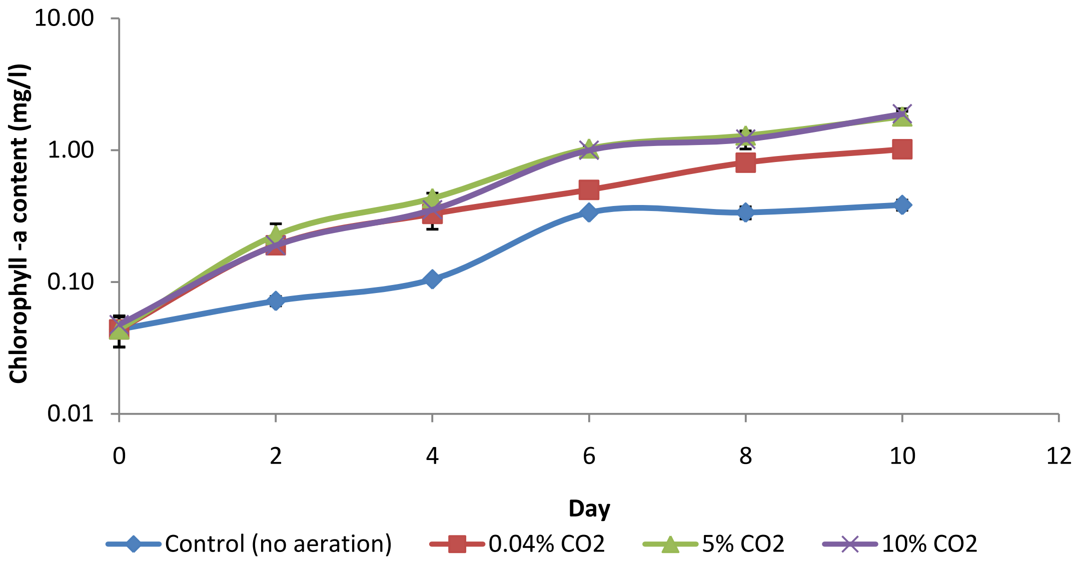


Figure 4.15: Semi logarithmic growth curves of a) *Chlorella vulgaris* UMACC 001 b) *Chlorella* UMACC 275 c) *Scenedesmus quadricauda* UMACC 041 and d) *Oocystis* UMACC 074 based on chlorophyll a concentration. Data represents the means ±S.D.

4.2.2.4 Specific growth rates of selected strains based on chlorophyll-a content

Based on the chlorophyll a content, the specific growth rates of the strains at different levels was calculated. Of the four strains studied, *Chlorella vulgaris* UMACC 001 produced highest specific growth rate at 5% CO_2, 0.3707±0.0171 day^{-1} (Table 4.6). *Chlorella* UMACC 275 produced higher (μ), 0.3419±0.0256 day^{-1} at 10% CO_2. *Scenedesmus quadricauda* UMACC 041 attained higher growth rate at 5% CO_2, 0.3354±0.0382 day^{-1}. For *Oocystis* UMACC 074, at 10% CO_2 showed higher growth rate of 0.3222±0.0276 day^{-1}. All the four strains showed relatively lower (μ) at ambient level compared with 5% CO_2 and 10% CO_2.

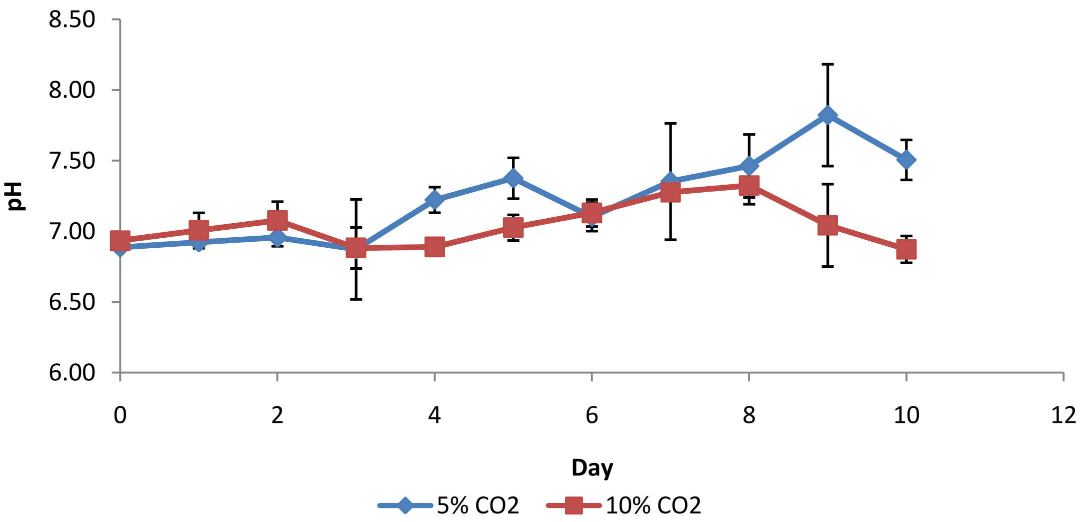
Table 4.6: Specific growth rate of *Chlorella vulgaris* UMACC 001, *Chlorella* UMACC 275, *Scenedesmus quadricauda* UMACC 041 and *Oocystis* UMACC 074 based on chlorophyll a concentration

Treatment	*Chlorella vulgaris* UMACC 001(Specific growth rate, µ day^{-1})	*Chlorella* UMACC 275 (Specific growth rate, µ day^{-1})	*Scenedesmus quadricauda* UMACC 041 (Specific growth rate, µ day^{-1})	*Oocystis* UMACC 074 (Specific growth rate, µ day^{-1})
Control (no aeration)	0.1280±0.0017	0.1245±0.0145	0.1240±0.0179	0.1156±0.0057
0.04% CO_2	0.2005±0.0169	0.1713±0.0661	0.1644±0.0213	0.1525±0.0210
5% CO_2	0.3707±0.0171	0.2620±0.0194	0.3354±0.0382	0.2987±0.0432
10% CO_2	0.3305±0.0252	0.3419±0.0256	0.3035±0.0033	0.3222±0.0276

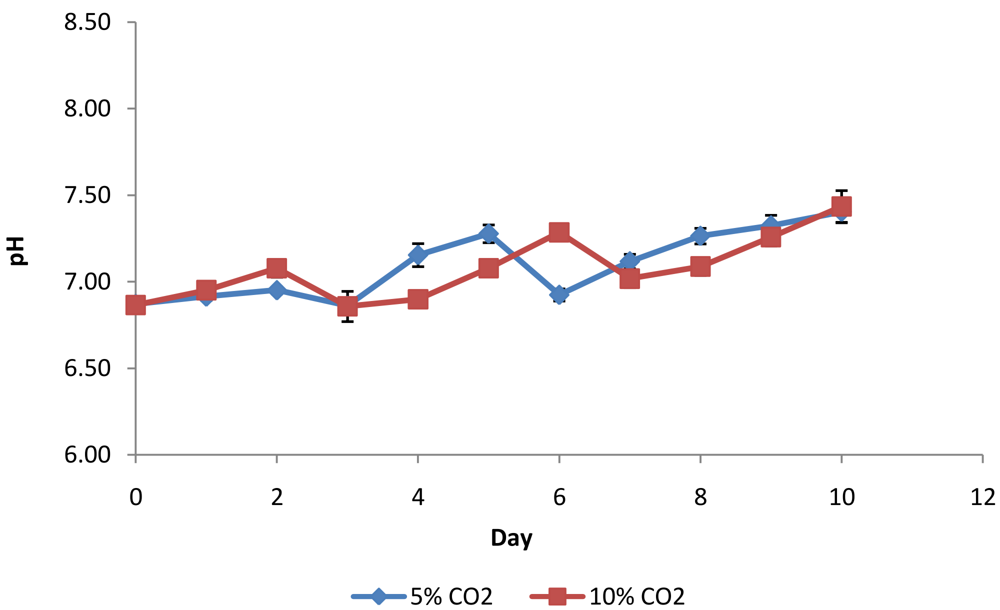
4.2.2.5 pH of culture medium

Based on the Figure 4.16, the pH of each species varies at 5% CO_2 and 10% CO_2. Overall, the range of pH for all the species was between 6.2 - 7.8. For *Chlorella vulgaris* UMACC 001, *Chlorella* UMACC 275 and *Oocystis* UMACC 074 the change of pH seems to be higher at 5% CO_2 compared with 10% CO_2. Whereas, the changes in pH was higher at 10% CO_2 for *Scenedesmus quadricauda* UMACC 041.

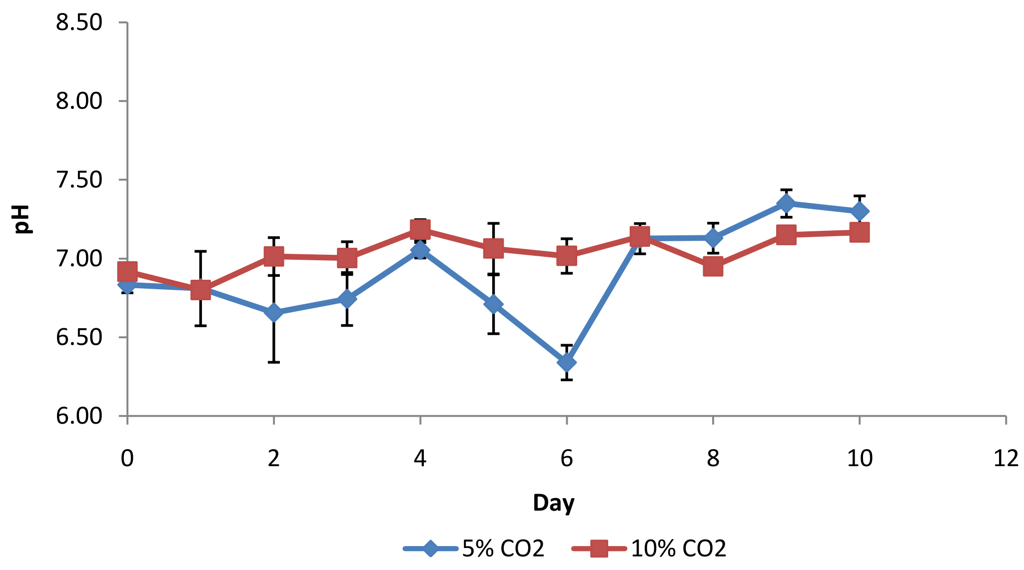
a) *Chlorella vulgaris* UMACC 001



b) *Chlorella* UMACC 275



c) *Scenedesmus quadricauda* UMACC 041



d) *Oocystis* UMACC 074

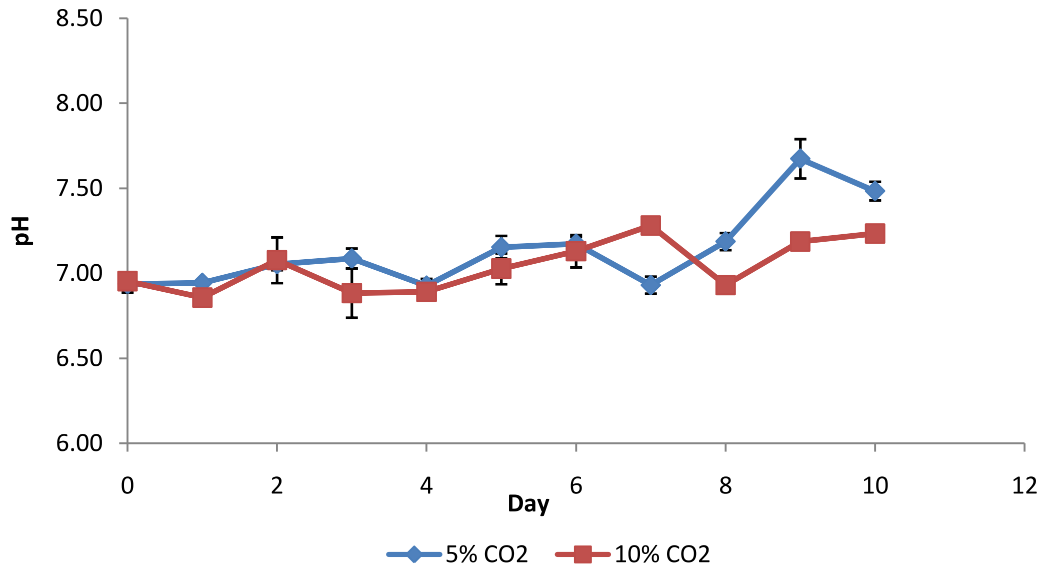


Figure 4.16: pH of a) *Chlorella vulgaris* UMACC 001 b) *Chlorella* UMACC 275 c) *Scenedesmus quadricauda* UMACC 041 and d) *Oocystis* UMACC 074 at 5% CO_2 and 10% CO_2. Data represents the means ±S.D.

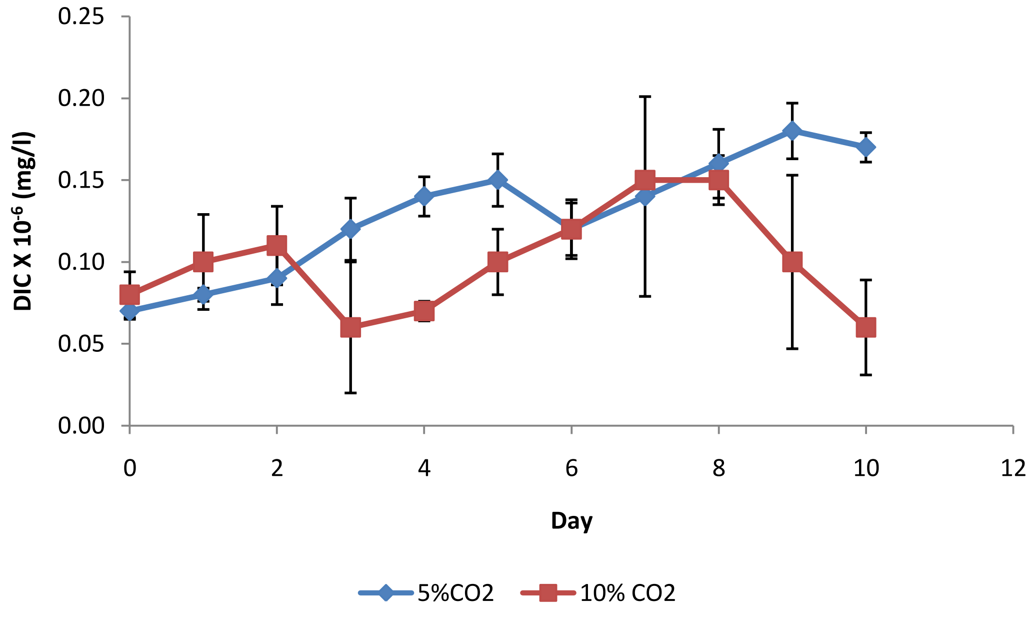
4.2.2.6 Amount of Dissolved Inorganic Carbon (DIC)

Based on pH, the amount of dissolved CO_2 was calculated for 5% and 10% CO_2 as described in section 3.7.3.

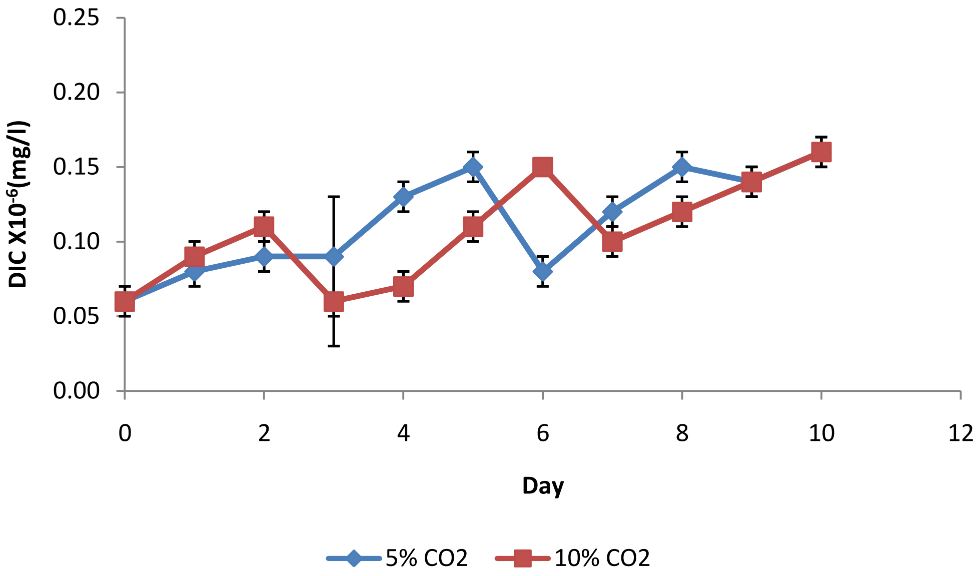
The amount of dissolved carbon dioxide in culture medium was similar for all the strains ranging from 0.00000004 to 0.00000016 mg/L (Figure 4.17). For *Chlorella vulgaris* UMACC 001, the dissolved CO_2 found to increase from day 3 in both levels (5% CO_2 and 10% CO_2) and drops on after day 8. Whereas, for *Chlorella* UMACC 275, the dissolved CO_2 fluctuating and after day 7, the amount of CO_2 seems to be increased at both levels. For *Scenedesmus quadricauda* UMACC 041 and *Oocystis* UMACC 074, the amount of dissolved CO_2 fluctuated at both levels.

Although pH was used to estimate the DIC levels, it must be recorded that this may not represent the true DIC content as a culture is complex, with many processes occurring in addition to photosynthesis. This is at best an indicator of DIC levels when 5% and 10% CO_2 introduced into the cultures.

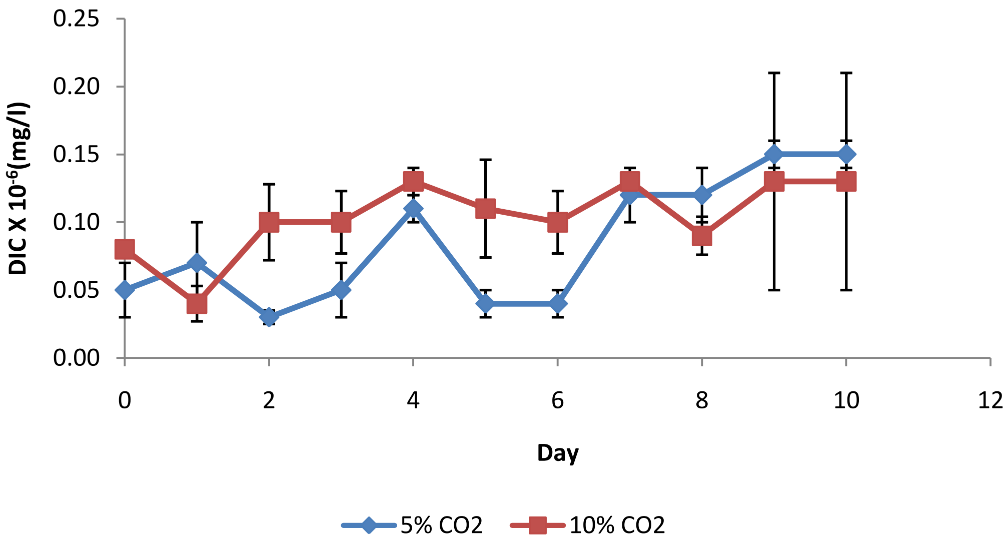
a) *Chlorella vulgaris* UMACC 001



b) *Chlorella* UMACC 275



c) *Scenedesmus quadricauda* UMACC 041



d) *Oocystis* UMACC 074

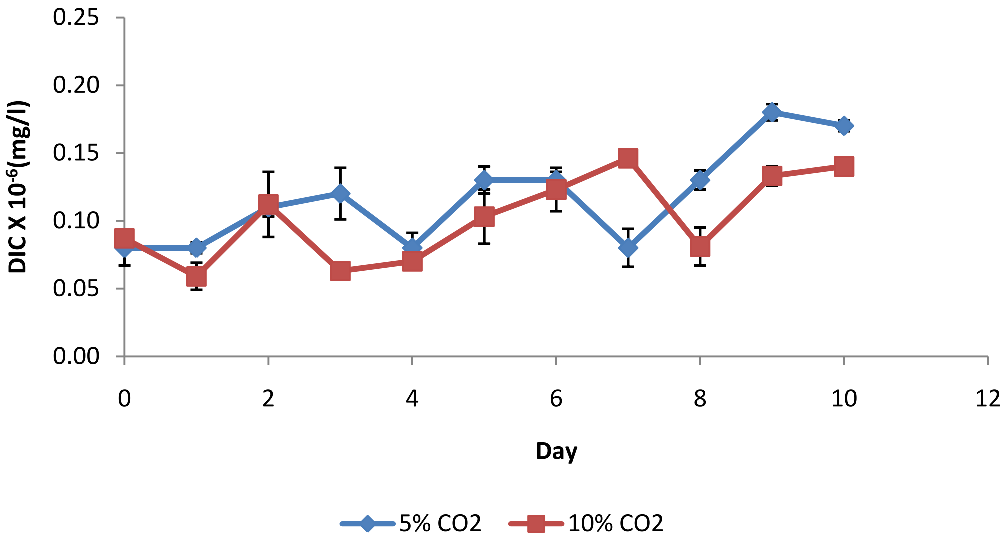
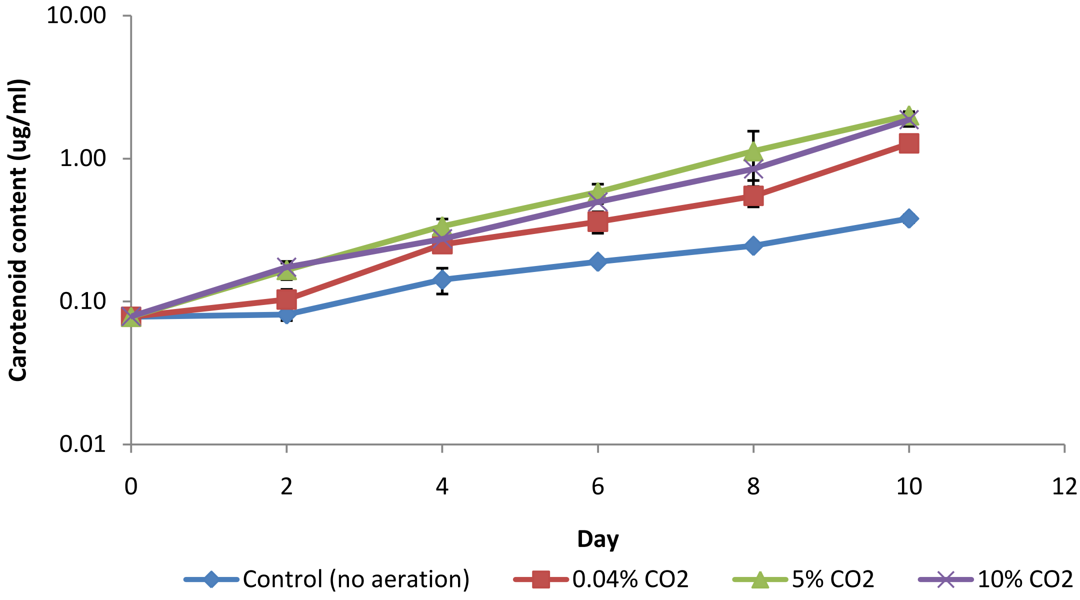


Figure 4.17: Amount of dissolved CO_2 of a) *Chlorella vulgaris* UMACC 001 b)*Chlorella* UMACC 275 c) *Scenedesmus quadricauda* UMACC 041 and d) *Oocystis* UMACC 074 at 5% and 10% CO_2. Data represents the means ±S.D.

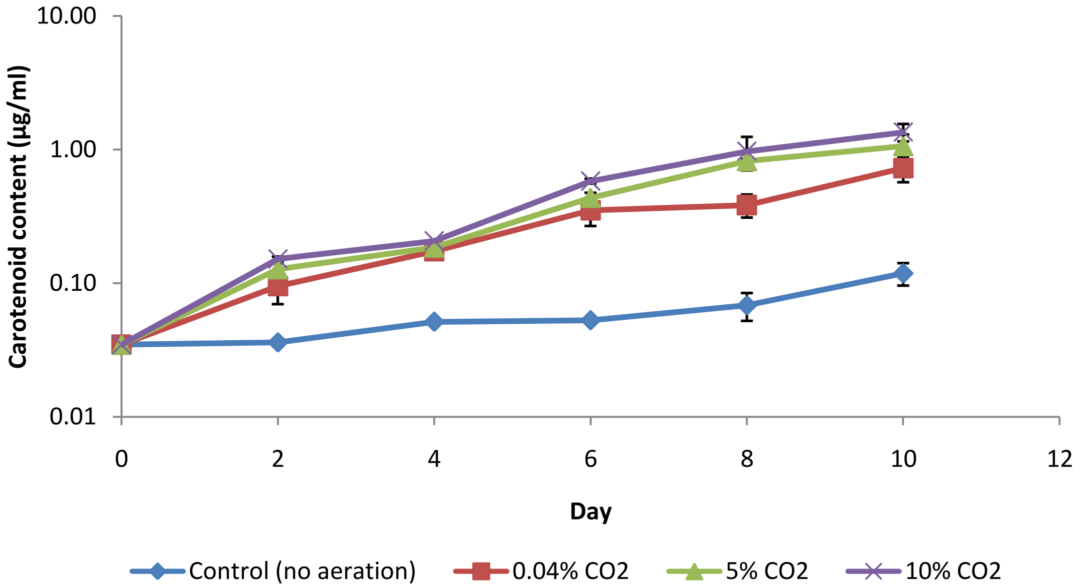
4.2.3 Carotenoid Content

For the carotenoid content, the highest concentration was observed in *Chlorella vulgaris* UMACC 001 at 5% CO_2, 2.007±0.117µg/ml followed by 1.868±0.192µg/ml ($p<0.05$) at 10% CO_2 on day 10 (Figure 4.18). *Chlorella* UMACC 275 attained higher carotenoids at 10% CO_2, 1.351±0.201µg/ml ($p<0.05$) on day 10. The other two strains *Scenedesmus quadricauda* UMACC 041 and *Oocystis* 074 achieved higher carotenoid content at 5% CO_2 1.351±0.177µg/ml ($p<0.05$) and 0.795±0.128µg/ml ($p<0.05$) respectively. Lowest carotenoids was observed in control and ambient condition in all the four strains.

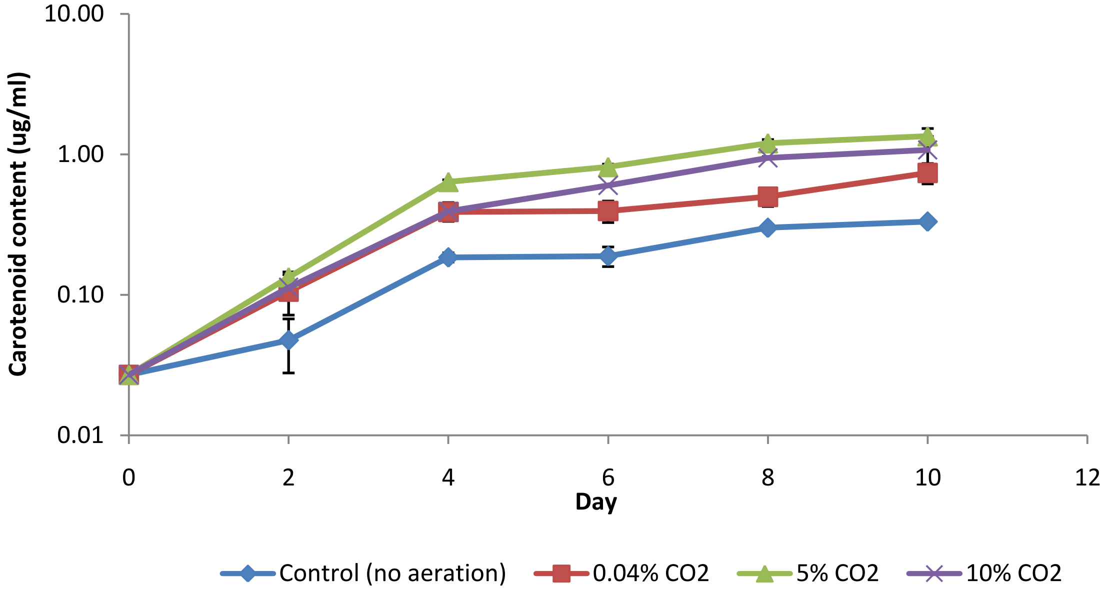
a) *Chlorella vulgaris* UMACC 001



b) *Chlorella* UMACC 275



c) *Scenedesmus quadricauda* UMACC 041



d) *Oocystis* UMACC 074

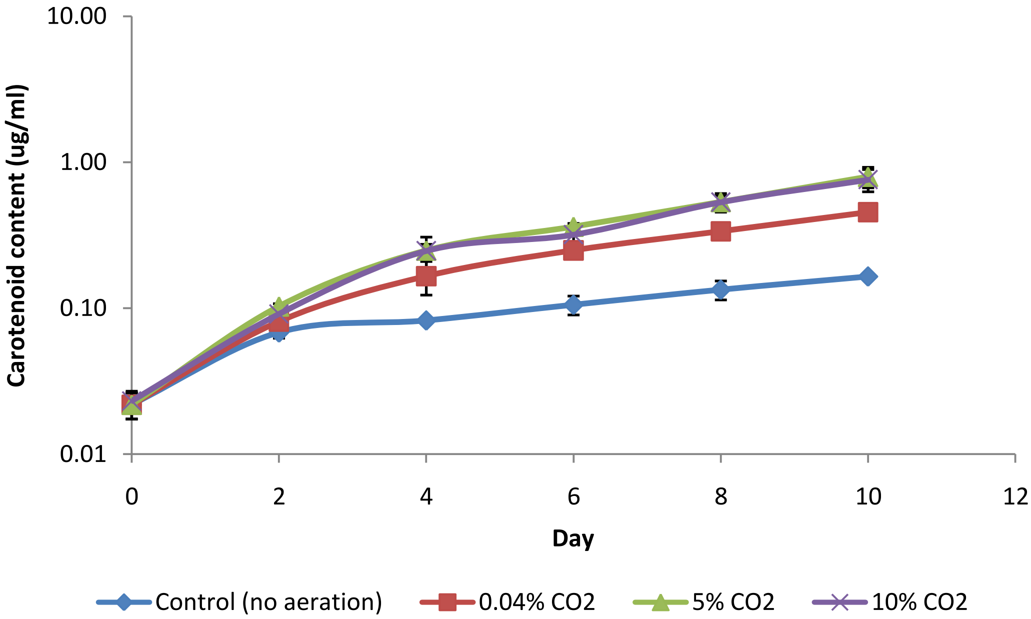
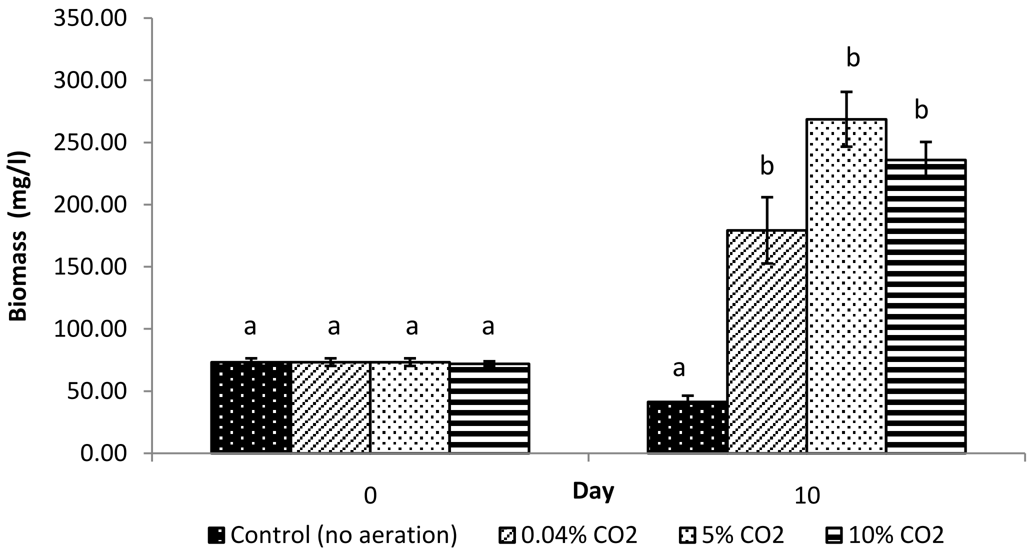


Figure 4.18: Semi logarithmic growth curves of a) *Chlorella vulgaris* UMACC 001 b) *Chlorella* UMACC 275 c) *Scenedesmus quadricauda* UMACC 041 and d) *Oocystis* UMACC 074 based on carotenoid content. Data represents the means ±S.D.

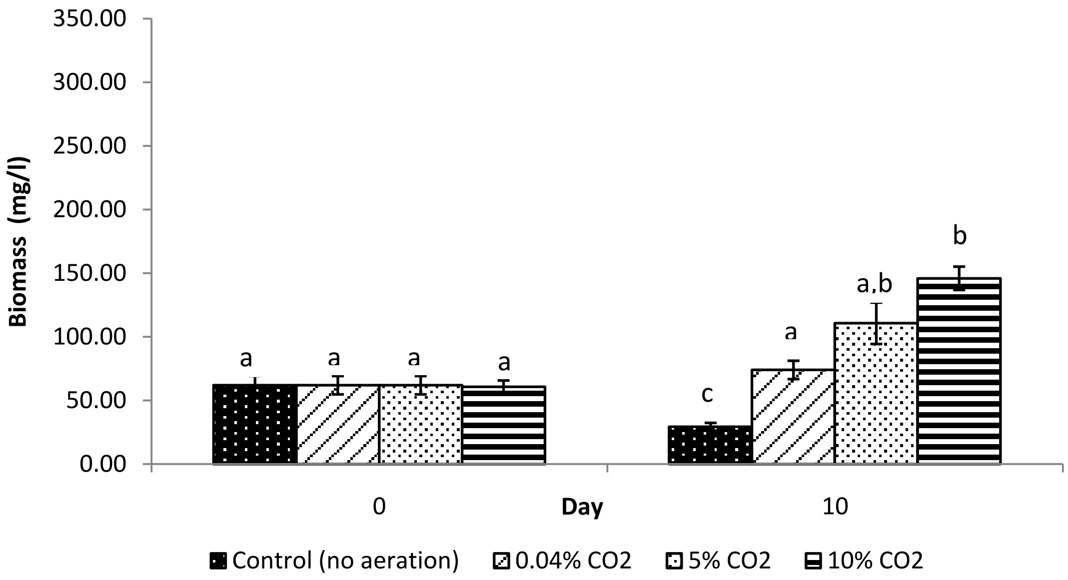
4.2.4 Biomass Production

Of the four strains, *Chlorella vulgaris* UMACC 001 produced highest biomass content at 5% CO_2, 268.67±22.030mg/L on day 10. At 10% CO_2, the biomass production was about 236±14.422mg/L (Figure 4.19). *Chlorella* UMACC 275 attained higher biomass 146±9.165mg/L at 10% CO_2 ($p<0.05$). For *Scenedesmus quadricauda* UMACC 041 the significant biomass production was 257.33±56.757mg/L ($p<0.05$) at 5% CO_2. In 10% CO_2, the biomass production was produced at 196±6.928mg/L ($p<0.05$). The biomass attained by *Oocystis* UMACC 074 at 5% CO_2 was 170±11.14mg/L that was not significantly different ($p<0.05$). Based on the results obtained, *Chlorella vulgaris* UMACC 001 was attained highest biomass at 5% CO_2 of all the four stains and followed by *Scenedesmus quadricauda* UMACC 041. At ambient condition, all the strains produced lower biomass compared with strains treated with 5% and 10% CO_2. The non-aerated cultures produced relatively low biomass.

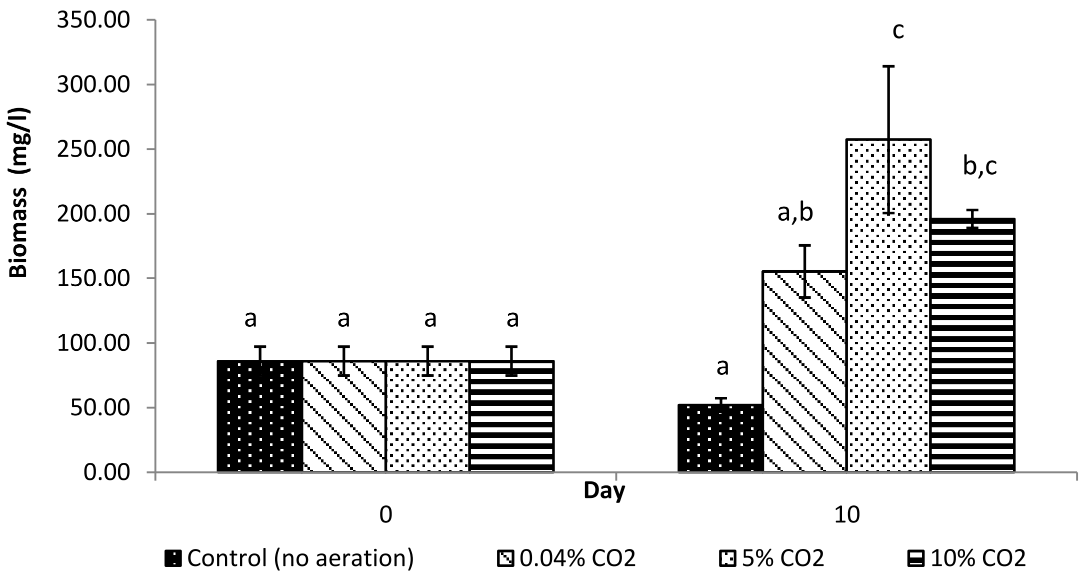
a) *Chlorella vulgaris* UMACC 001



b) *Chlorella* UMACC 275



c) *Scenedesmus quadricauda* UMACC 041



d) *Oocystis* UMACC 074

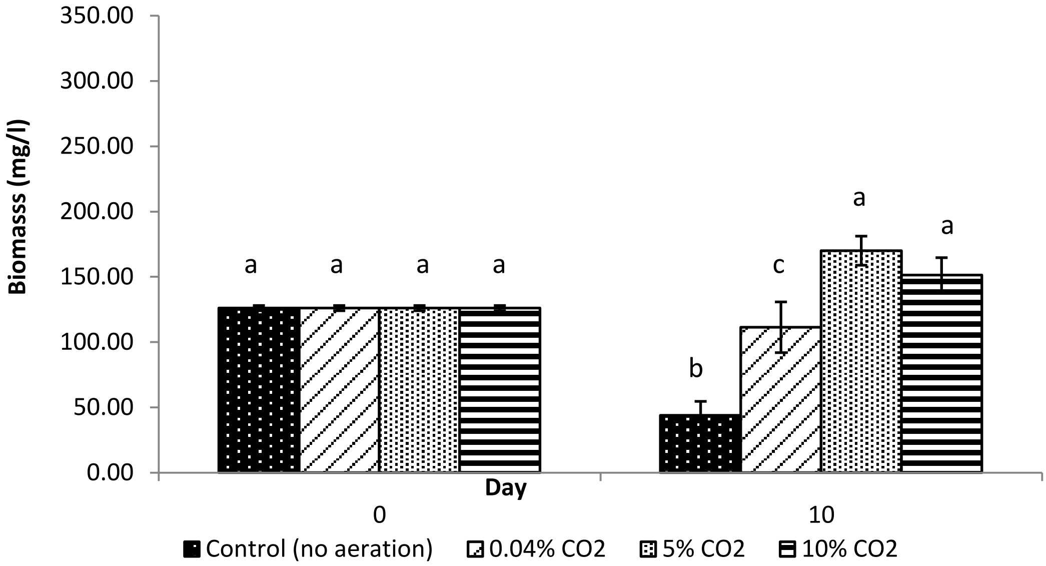


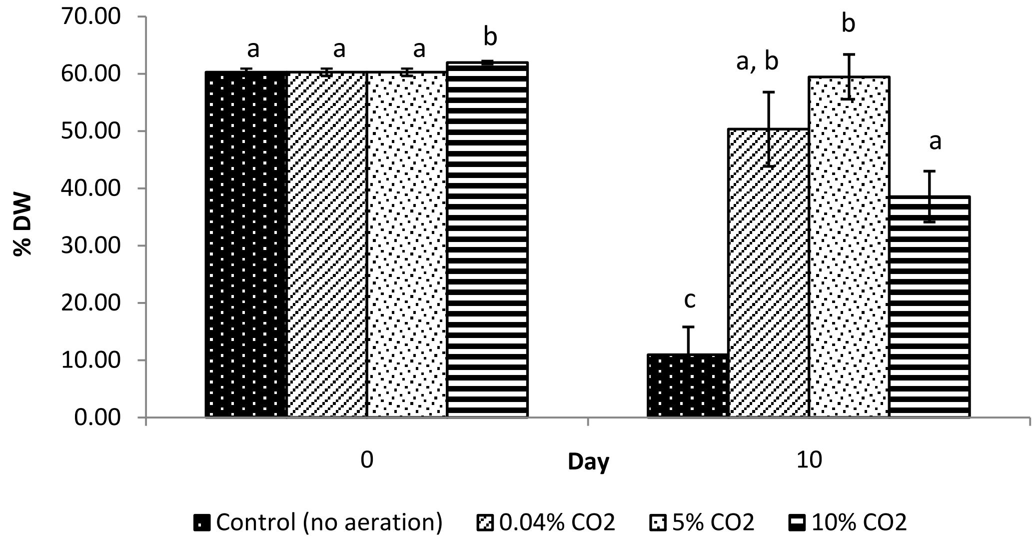
Figure 4.19: Biomass production of a) *Chlorella vulgaris* UMACC 001 b) *Chlorella* UMACC 275 c) *Scenedesmus quadricauda* UMACC 041 and d) *Oocystis* UMACC 074. Data represents the means ±S.D. Bar charts with different alphabets denote significant difference at $p < 0.05$.

4.2.5 Biochemical Composition

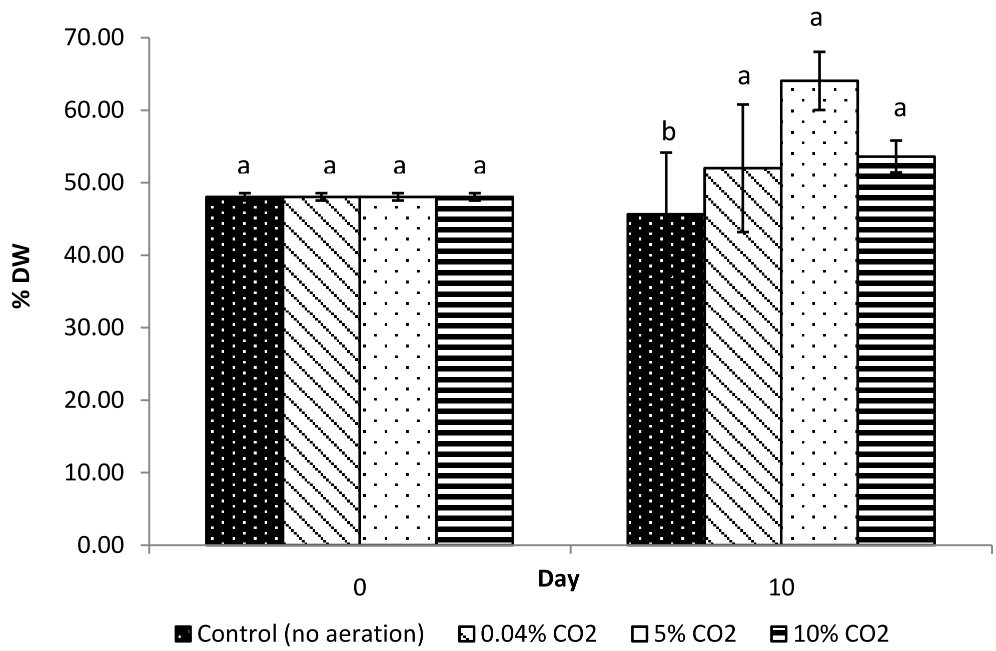
4.2.5.1 Proteins

The highest protein content was observed in the culture that was treated with 5% CO_2 in *Chlorella* UMACC 275 about 64.01±4.009% DW (Figure 4.20). This was followed by *Chlorella vulgaris* UMACC 001, 59.44±3.905% DW, *Oocystis* UMACC 074, 54.68±1.529% DW and *Scenedesmus quadricauda* UMACC 041, 52.80±2.001% DW ($p< 0.05$) respectively. The cultures treated with 10% CO_2 produced lower protein content compared to 5% of CO_2 as shown in the Figure 4.19. At 10% CO_2, *Chlorella* UMACC 275 again produced higher protein content 53.59±2.200% DW ($p< 0.05$), followed by *Oocystis* UMACC 074, 52.94±2.934% DW ($p<0.05$), *Scenedesmus quadricauda* UMACC 041, 48.77±1.622% DW ($p<0.05$) and *Chlorella vulgaris* UMACC 001 about 38.54±4.440% DW ($p<0.05$). The protein content seems to be higher at 0.04% CO_2 in *Chlorella vulgaris* UMACC 001 compared to 10% CO_2.

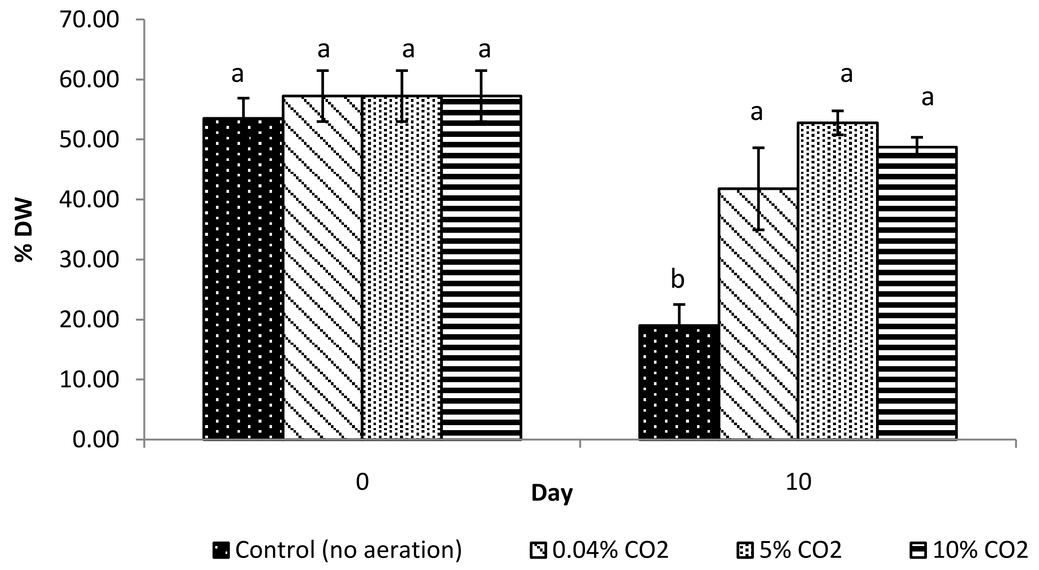
a) *Chlorella vulgaris* UMACC 001



b) *Chlorella* UMACC 275



c) *Scenedesmus quadricauda* UMACC 041



d) *Oocystis* UMACC 074

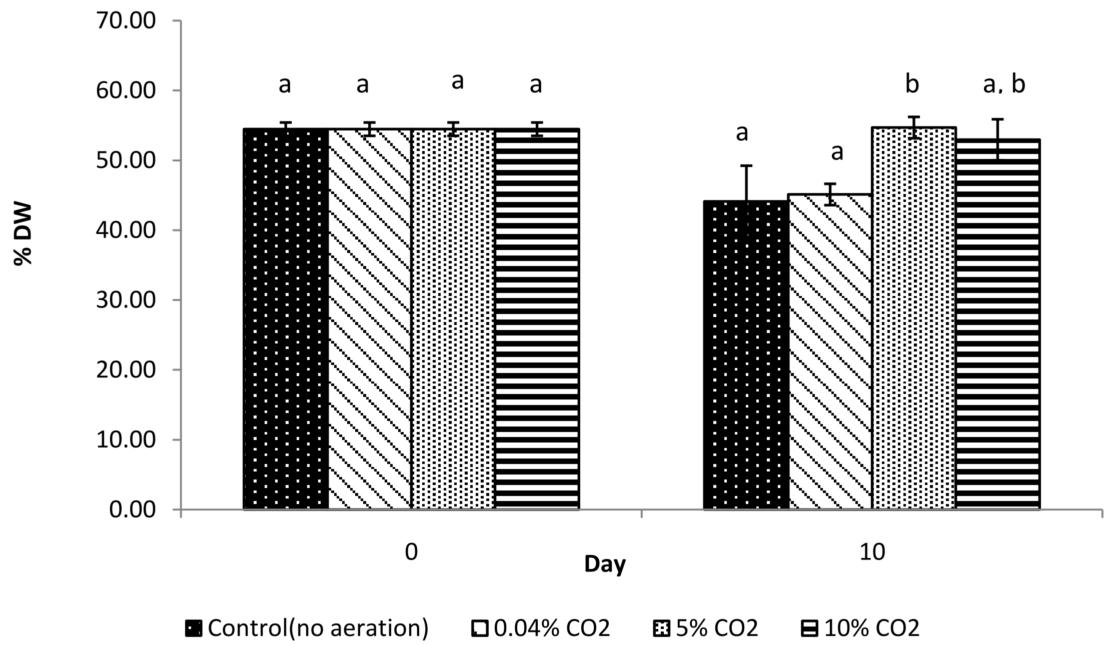
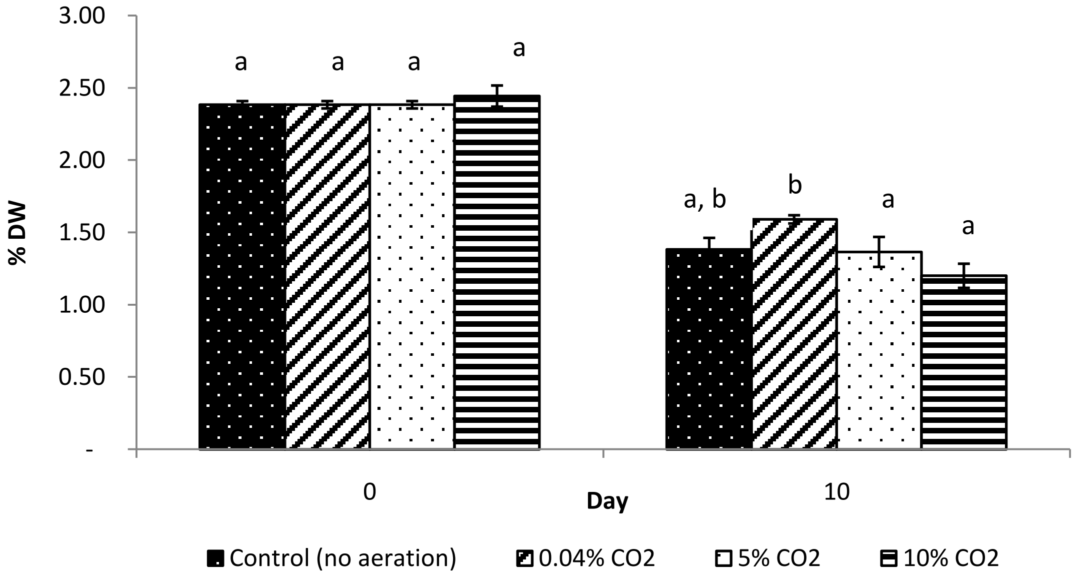


Figure 4.20: Protein content of a) *Chlorella vulgaris* UMACC 001 b) *Chlorella* UMACC 275 c) *Scenedesmus quadricauda* UMACC 041 and d) *Oocystis* UMACC 074 based on total dry weight. Data represents the means ±S.D. Bar charts with different alphabets denote significant difference at $p<0.05$.

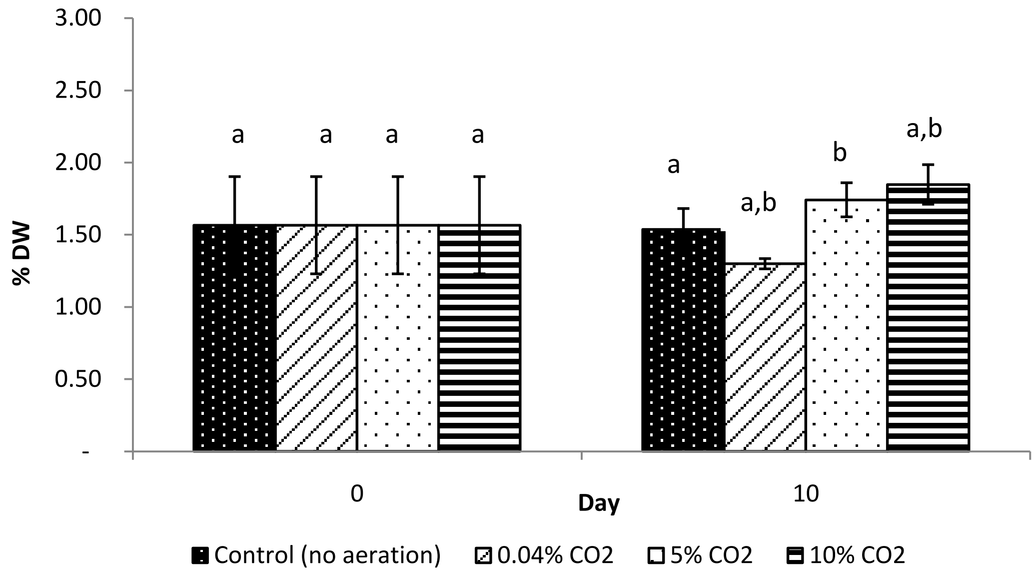
4.2.5.2 Carbohydrates

For the carbohydrate content, all the strains produced almost same carbohydrate content based on the Figure 4.21. At 5% CO_2, the higher carbohydrate content in *Chlorella* UMACC 275 was 1.74±0.118% DW ($p< 0.05$). At 10% CO_2, the carbohydrate content was 1.85±0.137% DW ($p<0.05$). However, *Oocystis* UMACC 074 attained highest carbohydrate content at 0.04% CO_2 without aeration about 3.34±0.202% DW ($p<0.05$). The other two strains attained lower carbohydrate content in all the treatments.

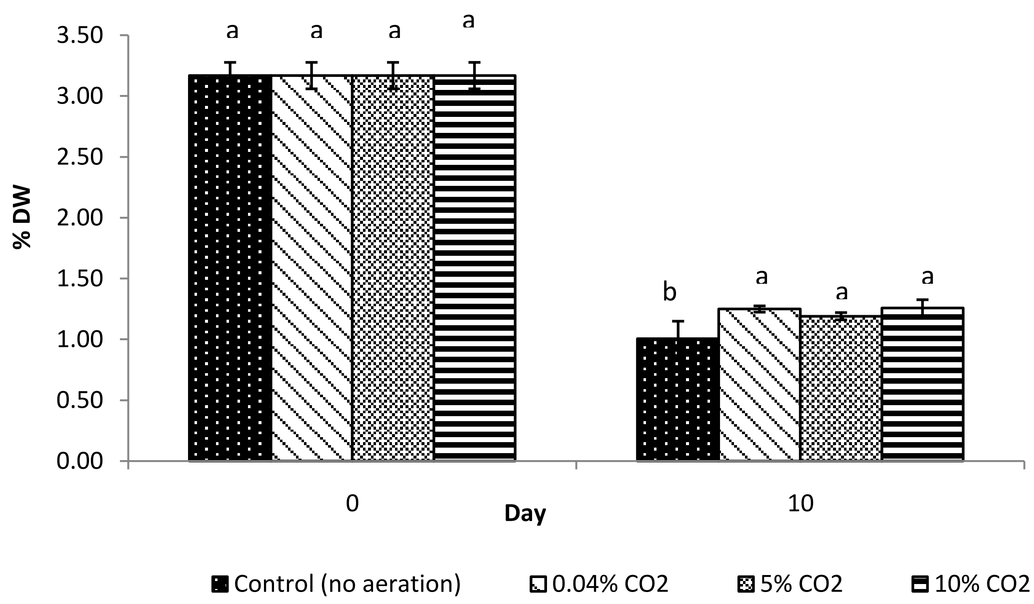
a) *Chlorella vulgaris* UMACC 001



b) *Chlorella* UMACC 275



c) *Scenedesmus quadricauda* UMACC 041



d) *Oocystis* UMACC 074

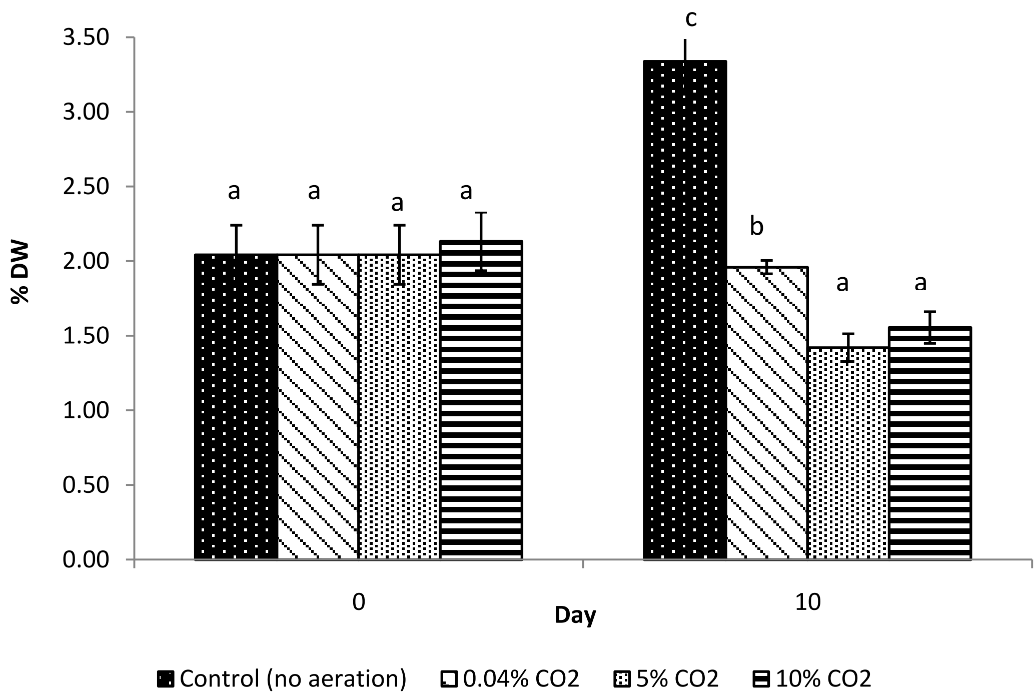
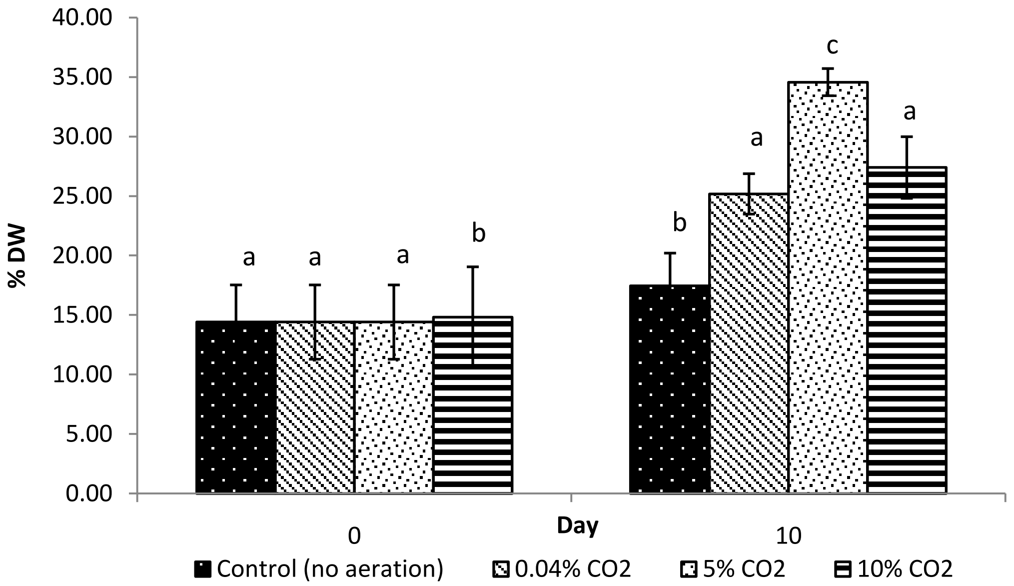


Figure 4.21: Carbohydrate content of a) *Chlorella vulgaris* UMACC 001 b) *Chlorella* UMACC 275 c) *Scenedesmus quadricauda* UMACC 041 and d) *Oocystis* UMACC 074 based on total dry weight. Data represents the means ±S.D. Bar charts with different alphabets denote significant difference at $p < 0.05$.

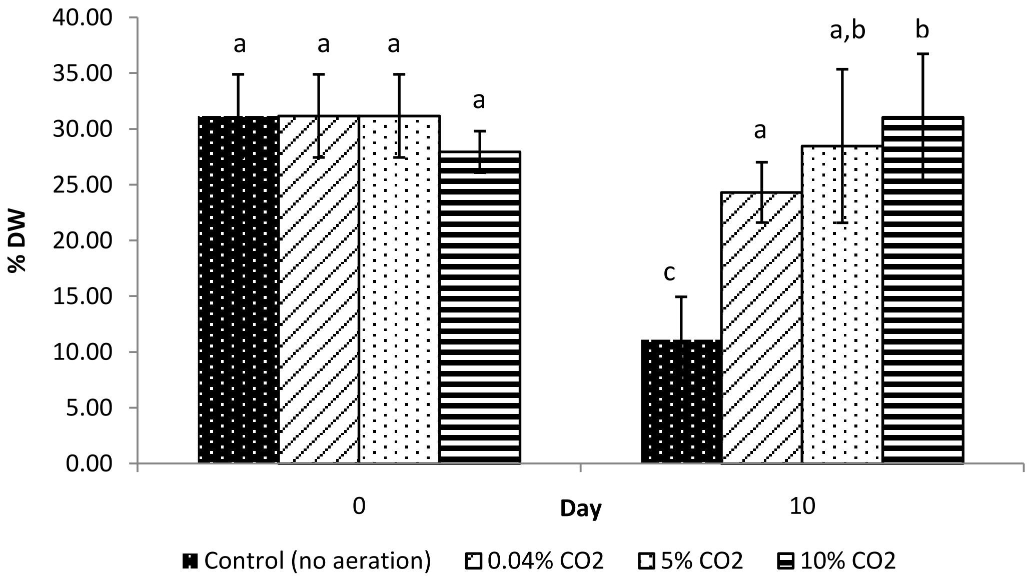
4.2.5.3 Lipids

In this experiment, the highest lipid content was obtained in *Chlorella vulgaris* UMACC 001 34.58±1.140% of DW at 5% CO_2. Besides, the lipid content of *Chlorella* UMACC 275, *Scenedesmus quadricauda* UMACC 041 and *Oocystis* UMACC 074 at 5% CO_2 were 28.48±6.884% DW, 26.10±1.951% DW and 18.82±2.35% DW respectively (Figure 4.22). However, the lipid content of *Chlorella* UMACC 275 is significantly higher at 10% CO_2 about 31.05±5.703% DW ($p<0.05$) compared to other three strains. At 10% CO_2, the lipid content of *Chlorella vulgaris* UMACC 001, *Scenedesmus quadricauda* UMACC 041 and *Oocystis* UMACC 074 were 27.40±2.589% DW, ($p<0.05$), 21.77±1.559% DW ($p<0.05$) and 15.79±4.74% DW. Based on the results obtained, all the strains obtained higher lipid content at 5% CO_2 compared with 10% CO_2 and 0.04% CO_2.

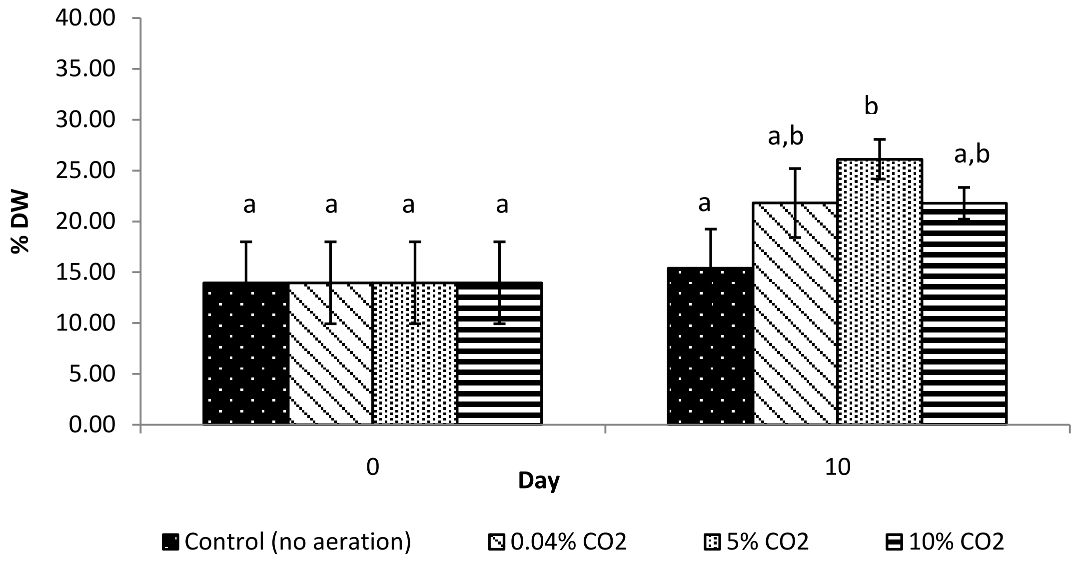
a) *Chlorella vulgaris* UMACC 001



b) *Chlorella* UMACC 275



c) *Scenedesmus quadricauda* UMACC 041



d) *Oocystis* UMACC 074

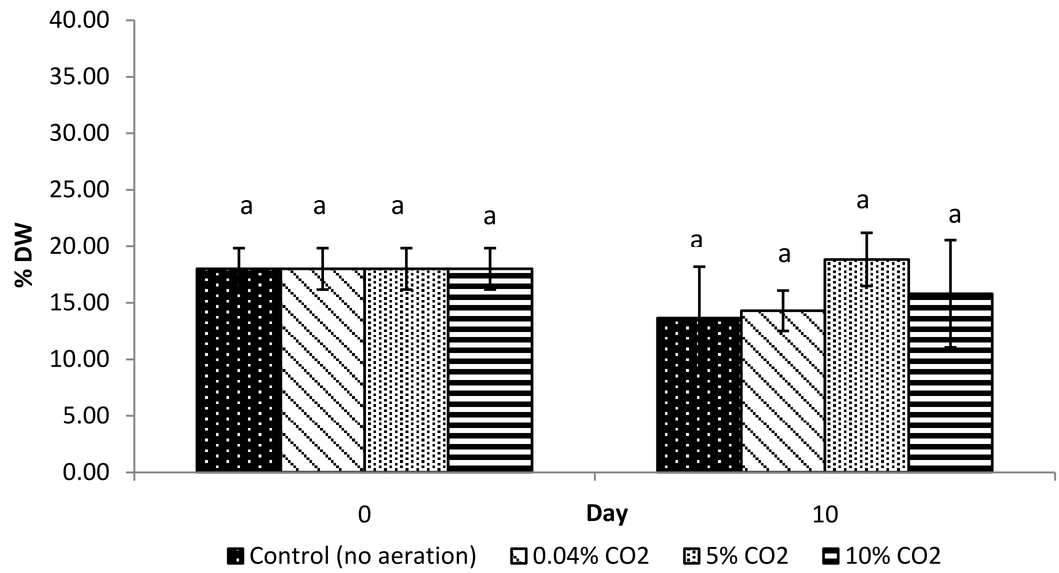


Figure 4.22: Lipid content of a) *Chlorella vulgaris* UMACC 001 b) *Chlorella* UMACC 275 c) *Scenedesmus quadricauda* UMACC 041 and d) *Oocystis* UMACC 074 based on total dry weight. Data represents the means ±S.D. Bar charts with different alphabets denote significant difference at $p < 0.05$.

4.2.6 Fatty Acid Composition

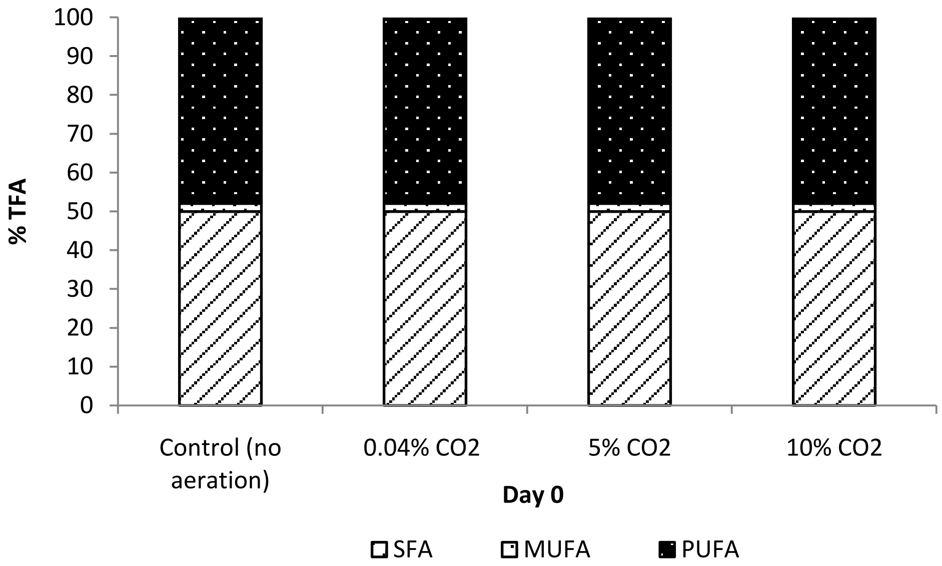
Based on Figure 4.23, the highest PUFA content of *Chlorella vulgaris* UMACC 001 was obtained at 10% CO_2 about 70.7% TFA while the highest SFA content was obtained in 0.04% CO_2 without aeration. Based on this figure, the PUFA content increased as the level of carbon dioxide increased while the SFA decreased. The amount of MUFA was relatively low compared with the other fatty acid which was about 1-5% TFA. From the Table 4.7, the 18:3 attained the high amount of 46.13% TFA at 10% CO_2. Based on this table, the amount of 18:2 increased on day 10 in all the treatments. For SFA, higher amount of 16:0 was produced for all the treatments.

For *Chlorella* UMACC 275 (Figure 4.24), the fatty acid distribution is similar to *Chlorella vulgaris* UMACC 001 where the highest PUFA was 63.8% TFA at 10% CO_2 and 59.9% TFA at 5% CO_2. The higher SFA content was observed in 0.04% CO_2, 49.4% TFA. The amount of MUFA is higher at 10% CO_2, about 11.4% TFA. From the Table 4.8, 18:2 and 18:3 recorded higher PUFA content in all the treatments of carbon dioxide.

The amount of PUFA in *Scenedesmus quadricauda* UMACC 041 was found to be almost the same in which the highest was produced at 10% CO_2, 73.4% TFA . The SFA content is lower in all the treatments compared with other strains. The amount of SFA is within the range of 22-29% TFA in Figure 4.25. The same trend was observed in *Oocystis* UMACC 074 (Figure 4.26) where MUFA<SFA< PUFA in all treatments. For SFA content, 18:0 decreased on day 10 for both *Scenedesmus quadricauda* UMACC 041 and *Oocystis* UMACC 074 (Table 4.9 and Table 4.10).

Overall, the highest PUFA distribution was observed in *Scenedesmus quadricauda* UMACC 041 followed by *Chlorella vulgaris* UMACC 001 at 10% CO_2. High amount of SFA produced in 0.04% CO_2 in all the strains. The amount of MUFA is relatively low in all the strains.

a) *Chlorella vulgaris* UMACC 001- Day 0



b) *Chlorella vulgaris* UMACC 001 – Day 10

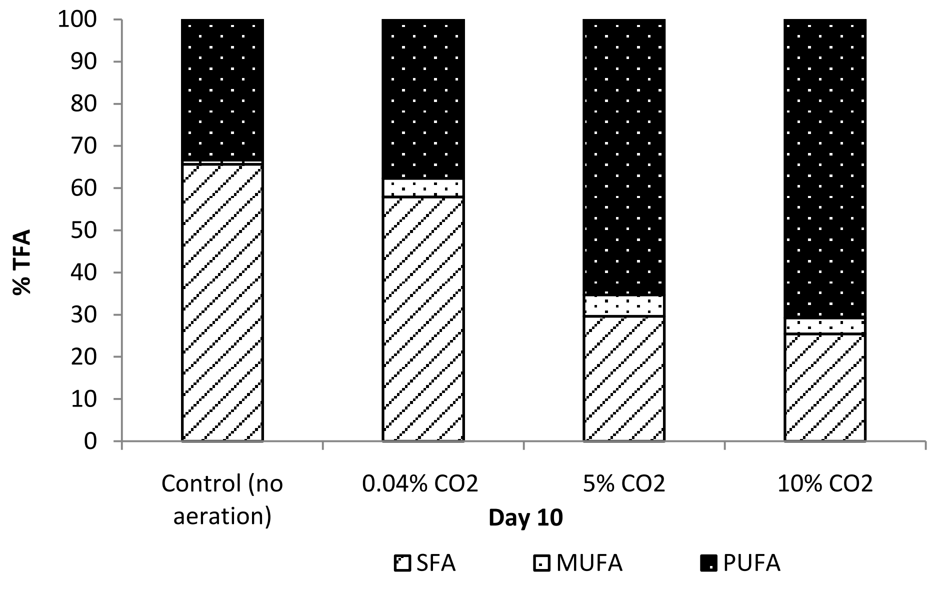
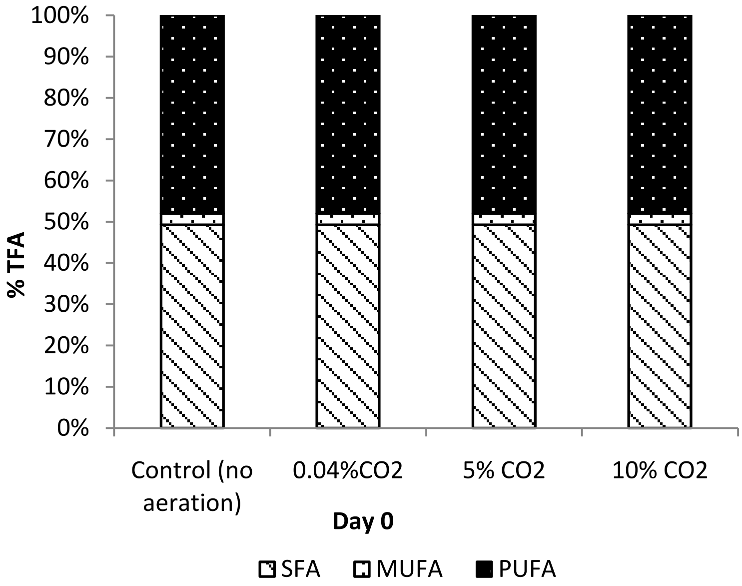


Figure 4.23: Distribution of saturated, monounsaturated and polyunsaturated fatty acids in *Chlorella vulgaris* UMACC 001 on a) day 0 b) day 10 at different level of carbon dioxide

a) *Chlorella* UMACC 275 – Day 0



b) *Chlorella* UMACC 275 – Day 10

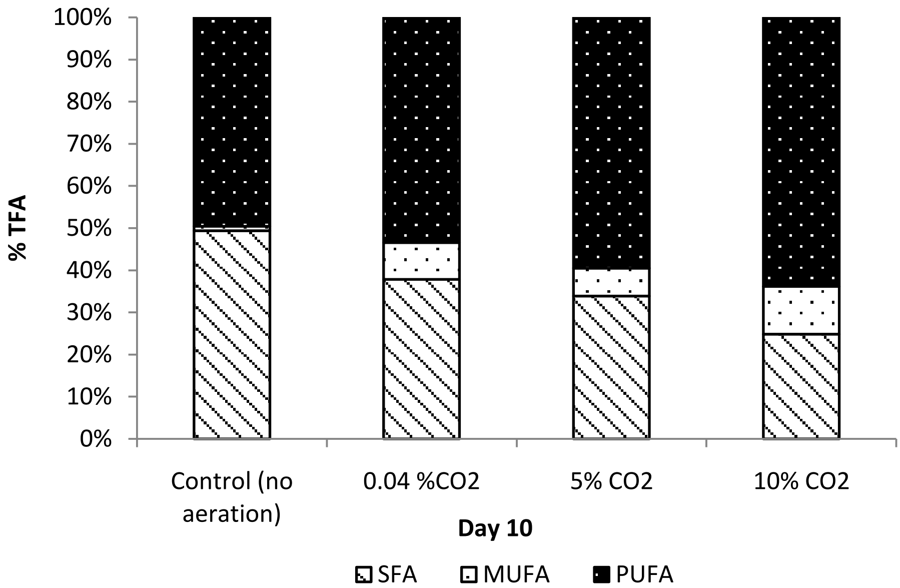
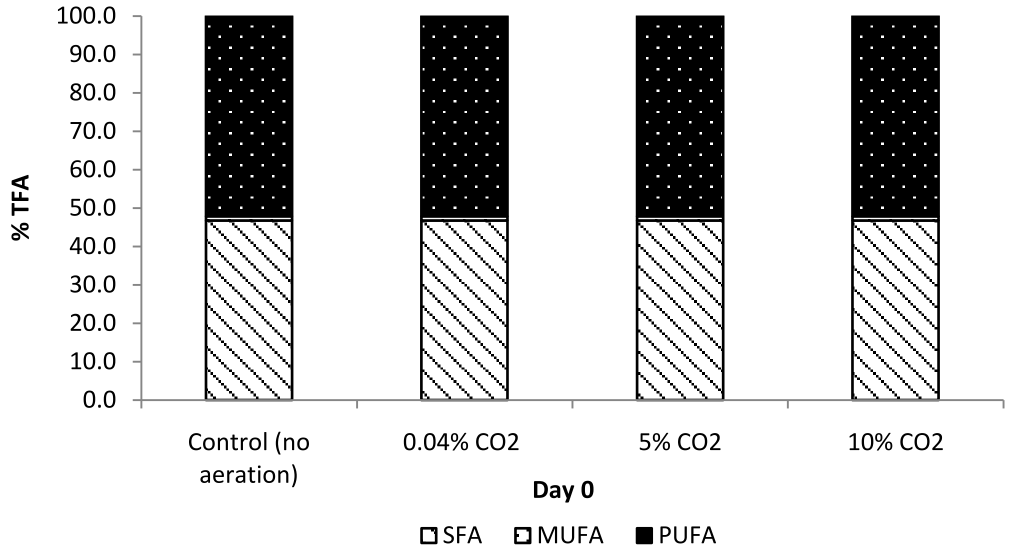


Figure 4.24: Distribution of saturated, monounsaturated and polyunsaturated fatty acids in *Chlorella* UMACC 275 on a) day 0 b) day 10 at different level of carbon dioxide

a) *Scenedesmus quadricauda* UMACC 041 – Day 0



b) *Scenedesmus quadricauda* UMACC 041- Day 10

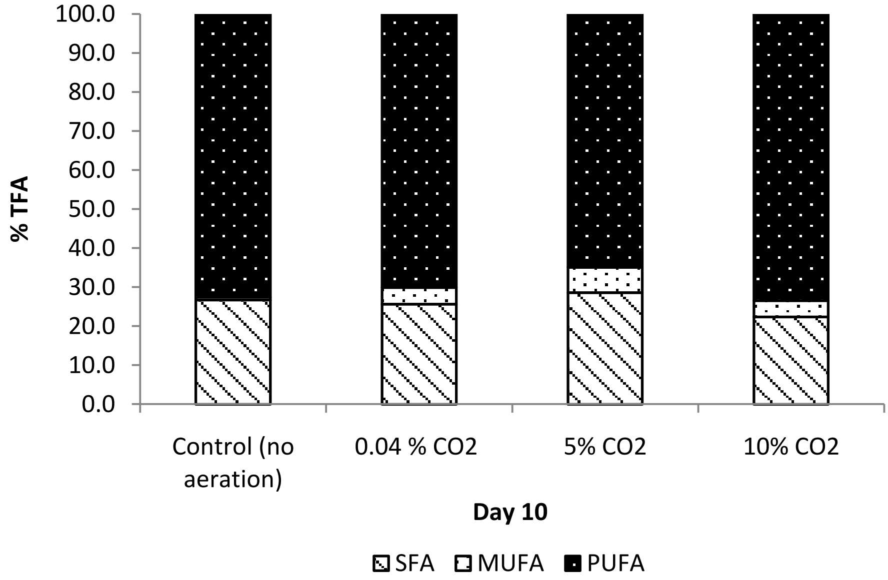
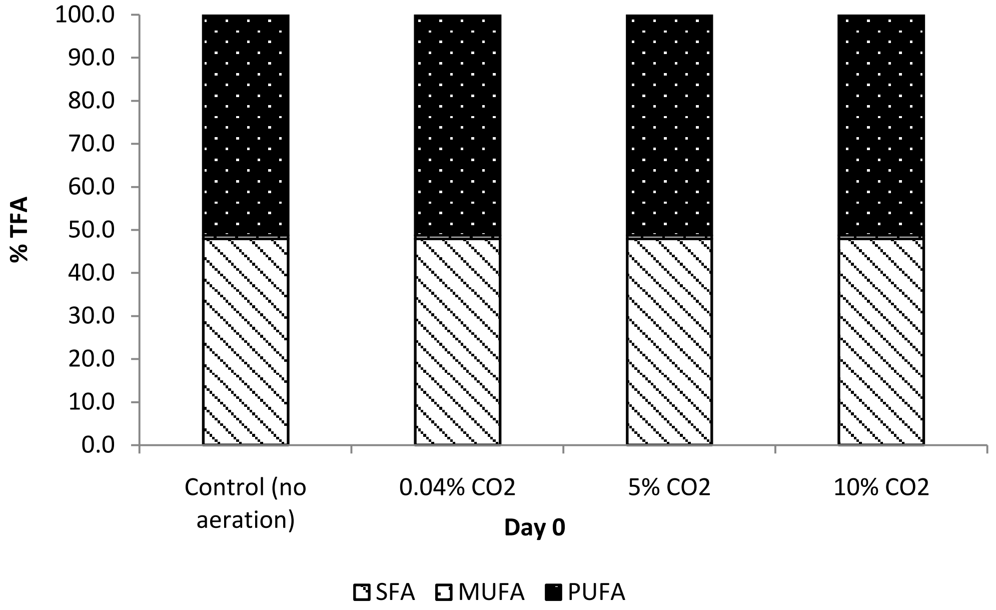


Figure 4.25: Distribution of saturated, monounsaturated and polyunsaturated fatty acids in *Scenedesmus quadricauda* UMACC 041 on a) day 0 b) day 10 at different level of carbon dioxide

a) *Oocystis* UMACC 074 – Day 0



b) *Oocystis* UMACC 074 – Day 10

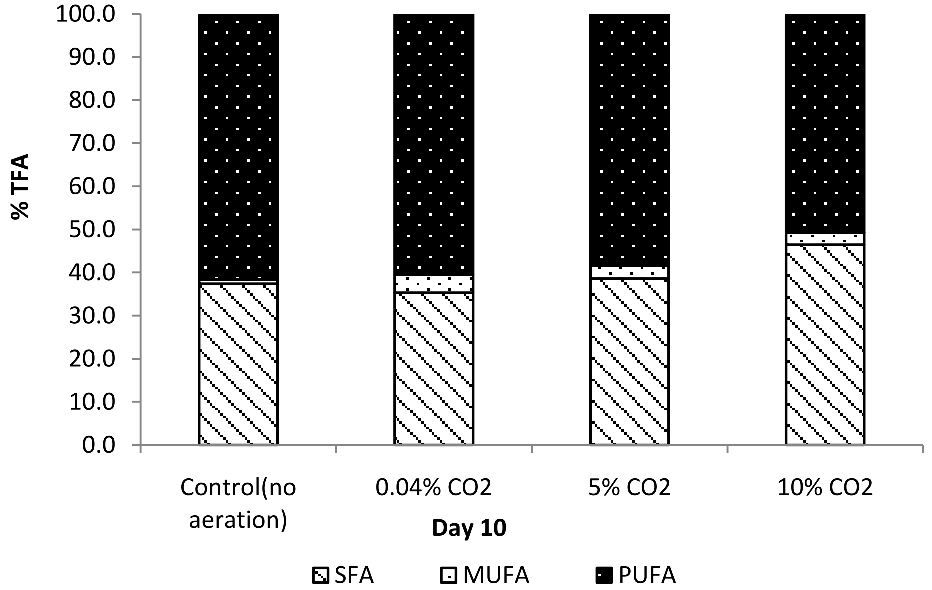


Figure 4.26: Distribution of saturated, monounsaturated and polyunsaturated fatty acids in *Oocystis* UMACC 074 on a) day 0 b) day 10 at different level of carbon dioxide

Table 4.7: Distribution of saturated, monounsaturated and polyunsaturated fatty acids in *Chlorella vulgaris* UMACC 001 on day 0 and day 10

Fatty acid	Day 0				Day 10			
	Control (no aeration)	**0.04% CO_2**	**5% CO_2**	**10% CO_2**	**Control (no aeration)**	**0.04% CO_2**	**5% CO_2**	**10% CO_2**
Saturated								
14:0	2.3	2.3	2.3	2.3	2.22	1.59	3.06	1.79
16:0	31.1	31.1	31.1	31.1	41.67	41.56	23.96	19.81
18:0	16.6	16.6	16.6	16.6	21.7	14.76	2.59	3.84
Sum	**50.0**	**50.0**	**50.0**	**50.0**	**65.59**	**57.91**	**29.61**	**25.44**
Monounsaturated								
16:1	1.5	1.5	1.5	1.5	0.98	4.37	4.27	3.82
18:1	0.5	0.5	0.5	0.5	0.00	0.00	0.80	0.00
Sum	**2.1**	**2.1**	**2.1**	**2.1**	**0.98**	**4.37**	**5.07**	**3.82**
Polyunsaturated								
16:2	11.6	11.6	11.6	11.6	0.00	0.00	6.95	0.30
16:3	7.4	7.4	7.4	7.4	1.13	12.98	14.67	14.74
18:2	9.1	9.1	9.1	9.1	16.35	14.86	13.69	9.57
18:3	19.8	19.8	19.8	19.8	15.95	9.89	30.01	46.13
Sum	**47.9**	**47.9**	**47.9**	**47.9**	**33.43**	**37.72**	**65.32**	**70.74**

Table 4.8: Distribution of saturated, monounsaturated and polyunsaturated fatty acids in *Chlorella* UMACC 275 on day 0 and day 10

Fatty acid	Day 0				Day 10			
	Control(no aeration)	**0.04% CO_2**	**5% CO_2**	**10% CO_2**	**Control(no aeration)**	**0.04% CO_2**	**5% CO_2**	**10% CO_2**
Saturated								
14:0	2.5	2.5	2.5	2.5	2.25	2.05	2.16	2.53
16:0	30.9	30.9	30.9	30.9	40.48	33.04	28.47	19.62
18:0	15.9	15.9	15.9	15.9	6.66	2.71	3.27	2.65
Sum	**49.3**	**49.3**	**49.3**	**49.3**	**49.39**	**37.80**	**33.90**	**24.80**
Monounsaturated								
16:1	1.5	1.5	1.5	1.5	1.02	8.80	6.55	11.38
18:1	1.2	1.2	1.2	1.2	0.00	0.00	0.00	0.00
Sum	**2.7**	**2.7**	**2.7**	**2.7**	**1.02**	**8.80**	**6.55**	**11.38**
Polyunsaturated								
16:2	0.0	0.0	0.0	0.0	0.00	0.00	0.00	5.90
16:3	17.2	17.2	17.2	17.2	0.00	11.80	28.56	17.37
18:2	19.1	19.1	19.1	19.1	28.03	22.40	21.87	20.98
18:3	11.8	11.8	11.8	11.8	21.56	19.21	9.12	19.57
Sum	**48.1**	**48.1**	**48.1**	**48.1**	**49.59**	**53.41**	**59.55**	**63.82**

Table 4.9: Distribution of saturated, monounsaturated and polyunsaturated fatty acids in *Scenedesmus quadricauda* UMACC 041 on day 0 and day 10

Fatty acid	Day 0				Day 10			
	Control(no aeration)	**0.04% CO_2**	**5% CO_2**	**10% CO_2**	**Control (no aeration)**	**0.04% CO_2**	**5% CO_2**	**10% CO_2**
Saturated								
14:0	1.4	1.4	1.4	1.4	3.0	1.1	1.20	1.0
16:0	28.1	28.1	28.1	28.1	18.5	19.3	21.95	18.5
18:0	17.1	17.1	17.1	17.1	5.2	5.2	5.47	2.8
Sum	**46.7**	**46.7**	**46.7**	**46.7**	**26.7**	**25.7**	**28.6**	**22.4**
Monounsaturated								
16:1	0.6	0.6	0.6	0.6	0.8	3.7	3.0	2.0
18:1	0.5	0.5	0.5	0.5	0.0	0.5	3.5	2.2
Sum	**1.1**	**1.1**	**1.1**	**1.1**	**0.8**	**4.2**	**6.5**	**4.2**
Polyunsaturated								
16:2	12.6	12.6	12.6	12.6	0.5	0.9	1.83	0.29
16:3	7.7	7.7	7.7	7.7	16.9	17.8	33.84	20.19
18:2	9.3	9.3	9.3	9.3	17.9	16.6	23.49	11.86
18:3	22.6	22.6	22.6	22.6	37.2	34.9	5.77	41.08
Sum	**52.2**	**52.2**	**52.2**	**52.2**	**72.5**	**70.2**	**64.9**	**73.4**

Table 4.10: Distribution of saturated, monounsaturated and polyunsaturated fatty acids in *Oocystis* UMACC 074 on day 0 and day 10

Fatty acid	Day 0				Day 10			
	Control (no aeration)	**0.04% CO_2**	**5% CO_2**	**10% CO_2**	**Control(no aeration)**	**0.04% CO_2**	**5% CO_2**	**10% CO_2**
Saturated								
14:0	2.2	2.2	2.2	2.2	2.55	2.30	2.01	1.96
16:0	28.8	28.8	28.8	28.8	31.75	25.60	26.18	32.30
18:0	16.8	16.8	16.8	16.8	3.11	7.43	10.43	12.21
Sum	**47.9**	**47.9**	**47.9**	**47.9**	**37.41**	**35.34**	**38.63**	**46.47**
Monounsaturated								
16:1	0.4	0.4	0.4	0.4	0.98	4.22	3.02	2.81
18:1	0.4	0.4	0.4	0.4	0.00	0.00	0.00	0.00
Sum	**0.8**	**0.8**	**0.8**	**0.8**	**0.98**	**4.22**	**3.02**	**2.81**
Polyunsaturated								
16:2	12.6	12.6	12.6	12.6	0.00	0.00	1.65	0.00
16:3	8.1	8.1	8.1	8.1	14.27	12.87	11.69	13.08
18:2	10.2	10.2	10.2	10.2	17.83	17.63	15.46	16.24
18:3	20.5	20.5	20.5	20.5	29.52	29.95	29.55	21.41
Sum	**51.3**	**51.3**	**51.3**	**51.3**	**61.62**	**60.44**	**58.35**	**50.72**

CHAPTER 5

DISCUSSION

In this research, ten microalgae strains namely *Chlorella vulgaris* UMACC 001, *Chlorella vulgaris* UMACC 103, *Chlorococcum miniatum* UMACC 102, *Oocystis* UMACC 074, , *Scenedesmus quadricauda* UMACC 041, *Scenedesmus obtusus* UMACC 100, *Chlorella vulgaris* UMACC 245, *Chlorella vulgaris* UMACC 253, *Chlorella* UMACC 255 and *Chlorella* UMACC 275 were investigated for their biomass, specific growth rate(μ) and biochemical composition under laboratory conditions. Almost all the strains were from Chlorophyta. Of the strains screened, four freshwater strains namely *Chlorella vulgaris* UMACC 001, *Chlorella* UMACC 275, *Scenedesmus quadricauda* UMACC 041 and *Oocystis* UMACC 074 were selected for their capacity to produce higher biomass, high lipid content and high specific growth rate for studies under elevated levels of carbon dioxide. Growth parameter and biochemical composition are the two main aspects that need to be considered in order to determine the potency of a strain that is reliable for commercial purposes and also for sustainable biodiesel production (Araujo and Garcia, 2005).

5.1 GROWTH AND BIOCHEMICAL CHARACTERIZATION OF SELECTED MICROALGAE

All the ten freshwater and marine strains were analysed with regards to their growth rate and biochemical composition because the cultivation of algae for energy production needs efficient methods to quantify and characterize the biomass composition of the microalgae species (Odlare et al., 2011). The growth parameter was determined by measuring OD_{620nm}, cell count and chlorophyll a content for every two days in order to monitor and compare the growth trend and biomass of each strain.

According to Odlare et al. (2011), measuring optical density with spectrophotometer is one of the easier and cheaper method to monitor the growth of microalgae during the cultivation time. Besides, the measurement of chlorophyll a, the main pigment for the conversion of light energy to chemical energy enables to determine the cell density. This pigment also influences the photosynthetic rate of particular algal species (Chen et al., 2011). In addition, the measurement of chlorophyll a enables better understanding to link the cell growth and lipid accumulation (Lv et al., 2010).

Based on the findings, *Chlorella vulgaris* UMACC 001 attained higher cell density compared with other strains. However, the highest chlorophyll a content was observed in *Chlorella* UMACC 275 2.461±0.298mg/L which was similar to *Chlorella vulgaris* UMACC 001, 2.414 ±0.438mg/L. According to Amutha and Murugesan (2011), *Chlorella* has high capability of producing higher chlorophyll during the late exponential phase as the cells attained maximal growth at this period and possess higher photosynthetic rate that can increase the biomass.

Based on the results obtained, all the microalgae, both freshwater and marine strains possessed short lag period and longer exponential phase. It shows that the cells were adapted to the culture medium in a short time with the available nutrients and growth factors such as light intensity, temperature, and pH of the medium. Furthermore, all the cultures in this study were enriched with continuous aeration that allows homogenization of nutrients that to be uptake by microalgae cells and promotes the growth to produce higher biomass (Mata et al., 2011; Scarsella et al., 2011). Generally, marine microalgae attain lower growth rate and biomass under normal condition unless supplied with additional organic carbon sources such as glucose and acetate or inorganic carbon source that may increase their biomass and lipid accumulation (Feng, et al., 2005).

The specific growth rate, μ, for each strain was calculated during exponential phase based on the chlorophyll a content that determines the photosynthetic activity of microalgal cells (Chiu et al., 2008). During exponential phase, the microalgal cells have adapted to the environment resulting in higher growth and photosynthetic rate. In this study, the highest specific growth rate 0.2433 ± 0.0168day^{-1} was observed in *Chlorella vulgaris* UMACC 001 (Table 4.1). This shows that *Chlorella vulgaris* UMACC 001 is a fast growing strain that suggest potential for large scale production. Another strain, *Chlorella* UMACC 275 achieved higher μ= 0.2202 ± 0.0108 day^{-1} compared with other strains. This proved that *Chlorella* strains are fast growing with higher specific growth rate and can attain higher biomass under photoautotrophic condition, allowing exploitation for various commercial purposes. According to Velea et al (2009), all the strains of *Chlorella* have higher growth potential giving rise to effective biomass production and economic viability.

Carotenoid production by microalgae is one of the important aspects as the carotenoid can be commercialized as food additives including colorants, antioxidants and vitamins. It has the ability to protect against oxygen free radicals that can be applied for the therapeutic applications (Salquero et al., 2003). Based on the findings, *Chlorella* UMACC 275 attained highest carotenoid content of 1.049±0.126 μg/ml and followed by *Chlorella vulgaris* UMACC 001, 1.023±0.183μg/ml respectively compared to other strains (Figure 4.5). Synthesis of high amount of carotenoids such as α-carotene, β-carotene, violaxanthin and neoxanthin in *Chlorella* gained significant attention in health market (Inbaraj et al., 2006).

Biomass production was another important aspect for the selection of the microalgal strains in this study. The highest biomass obtained in this study was on day 12 for all the strains in comparison with day 4 and day 8. It is because on day 4 and day 8, the algal cells are still actively growing by utilizing the available nutrients at this period of culture. According to Moazami et al (2011), fast growing microalgae are capable of producing maximum biomass within 8 to 10 days of cycle that is economical for large scale production.

Based on the Figure 4.6, *Chlorella vulgaris* UMACC 001 produced highest biomass 240.7±5.774mg/l DW followed by *Chlorella* UMACC 275, 190.67±45.622mg/L DW. According to Phukan et al., (2011), a *Chlorella* strain grown in BBM medium in 500ml flasks with (16:8) light and dark cycle (1200lux) attained biomass of 500mg/l DW. The higher biomass yield compared with the present study may due to the longer exposure period, as light controls algal metabolic activity that can lead to increase in cell density during autotrophic cultivation (Li et al., 2011). The *Chlorella* strains possess higher biomass production due to its ability to adapt the culture condition very fast that triggers rapid cell growth and increase the biomass. *Chlorella* is a potential strain for high biomass production with wide nutritional compounds that allows feasible large scale culture for commercial purposes (Day et al., 2009).

Scenedesmus quadricauda UMACC 041 is another potential strain produced high biomass of 170.67± 9.452mg/L DW in this study. This is in contrast to that reported for

Scenedesmus quadricauda which exhibited slow growth and low lipid accumulation under normal conditions without the supply of carbon sources such as glucose, acetate and CO_2 (Zhao et al., 2012). There was no change in the biomass of *Oocystis* UMACC 074 compared with day 0 and day 12 while *Chlorococcum miniatum* UMACC 102 possessed reduction in biomass that proves this strain is not feasible to be cultured in large scale system.

Protein is the most abundant biochemical in microalgae that contributes to its high nutritional value (Fernandez et al., 2006). In this study, *Scenedesmus obtusus* UMACC 100 and *Chlorococcum miniatum* UMACC 102 (Figure 4.7) produced the highest amount of protein 87.87±11.161% DW and 85.0 ± 3.418% DW respectively on day 4 of the culture. However, the protein content in all the strains decreased by the end of the cycle, probably due to nutrient depletion by the algal cells. Generally, the protein content has an important influence on the nutritional value of microalgae in association with fatty acids and carbohydrate, and had either a primary role in determining nutritional value or a secondary 'modifying' role depending on the composition of the microalgae (Fernandez et al., 2006).

According to Fernandez et al, (2006), the major biochemicals in microalgae are protein 30–40% DW, lipid 10–20% DW and carbohydrate 5–15% DW, that vary from one species to another. The carbohydrate content is lower than protein and lipid. However, the carbohydrate content in this study was relatively low for all the strains that is below 3% DW. *Chlorella* UMACC 275 contained higher amount of carbohydrate, 2.38±0.348% DW (Figure 4.8). The lower carbohydrate content may due to the nutrient limitation (nitrogen limitation) or nutrient depletion that create stress to the microalgae cells and causes carbon partioning from carbohydrates or proteins and converts into lipid (Feng et al., 2011). Besides, low light intensity (42 µmolm^{2}s^{-1}) used in this study may have caused reduction in the accumulation of carbohydrate in microalgae cells. A study reported that increasing of irradiance from 75 to 300µmolm^{2}s^{-1} increase the polysaccharide content in *Porphyridium* and *Porphyridium aerugineum*. It was also shown that carbohydrate synthesis in *Spirulina platensis* grown outdoors was significantly higher on sunny days than on cloudy days (Dragone et al., 2011).

The lipid content for freshwater and marine strains have the same trend with the protein content where the strains produced higher lipid content on day 4 and day 8 (Figure 4.9). The results show that all the *Chlorella* (both from marine and freshwater) basically produced higher amount of lipid than the other strains. The highest lipid content can be observed in *Chlorella* UMACC 275, 38.7±4.619 % DW on day 4 followed by *Chlorella vulgaris* UMACC 001, 31.5±3.208% DW on day 8. According to Widjaja (2009), freshwater *Chlorella vulgaris* is a good candidate for the biofuel production as it can yield high lipid content with its higher growth rate. Another study mentioned that *Chlorella vulgaris* act as the possible commercial strain for lipid production due to its faster growth and easy cultivation for biofuel feedstock (Lv et al., 2010). In this study, the marine strains attained lower lipid content compared with the freshwater strains. Generally marine microalgae are one known as one of the major lipid producers. The lower lipid production by them in this study may due to the insufficient nutrients and other abiotic condition that gives lower lipid productivity.

Besides, addition of organic sources such as glucose, acetate and sodium thiosulphate (heterotrophic condition) to culture medium, significantly enhance the lipid production in *Chlorella* (Feng et al., 2005). According Liu et al, (2011), the lipid content of *Chlorella zofingiensis* grown in heterotrophic condition is higher compared with photoautotrophic cultivation. Based on their study, *Chlorella zofingiensis* was grown in two different conditions in which 500ml of the medium allowed to grow under continuous illumination of 30µmolm^{2}s^{-1} for photoautotrophic condition and glucose was added without light supply for heterotrophic growth in batch cultures. The lipid accumulation in heterotrophic condition was 51.5% DW and 25.8% DW in photoautotrophic condition that proves addition of carbon source enhance lipid production. Microalgae have the ability to utilize both organic such as glucose, acetate and inorganic carbon sources for their optimal growth and higher accumulation of lipid in their cell wall (Huang et al., 2010).

In this study, the lipid content of both *Scenedesmus quadricauda* UMACC 041 and *Scenedesmus obtusus* UMACC 100 was comparatively lower, that is between 8-20.2% DW. *Scenedesmus* is known as one of the potential species for biofuel production that can yield about 30-50% lipid of total biomass in which addition of nutrients produces higher lipid production (Xin et al., 2010).

The lipid production is also closely related to nitrogen source such as ammonium, nitrate and urea. Limited nitrogen source can increase the lipid accumulation in cell wall in which it can utilize the sources during log phase (Converti et al., 2009; Hsieh and Wu, 2009). A study supported that depletion of nitrogen source can increase the lipid content in a few *Chlorella* strains such as *Chlorella emersonii* (63% DW), *Chlorella minutissima* (56% DW), *Chlorella vulgaris* (57.9% DW), *Chlorella luteoviridis* (28.8% DW), *Chlorella capsulata* (11.4% DW), and *Chlorella pyrenoidosa* (29.2% DW) (Zeng et al., 2011). This enables the strains to serve promising sources of bioenergy.

Oocystis UMACC 074 produced the highest SFA content of 70.4% TFA followed by *Scenedesmus obtusus* UMACC 100 about 69.4% TFA (Figure 4.10) (Table 4.2). All the

Chlorella strains were also attained high amount of SFA within the range of 30-70% TFA. A study reported that *Chlorella* and *Scenedesmus* grown in 16:8 light-dark cycles with 50 µmolm^{2}s^{-1} irradiance attained SFA 24.4% TFA and 22.7% TFA respectively (Kaur et al., 2012). Based on their study, SFA widely used for biofuel feedstock can be increased with prolonged cultivation. SFA have higher melting points and crystallize at a higher temperature. Therefore, biodiesel fuels derived from fats or oils with significant amounts of SFA display better cold flow properties for sustainable biofuel production.

Both the freshwater and marine *Chlorella* (UMACC 001, UMACC 103, UMACC 245, UMACC 253 and UMACC 255), produced highest PUFA content at the late stage of the cycle compared with SFA and MUFA (day 8 and day 12) in which the highest PUFA content was recorded in *Chlorella vulgaris* UMACC 245, about 84.2% TFA and lower MUFA of 6.6% TFA. It was reported that *Chlorella* that grown in autotrophic condition produced high PUFA (49.6% TFA) but lower MUFA (20.1% TFA) which is consistent with this study (Liu et al., 2011). Another study also reported that high amount of PUFA was observed in *Chlorella* (54.9% TFA) that was grown under autotrophic cultivation with 16:8 light-dark cycles and 50 µmolm^{2}s^{-1} irradiance (Kaur et al., 2012). It was shown that microalgae are important sources of PUFA such as eicosapentaenonic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA). Enhanced culture medium with carbon source can possibly increase the amount of PUFA especially for marine *Chlorella* that is a potential strain for EPA production for use as artificial food in aquaculture (Petkov and Garcia, 2007; Feng et al., 2005). It is known that *Chlorella* is not only highly potential for producing chlorophyll, carotenoids and minerals but is also a good source of PUFA. The PUFA obtained from microalgae are widely utilized for medical purposes especially in treating cardiovascular diseases (Otles and Pire, 2001).

Since all the cultures were supplied with continuous aeration although no additional CO_2 was supplied throughout the cycle, the algal cells had higher growth rate and biomass production compared to control without aeration. Aeration results in mixing of the cells with the nutrients allowing more efficient absorption of the nutrients leading to increased rate of reaction, higher specific growth rate and biomass production. In addition, gradients of nutrients and inhibitory organic compounds are eliminated.

Based on the results of this selection study, four strains namely *Chlorella vulgaris* UMACC 001, *Scenedesmus quadricauda* UMACC 041, *Chlorella* UMACC 275 and *Oocystis* UMACC 074 were selected. They had the higher μ, biomass and lipid content.

5.2 EFFECT OF ELEVATED LEVELS OF CARBON DIOXIDE ON MICROALGAE GROWTH AND BIOCHEMICAL COMPOSITION

Four microalgae, *Chlorella vulgaris* UMACC 001, *Scenedesmus quadricauda* UMACC 041, *Chlorella* UMACC 275 and *Oocystis* UMACC 074 were selected from the preliminary study and grown in different levels of CO_2 under photoautotrophic cultivation. Although heterotrophic cultivation can produce higher lipid content and biomass productivity, the cost of additional feedstock (glucose, acetate and sodium thiosulphate) is higher and the energy transformation efficiency is low (Xin et al., 2010). Heterotrophic cultures cannot produce light-induced metabolites like pigments. Mixotrophic cultures can increase the cell density and lipid accumulation with supply of organic and inorganic carbon simultaneously. However, excess carbon concentration can inhibit the metabolism of organic compounds (Zhao et al., 2012). Therefore, photoautotrophic cultivation was used in this study for cultivation of microalgae strains. Photoautotrophic cultivation is most commonly used as it is inexpensive for large scale system. It also allows direct use of light and CO_2 as all the strains in this study were photosynthetic that can efficiently convert light energy into chemical energy (biomass) (Garcia et al., 2011). An advantage of photoautotrophy is seen through the direct utilization of CO_2 from flue gas and other sources promoting biofixation that can mitigate high amount of CO_2 from the atmosphere.

Based on the findings, all the strains that treated with 5% and 10% CO_2, showed higher cell density compared with ambient condition and culture without continuous aeration based on the optical density, cell count, chlorophyll content and biomass (Figure 4.13, Figure 4.14, Figure 4.15 and Figure 4.19). The growth rate was influenced by the DIC (CO_2 or HCO_3^-) present in the culture medium as the microalgae utilize it for photosynthesis. The presence of high DIC in the culture medium stimulates the carbon concentrating mechanism (CCM) in microalgae allowing for higher photosynthetic activity and biofixation (Ghoshal et al., 2002). In a culture medium, CO_2 combines with H_2O to form H_2CO_3 which dissociates into HCO_3^- and H^+ depending on the pH of medium. It is known that HCO_3^- is the predominant form of inorganic carbon at alkaline pH that is utilized by algal cells through active transport (Zhao et al., 2011; Huertas et al., 2000). In this study, all the cultures were not buffered and had pH ranging from 6.2-8.0 that may allow the formation of more HCO_3^- species (Figure 4.16). The changes in pH determine the DIC in the culture medium (Figure 4.17). A previous study reported a *Chlorella* that grown in a photobioreactor showed higher growth at pH 6.8 under photoautotrophic cultivation (Kao et al., 2012). From their study, the major DIC at pH 6.7 was HCO_3^-. From this it is known that pH affects the bioavailability of nutrients (DIC) and transport of substrates across cytoplasmic membrane of microalgae where decreased pH would suppress the microalgal growth. The pH also affects the enzyme activity and electron transport in photosynthesis and respiration that affects the cell density.

In this study, the highest growth rate was observed in *Chlorella vulgaris* UMACC 001 at 5% CO_2 based on the optical density, cell count, chlorophyll content (Figure 4.13, Figure 4.14 and Figure 4.15) compared with other three strains. The chlorophyll-a content significantly increased to 4.35±0.325mg/l ($p<0.05$) on day 10. In addition to that, *Oocystis* UMACC 074 which was a slow growing strain also possessed high chlorophyll content at 5% CO_2 compared with ambient condition. The level of DIC plays a major role in the regulation of chlorophyll pigment concentration (Huertas et al., 2000). High amount of DIC enhance the incorporation of ribulose with carbon species (high carboxylating activity) and increase the carbon fixation in photosynthesis which can be monitored from increasing chlorophyll content. This shows that supply of high carbon dioxide levels in the culture medium enhances the photosynthetic pigments of microalgae cells (Huertas et al., 2000). According to Morais and Costa (2007), addition of carbon dioxide in culture medium stimulates the growth of the cells and can be a limiting factor of the microorganisms.

Based on the Table 4.6, *Chlorella vulgaris* UMACC 001 attained the highest specific growth rate (μ) 0.3707±0.0171 day^{-1} at 5% CO_2 and *Chlorella* UMACC 275 attained higher μ at 10% CO_2, 0.3419±0.0256 day^{-1}. A study reported that *Chlorella* that grown in a photobioreactor at 5% CO_2 (continuous supply) with the flow rate of 200ml/min and volume of 800ml in batch system attained μ of 0.343 day^{-1} (Chiu et al., 2008). The μ obtained is comparably lower with the present study. This may due to the dense culture in photobioreactor that causes low light penetration into the medium and inadequate supply of CO_2 as the supply of CO_2 was not continuous in this study. However, higher flow rate may also influence the growth rate of microalgae as it can reduce the pH that subsequently reduces the growth. According to Widjaja (2009), the growth of *Chlorella vulgaris* increased until the flow rate of 50ml/min of pure CO_2

(without addition of compress air) in 4L culture and decreased growth rate was observed at high flow rate, 200ml/min of pure CO_2. High CO_2 can reduce the pH of the culture medium because unused CO_2 can be converted into H_2CO_3 and can inhibit the growth of algal cells.

The specific growth rate of *Scenedesmus quadricauda* UMACC 041 at 5% CO_2 0.3354±0.0382day^{-1} seems to be higher than at 10% CO_2 0.3035±0.0033day^{-1}. According to Yoo et al, (2010), *Scenedesmus* can grow best at 10% CO_2 as it can attain high biomass and lipid content at elevated levels of CO_2 which is consistent with this study. Among the four species, *Oocystis* UMACC 074 showed good growth at 10 % CO_2 with the specific growth rate of 0.3222±0.0276 day^{-1}. Generally, *Oocystis* is considered not a fast growing species at ambient condition. Despite this, addition of inorganic carbon source enhances the growth rate leading to high biomass and lipid content.

Besides, the carotenoid content was found to be 1.5 times higher in 5% CO_2 in comparison with the ambient condition (Figure 4.25). According to Chinnasamy et al., (2009), the carotenoid content produced at 6% CO_2 was 2.04 times higher than the ambient condition which is close to the value obtained in this study. Another study reported that *Chlorella vulgaris* can grow best at aerated 5% CO_2 and achieved lower growth rate or can inhibit the growth at high concentration of carbon dioxide (Ryu et al., 2009). *Chlorella vulgaris* has higher potency to produce carotenoids such as canthaxanthin and astaxanthin that has pharmacological significant. The more the CO_2 incorporated with the algal cells, the more it synthesizes carotenoid that is economically viable.

Enrichment of CO_2 had influenced the biomass of all the four strains studied compared with the ambient condition. It is because the addition of CO_2 in culture medium induces the cell adaptation for increased photosynthetic rate that can convert to high biomass. (Logothetis et al., 2004). Based on the Figure 4.19, *Chlorella vulgaris* UMACC 001 produced the highest biomass 268.67±22.030mg/L ($p>0.05$) at 5% CO_2 and 236±14.422mg/L ($p>0.05$) at 10% CO_2. While *Chlorella* UMACC 275, attained higher biomass at 10% CO_2, 146mg/l ($p<0.05$). A study showed that a *Chlorella* strain that grown in a photobioreactor with 600ml volume attained highest biomass 2020mg/l at 5% CO_2 (mixed with compressed air) on day 6 of culture period (Ryu et al., 2009). The higher biomass production may due to the high cell concentration in larger culture volume (photobioreactor) compared with the present study that used smaller culture volume. In a different study, *Chlorella* achieved its highest biomass within 6 days at 10% CO_2 (Radmann et al., 2011). Another different study proved that *Chlorella vulgaris* that grown in photobioreactor at 10% CO_2 attained 258.52mg/l which is almost same with the present study (Sasi, 2009). Some *Chlorella* even could exhibit good tolerance at higher levels of CO_2 (>10% CO_2) According to Tang et al, (2011), *Chlorella* pyrenoidosa that grown in 1L flask at 50% CO_2 attained biomass of 0.69g/l. From this, it was shown that *Chlorella* species are highly tolerant at elevated levels of CO_2 and produce high biomass by biofixation.

For *Scenedesmus quadricauda* UMACC 041, the significant biomass was 257.33±56.757mg/L ($p<0.05$) 5% $CO_{2.}$ A study mentioned that *Scenedesmus obliquus* and *Chlorella pyrenoidosa* grown at different carbon dioxide levels ranging from 0.04% to 50% CO_2 achieved the maximum biomass at 10% CO_2 (Tang et al., 2011). Based on this study, it is known that the slow growing strains can show better tolerance at elevated levels of CO_2 with high growth rate and biomass compared with the ambient condition.

The highest protein content in all the strains was observed in the culture treated with 5% CO_2 in *Chlorella* UMACC 275, *Chlorella vulgaris* UMACC 001, *Oocystis* UMACC 074 and *Scenedesmus quadricauda* UMACC 041 were 64.01±4.009% DW($p < 0.05$), 59.44±3.905% DW ($p < 0.05$), 54.68±1.529% DW ($p < 0.05$) and 52.80±2.001% DW ($p < 0.05$) (Figure 4.20) respectively.

From the findings, the carbohydrate content was relatively low which was below 2% DW in all the strains (Figure 4.21). As previously discussed, the lower content of carbohydrate may due to the utilization of carbon source by growing microalgae during night as the provision of CO_2 was not continuous. A study reported that, *Chlorella vulgaris* that grown in a bubble column photobioreactor at 10% CO_2, showed decrease in carbohydrate content because no light supplied during night and used its own carbohydrates to maintain their growth (Wijanarko et al., 2008).

Based on Figure 4.22, the highest lipid content was attained by *Chlorella vulgaris* UMACC 001, 34.58±1.140% of DW ($p < 0.05$) at 5% CO_2 while *Chlorella* UMACC 275 attained high lipid content of 31.05±5.703% DW ($p < 0.05$) at 10% CO_2. One study reported that *Chlorella pyrenoidosa* that grown in 1L flask attained lipid content of 20.65% DW at 5% CO_2, 24.25% DW at 10% CO_2, 26.02% DW at 30% CO_2 and 26.75% DW at 50% CO_2 (Tang et al., 2011). This amount is comparably lower with lipid content obtained in *Chlorella* in this study. Another study reported that a *Chlorella* strain that grown in a photobioreactor obtained lipid content of 25.2 ± 1.2% DW under flue gas and 26.4 ± 2.3% DW at 25% CO_2 in 6 days culture period (Chiu et al., 2011).

Besides, it is known that *Chlorella* is a reliable strain that could grow under elevated levels of carbon dioxide that can reduce the greenhouse gaseous and also for the sustainable biofuel feedstock as it can produce high lipid content and high biomass (Feng et al., 2011).

The lipid content of *Scenedesmus quadricauda* UMACC 041 and *Oocystis* UMACC 074 increased with the supply of CO_2 compared with the ambient condition (0.04% CO_2). At 5% CO_2, the lipid content of *Scenedesmus quadricauda* UMACC 041 was 26.10±1.951% DW ($p < 0.05$). Though these two species posses slow growth trend compared with the fast growing *Chlorella vulgaris* UMACC 001 and *Chlorella* UMACC 275, supply of carbon dioxide enables the cells to increase the lipid content. From this, it is known that the algal cells assimilate the inorganic or dissolved carbon dioxide in culture medium as nutrient source to accumulate higher lipid in cell walls by photosynthesis. Carbon dioxide concentration is one of the factors that affect the production of lipid in microalgae (Lv et al., 2010). Microalgal strains that can develop high lipid content become the promising source for biofuel production in the future (Miao and Wu, 2006).

Based on the GC analysis, high PUFA content was observed in all the four strains that were grown with carbon dioxide supply. *Chlorella vulgaris* UMACC 001, *Chlorella* UMACC 275 and *Scenedesmus quadricauda* UMACC 041 possessed their highest PUFA content at 10% CO_2 while *Oocystis* at 5% CO_2 (Table 4.7, Table 4.8, Table 4.9 and Table 4.10). The highest PUFA content was observed in *Scenedesmus quadricauda* UMACC 041 about 73.4%TFA and *Chlorella vulgaris* UMACC 001, *Chlorella* UMACC 275 were attained 70.7% TFA and 63.8% TFA. The addition of CO_2 enhances the lipid content that triggers the production of more PUFA compared with SFA and

MUFA. Besides, the SFA content is also seems to be higher in all the strains at elevated levels of CO_2 compared with ambient condition. The SFA amount in *Chlorella vulgaris* UMACC 001 ,*Chlorella* UMACC 275 and *Scenedesmus quadricauda* UMACC 041 were 29.61% TFA, 33.90% TFA and 28.6% TFA at 5% CO_2 (Table 4.7,Table 4.8 and Table 4.9). A study showed that the amount of SFA in *Chlorella* and *Scenedesmus* at 10% CO_2 was 25.3% TFA and 39% TFA (Yoo et al., 2010) which was comparably similar with the present study. According to Amini et al. (2011), *Chlorella* has good potential to accumulate more than 20% lipids and the fatty acids such as 18:1, 16:0 (palmitic acid) and 18:3 (linolenic acid) that can be further analysed for various commercial uses such as biofuel production. Besides, the SFA that detected in *Chlorella* can perform an excellent cetane number and oxidative stability that is viable for biodiesel feedstock. Another study explains that two strains of green microalgae namely *Scenedesmus obliquus* and *Chlorella pyrenoidosa* possess higher biofixation rate at 10% CO_2 and obtained higher lipid content with the fatty acids of C16–C18 which is consistent whit this study (Tang et al., 2011). Besides, it also reported that high level of carbon dioxide can allows accumulation of more lipids and fatty acids that have great potential for biofixation and biofuel production (Tang et al., 2011).

Although all the strains were supplied with inorganic carbon source, there are several factors may influence the production of lipid, efficiency of the photosynthetic pigments, biomass production, biomass consumption during respiration, insufficient carbon dioxide transfer, nutrient shortfall and photo inhibition. The factors are such as temperature, light intensity, cultivation density and nutrients may affect the productivity as the uptake of these resources varies from one species to another. The shading effects in dense cultures of photosynthetic microorganisms can also reduce the level of light reaching individual cells and thus prevent carbon dioxide fixation (Hende et al., 2012;

Converti et al., 2009; Morais and Costa, 2007). Though all the species supplied with same amount of light intensity, each species adapted to different light intensity as it uptake different rate of DIC that leads to different biofixation and photosynthetic rate (Yoo et al., 2010). It is because each strain has their own capability for the fixation of carbon sources to accumulate high biomass, lipids and other biochemical compounds (Hu and Gau, 2003).

Based on this study, all the three strains, *Chlorella vulgaris* UMACC 001 and *Scenedesmus quadricauda* UMACC 041 showed better growth at 5% CO_2 producing higher biomass, lipid, protein and specific growth rate. While *Chlorella* UMACC 275 and *Oocystis* UMACC 074 shown better growth at 10% CO_2 compared with ambient condition. This shows that high levels of CO_2 maximize the algal growth under natural conditions. Elevated levels of CO_2 also have good potential to enhance the photosynthetic rate that allows high biomass and high accumulation of lipid in microalgae cells (Hu and Gau, 2003). This high biomass from these strains is ideal for intensive agriculture and may be an excellent source for biodiesel production while favors the mitigation of CO_2 emitted from fossil fuel-fired power plants and other sources, thereby reduce the emissions of a major greenhouse gas.

5.3 APPRAISAL OF THE STUDY

Microalgae are known to be the most reliable renewable energy source that has high potential for sequestering carbon dioxide, a greenhouse that leads to global warming. They are extensively exploited for their high lipid production, maximum biomass production and other useful compounds that have great significance in industry and pharmaceuticals. Biomitigation of carbon dioxide by the microalgae is one of the promising ways of bioremediation as it can reduce the elevated level of carbon dioxide in the environment.

The significance of this study is that the *Chlorella vulgaris* UMACC 001 was shown to have to grow at elevated carbon dioxide at 5% and 10% CO_2 and produce high biomass. It also produced high lipid content with a wide array of SFA that can be used as biofuel feedstock. Besides, this photoautotrophic cultivation mode allows efficient energy transformation and cost effective in mitigating the increasing levels of carbon dioxide. The drawback in this study was that some strains attained low lipid accumulation compared with previous studies although high biomass was achieved. This may due to the insufficient supply of carbon dioxide as the provision of carbon dioxide in this study was for 12 hours only and the nutrients were not replenished in the batch culture. Modification of culture medium and continuous supply of carbon dioxide at elevated levels may induce high lipid accumulation with increased SFA content.

Microalgae have the potential to change the energy industry, providing a solution to transform the existing systems for biofuels production and enabling new applications of existing technologies, provided that one can improve its production cost to a point competitive with fossil fuels.

5.4 RECOMMENDED AREAS FOR FURTHER STUDIES

Microalgae are one of the promising microorganisms for the mitigation of carbon dioxide from the atmosphere as they can fix more carbon than the terrestrial plants. Therefore, it is vital to apply approaches in order to improve their productivity.

1. In order to maximize the algal growth and higher lipid accumulation, the cultures could be grown in batch culture using large scale system such as photobioreactor. Cultivation of microalgae in a photobioreactor allows for enhanced biomass production and high lipid accumulation. Besides lipid production, other biochemicals which are produced may be utilized as co-products.

2. Future studies may focus on growing the tolerant algal strains in higher levels of CO_2 to determine their tolerance and efficiency of biofixation as 10% CO_2 was the highest level in this study. This may be increase the tolerance of microalgae at higher levels of carbon dioxide and in addition to assessing growth rate and biochemical composition, stress biomarkers and photosynthetic efficiency may also be investigated.

3. Since *Chlorella vulgaris* UMACC 001 is a potential strain for high biomass and high lipid production, genetic modification of this strain should be investigated in future in order to manipulate the strain to produce more SFA, which is the preferred fatty acid for biofuel production.

CHAPTER 6

CONCLUSION

Ten strains of microalgae from the UMACC were screened for their growth performance and biochemical composition. Four strains were selected for further studies on their ability to grow at different levels of CO_2. Based on the findings of this study, the following conclusions can be made:

1. Based on the screening, *Chlorella vulgaris* UMACC 001, *Scenedesmus quadricauda* UMACC 041, *Chlorella* UMACC 275 and *Oocystis* UMACC 074 are the potential strains as they produced high amounts of biomass and lipids with high specific growth rate.

2. Enrichment of carbon dioxide enhanced the biomass and lipid accumulation of all strains. The highest biomass and lipid content were 240.67±5.77mg/l and 38.67± 4.62% DW respectively that were observed in *Chlorella vulgaris* UMACC 001. The maximum specific growth rate also was achieved by UMACC 001 0.2433 ± 0.0168day^{-1}.

3. The addition of carbon dioxide has greatly influence the lipid production and the composition of fatty acids. The highest lipid production was obtained in culture medium enriched with 5% CO_2. While the highest PUFA and SFA were attained by the cultures added with 10% CO_2. The highest PUFA content 73.4% TFA was observed in *Scenedesmus quadricauda* UMACC 041.

4. Based on the growth of microalgae at elevated carbon dioxide, *Chlorella vulgaris* UMACC 001 produced highest amounts of biomass and lipids at 5% CO_2. Taken together, this study showed that *Chlorella vulgaris* UMACC 001 could be a potential strain for CO_2 biofixation and biofuel production. Further studies are worthwhile to

assess the potential of this strain, especially using large scale culture systems such as photobioreactors and open ponds.

To answer the research questions:

1. The tropical microalgae *Chlorella vulgaris* UMACC 001, *Scenedesmus quadricauda* UMACC 041, *Oocystis* UMACC 074 and *Chlorella* UMACC 275 were shown to be able to grow when supplied up to 10% CO_2.

2. At elevated CO_2 level, there was an increase in lipid and protein content in *Chlorella vulgaris* UMACC 001, *Scenedesmus quadricauda* UMACC 041, *Oocystis* UMACC 074 and *Chlorella* UMACC 275. There was no significant changes ($p<0.05$) in carbohydrate content.

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APPENDIX A

Growth trends of preliminary screening of ten microalgae strains

a) Optical density (OD 620_{nm})

i) *Chlorella vulgaris* UMACC 001

Day	R1	R2	R3	Mean	STDEV
0	0.023	0.022	0.023	0.023	± 0.001
2	0.044	0.049	0.066	0.053	± 0.012
4	0.083	0.073	0.061	0.072	± 0.011
6	0.123	0.120	0.106	0.116	± 0.009
8	0.188	0.198	0.172	0.186	± 0.013
10	0.342	0.383	0.352	0.359	± 0.021
12	0.364	0.402	0.387	0.384	± 0.019

ii) *Chlorella* UMACC 275

Day	R1	R2	R3	Mean	STDEV
0	0.021	0.021	0.021	0.021	± 0.000
2	0.034	0.037	0.051	0.041	± 0.009
4	0.065	0.067	0.084	0.072	± 0.010
6	0.079	0.084	0.099	0.087	± 0.010
8	0.105	0.129	0.167	0.134	± 0.031
10	0.143	0.227	0.231	0.200	± 0.050
12	0.177	0.251	0.271	0.233	± 0.050

iii) *Scenedesmus quadricauda* UMACC 041

Day	R1	R2	R3	Mean	STDEV
0	0.021	0.022	0.023	0.022	± 0.001
2	0.055	0.042	0.057	0.051	± 0.008
4	0.064	0.063	0.065	0.064	± 0.001
6	0.080	0.080	0.083	0.081	± 0.002
8	0.098	0.116	0.115	0.110	± 0.010
10	0.182	0.192	0.188	0.187	± 0.005
12	0.188	0.206	0.204	0.199	± 0.010

iv) *Oocystis* UMACC 074

Day	R1	R2	R3	Mean	STDEV
0	0.021	0.021	0.021	0.021	± 0.000
2	0.072	0.065	0.064	0.067	± 0.004
4	0.076	0.067	0.067	0.070	± 0.005
6	0.086	0.091	0.093	0.090	± 0.004
8	0.086	0.091	0.093	0.090	± 0.004
10	0.087	0.097	0.099	0.094	± 0.006
12	0.094	0.108	0.111	0.104	± 0.009

v) *Scenedesmus obtusus* UMACC 100

Day	R1	R2	R3	Mean	STDEV
0	0.025	0.020	0.021	0.022	± 0.003
2	0.059	0.064	0.060	0.061	± 0.003
4	0.061	0.068	0.067	0.065	± 0.004
6	0.067	0.078	0.083	0.076	± 0.008
8	0.090	0.119	0.111	0.107	± 0.015
10	0.155	0.205	0.173	0.178	± 0.025
12	0.170	0.187	0.187	0.181	± 0.010

vi) *Chlorococcum miniatum* UMACC 102

Day	R1	R2	R3	Mean	STDEV
0	0.021	0.022	0.021	0.021	± 0.001
2	0.063	0.063	0.062	0.063	± 0.001
4	0.074	0.074	0.070	0.073	± 0.002
6	0.089	0.092	0.093	0.091	± 0.002
8	0.089	0.092	0.097	0.093	± 0.004
10	0.099	0.093	0.098	0.097	± 0.003
12	0.103	0.112	0.104	0.106	± 0.005

vii) *Chlorella vulgaris* UMACC 103

Day	R1	R2	R3	Mean	STDEV
0	0.024	0.023	0.023	0.023	± 0.001
2	0.053	0.046	0.050	0.050	± 0.004
4	0.064	0.066	0.072	0.067	± 0.004
6	0.084	0.075	0.069	0.076	± 0.008
8	0.109	0.110	0.115	0.111	± 0.003
10	0.190	0.200	0.215	0.202	± 0.013
12	0.276	0.285	0.254	0.272	± 0.016

viii) *Chlorella* UMACC 253

Day	R1	R2	R3	Mean	STDEV
0	0.022	0.025	0.024	0.024	± 0.002
2	0.063	0.063	0.062	0.063	± 0.001
4	0.068	0.057	0.056	0.060	± 0.007
6	0.071	0.061	0.072	0.068	± 0.006
8	0.108	0.094	0.116	0.106	± 0.011
10	0.172	0.179	0.200	0.184	± 0.015
12	0.193	0.172	0.218	0.194	± 0.023

ix) *Chlorella vulgaris* UMACC 245

Day	R1	R2	R3	Mean	STDEV
0	0.021	0.021	0.021	0.021	± 0.000
2	0.056	0.058	0.055	0.056	± 0.002
4	0.062	0.073	0.075	0.070	± 0.007
6	0.074	0.078	0.081	0.078	± 0.004
8	0.079	0.083	0.085	0.082	± 0.003
10	0.089	0.091	0.094	0.091	± 0.003
12	0.094	0.103	0.106	0.101	± 0.006

x) *Chlorella* UMACC 255

Day	R1	R2	R3	Mean	STDEV
0	0.021	0.021	0.021	0.021	0.000
2	0.080	0.086	0.088	0.085	0.004
4	0.165	0.173	0.163	0.167	0.005
6	0.185	0.188	0.191	0.188	0.003
8	0.191	0.192	0.195	0.193	0.002
10	0.189	0.193	0.189	0.190	0.002
12	0.205	0.194	0.211	0.203	0.009

b) Cell count

i) *Chlorella vulgaris* UMACC 001

Day	R1	R2	R3	Mean	STDEV
0	33	30	25	29	± 4
2	55	60	60	58	± 3
4	71	70	78	73	± 4
6	101	112	104	106	± 6
8	145	133	151	143	± 9
10	245	226	230	234	± 10
12	269	258	276	268	± 9

ii) *Chlorella* UMACC 275

Day	R1	R2	R3	Mean	STDEV
0	20	18	13	17	± 4
2	20	22	31	24	± 6
4	70	67	83	73	± 9
6	89	83	121	98	± 20
8	103	132	121	119	± 15
10	223	218	252	231	± 18
12	259	255	267	260	± 6

iii) *Scenedesmus quadricauda* UMACC 041

Day	R1	R2	R3	Mean	STDEV
0	17	18	14	16	± 2
2	25	27	29	27	± 2
4	31	29	37	32	± 4
6	40	47	39	42	± 4
8	72	55	63	63	± 9
10	86	71	78	78	± 8
12	94	86	89	90	± 4

iv) *Oocystis* UMACC 074

Day	R1	R2	R3	Mean	STDEV
0	6	8	10	8	± 2
2	22	22	21	22	± 1
4	31	26	27	28	± 3
6	35	31	29	32	± 3
8	38	34	32	35	± 3
10	42	36	39	39	± 3
12	48	41	51	47	± 5

v) *Scenedesmus obtusus* UMACC 100

Day	R1	R2	R3	Mean	STDEV
0	11	9	9	10	± 1
2	18	14	12	15	± 3
4	23	28	29	27	± 3
6	34	28	31	31	± 3
8	48	39	36	41	± 6
10	57	61	58	59	± 2
12	68	74	62	68	± 6

vi) *Chlorococcum miniatum* UMACC 102

Day	R1	R2	R3	Mean	STDEV
0	11	8	7	9	± 2
2	22	21	17	20	± 3
4	28	33	29	30	± 3
6	31	36	37	35	± 3
8	33	38	39	37	± 3
10	38	45	44	42	± 4
12	41	49	47	46	± 4

vii) *Chlorella vulgaris* UMACC 103

Day	R1	R2	R3	Mean	STDEV
0	11	14	14	13	± 2
2	25	25	26	25	± 1
4	31	33	40	35	± 5
6	73	68	66	69	± 4
8	79	73	87	80	± 7
10	101	90	94	95	± 6
12	121	104	115	113	± 9

viii) *Chlorella* UMACC 253

Day	R1	R2	R3	Mean	STDEV
0	14	13	11	13	± 2
2	17	17	16	17	± 1
4	28	24	25	26	± 2
6	47	40	51	46	± 6
8	62	64	49	58	± 8
10	89	93	81	88	± 6
12	96	102	104	101	± 4

ix) *Chlorella vulgaris* UMACC 245

Day	R1	R2	R3	Mean	STDEV
0	13	13	9	12	± 2
2	14	19	18	17	± 3
4	28	30	36	31	± 4
6	34	32	38	35	± 3
8	37	35	42	38	± 4
10	45	41	51	46	± 5
12	51	47	55	51	± 4

x) *Chlorella* UMACC 255

Day	R1	R2	R3	Mean	STDEV
0	11	15	14	13	± 2
2	40	42	50	44	± 5
4	50	65	77	64	± 14
6	52	67	79	66	± 14
8	59	69	81	70	± 11
10	63	75	78	72	± 8
12	73	77	81	77	± 4

c) Chlorophyll a content

i) *Chlorella vulgaris* UMACC 001

Day	Sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
0	R1	0.032	0.019	0.014	0.344	344.350	0.344	0.328	0.018
	R2	0.028	0.011	0.006	0.310	309.550	0.310		
	R3	0.031	0.020	0.015	0.331	331.300	0.331		
2	R1	0.032	0.016	0.007	0.349	349.260	0.349	0.347	0.012
	R2	0.031	0.018	0.009	0.335	334.760	0.335		
	R3	0.033	0.018	0.008	0.358	358.100	0.358		
4	R1	0.072	0.028	0.014	0.797	796.560	0.797	0.824	0.032
	R2	0.078	0.033	0.018	0.859	859.050	0.859		
	R3	0.074	0.030	0.016	0.817	816.860	0.817		
6	R1	0.090	0.037	0.020	0.993	992.730	0.993	1.059	0.079
	R2	0.104	0.043	0.024	1.147	1146.710	1.147		
	R3	0.094	0.039	0.021	1.036	1036.370	1.036		
8	R1	0.082	0.036	0.022	0.901	900.960	0.901	1.125	0.199
	R2	0.108	0.044	0.022	1.192	1192.080	1.192		
	R3	0.116	0.046	0.023	1.282	1282.120	1.282		
10	R1	0.135	0.053	0.026	1.493	1492.930	1.493	1.590	0.084
	R2	0.148	0.059	0.030	1.635	1635.310	1.635		
	R3	0.149	0.063	0.032	1.641	1641.390	1.641		
12	R1	0.251	0.105	0.053	2.767	2766.630	2.767	2.414	0.438
	R2	0.232	0.101	0.057	2.551	2550.910	2.551		
	R3	0.174	0.069	0.036	1.923	1922.970	1.923		

ii) *Chlorella* UMACC 275

Day	Sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
	R1	0.017	0.010	0.046	0.178	177.660	0.178		
	R2	0.015	0.009	0.038	0.157	156.890	0.157		
0	R3	0.017	0.011	0.046	0.176	176.350	0.176	0.170	0.012
	R1	0.043	0.017	0.014	0.47457	474.57	0.475		
	R2	0.051	0.021	0.013	0.56227	562.27	0.562		
2	R3	0.041	0.015	0.007	0.45497	454.97	0.455	0.497	0.057
	R1	0.049	0.017	0.008	0.54501	545.01	0.545		
	R2	0.053	0.029	0.015	0.57471	574.71	0.575		
4	R3	0.049	0.019	0.009	0.54225	542.25	0.542	0.554	0.018
	R1	0.091	0.047	0.028	0.99011	990.11	0.990		
	R2	0.094	0.041	0.022	1.03361	1033.61	1.034		
6	R3	0.087	0.036	0.021	0.9591	959.1	0.959	0.994	0.037
	R1	0.109	0.046	0.023	1.201	1200.920	1.201		
	R2	0.102	0.043	0.021	1.124	1123.930	1.124		
8	R3	0.096	0.041	0.019	1.057	1057.230	1.057	1.127	0.072
	R1	0.131	0.051	0.025	1.449	1449.290	1.449		
	R2	0.144	0.057	0.028	1.592	1591.810	1.592		
10	R3	0.145	0.058	0.028	1.602	1602.100	1.602	1.548	0.085
	R1	0.192	0.079	0.047	2.117	2117.130	2.117		
	R2	0.238	0.100	0.055	2.622	2622.100	2.622		
12	R3	0.240	0.101	0.056	2.644	2643.850	2.644	2.461	0.298

iii) *Scenedesmus quadricauda* UMACC 041

Day	Sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
	R1	0.020	0.005	0.001	0.225	225.310	0.225		
	R2	0.024	0.009	0.004	0.266	266.050	0.266		
0	R3	0.021	0.005	0.001	0.237	236.910	0.237	0.243	0.021
	R1	0.032	0.009	0.000	0.359	359.410	0.359		
	R2	0.031	0.007	0.001	0.350	350.290	0.350		
2	R3	0.034	0.011	0.006	0.379	379.150	0.379	0.363	0.015
	R1	0.038	0.019	0.011	0.414	414.370	0.414		
	R2	0.042	0.021	0.012	0.458	458.010	0.458		
4	R3	0.049	0.021	0.011	0.539	539.350	0.539	0.471	0.063
	R1	0.078	0.030	0.018	0.863	862.980	0.863		
	R2	0.084	0.033	0.020	0.928	928.370	0.928		
6	R3	0.087	0.029	0.015	0.969	969.110	0.969	0.920	0.054
	R1	0.076	0.034	0.021	0.834	834.120	0.834		
	R2	0.077	0.031	0.017	0.850	850.210	0.850		
8	R3	0.064	0.024	0.012	0.709	709.280	0.709	0.798	0.077
	R1	0.072	0.028	0.014	0.797	796.560	0.797		
	R2	0.077	0.030	0.015	0.852	851.800	0.852		
10	R3	0.090	0.035	0.018	0.996	995.630	0.996	0.881	0.103
	R1	0.086	0.031	0.015	0.955	954.890	0.955		
	R2	0.112	0.048	0.028	1.232	1232.400	1.232		
12	R3	0.087	0.039	0.024	0.955	954.750	0.955	1.047	0.160

iv) *Oocystis* UMACC 074

Day	Sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
	R1	0.030	0.014	0.010	0.328	328.260	0.328		
	R2	0.021	0.007	0.004	0.234	233.870	0.234		
0	R3	0.029	0.012	0.008	0.320	319.560	0.320	0.294	0.052
	R1	0.009	0.003	0.001	0.100	100.330	0.100		
	R2	0.005	0.002	0.002	0.055	55.100	0.055		
2	R3	0.006	0.001	0.001	0.068	68.150	0.068	0.075	0.023
	R1	0.009	0.008	0.004	0.093	93.360	0.093		
	R2	0.010	0.009	0.004	0.104	103.650	0.104		
4	R3	0.012	0.008	0.003	0.128	128.300	0.128	0.108	0.018
	R1	0.036	0.013	0.010	0.399	399.170	0.399		
	R2	0.033	0.011	0.007	0.367	367.410	0.367		
6	R3	0.039	0.008	0.005	0.441	441.220	0.441	0.403	0.037
	R1	0.033	0.011	0.005	0.368	367.690	0.368		
	R2	0.032	0.010	0.004	0.358	357.540	0.358		
8	R3	0.036	0.009	0.004	0.405	405.250	0.405	0.377	0.025
	R1	0.035	0.011	0.005	0.391	390.890	0.391		
	R2	0.036	0.013	0.005	0.400	399.870	0.400		
10	R3	0.037	0.013	0.006	0.411	411.330	0.411	0.401	0.010
	R1	0.035	0.009	0.005	0.394	393.510	0.394		
	R2	0.032	0.011	0.005	0.356	356.090	0.356		
12	R3	0.038	0.014	0.003	0.422	422.040	0.422	0.391	0.033

v) *Scenedesmus obtusus* UMACC 100

Day	Sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
	R1	0.032	0.016	0.008	0.349	349.120	0.349		
	R2	0.026	0.011	0.013	0.285	285.370	0.285		
0	R3	0.028	0.013	0.009	0.307	306.510	0.307	0.314	0.032
	R1	0.068	0.028	0.014	0.750	750.160	0.750		
	R2	0.067	0.025	0.010	0.743	743.050	0.743		
2	R3	0.065	0.022	0.007	0.724	724.200	0.724	0.739	0.013
	R1	0.053	0.024	0.013	0.582	581.540	0.582		
	R2	0.053	0.023	0.012	0.583	582.990	0.583		
4	R3	0.056	0.027	0.017	0.612	611.850	0.612	0.592	0.017
	R1	0.071	0.029	0.015	0.784	783.510	0.784		
	R2	0.084	0.034	0.018	0.927	927.340	0.927		
6	R3	0.078	0.031	0.015	0.862	862.090	0.862	0.858	0.072
	R1	0.044	0.024	0.012	0.477	477.280	0.477		
	R2	0.053	0.021	0.011	0.586	585.750	0.586		
8	R3	0.069	0.031	0.017	0.757	757.410	0.757	0.607	0.141
	R1	0.066	0.028	0.013	0.727	727.100	0.727		
	R2	0.065	0.028	0.014	0.715	715.360	0.715		
10	R3	0.070	0.030	0.016	0.770	770.460	0.770	0.738	0.029
	R1	0.094	0.043	0.025	1.031	1030.570	1.031		
	R2	0.088	0.044	0.027	0.959	959.380	0.959		
12	R3	0.077	0.032	0.021	0.848	848.340	0.848	0.946	0.092

vi) *Chlorococcum miniatum* UMACC 102

Day	Sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
0	R1	0.042	0.019	0.013	0.460	460.490	0.460	0.440	0.018
	R2	0.039	0.016	0.014	0.429	429.480	0.429		
	R3	0.039	0.016	0.013	0.430	429.620	0.430		
2	R1	0.020	0.006	0.002	0.224	223.860	0.224	0.244	0.037
	R2	0.026	0.011	0.002	0.287	286.910	0.287		
	R3	0.020	0.007	0.005	0.222	222.130	0.222		
4	R1	0.019	0.009	0.005	0.208	207.910	0.208	0.198	0.014
	R2	0.017	0.011	0.009	0.182	181.530	0.182		
	R3	0.019	0.011	0.008	0.205	204.870	0.205		
6	R1	0.041	0.010	0.006	0.462	461.660	0.462	0.442	0.018
	R2	0.038	0.009	0.004	0.428	428.450	0.428		
	R3	0.039	0.013	0.005	0.435	434.670	0.435		
8	R1	0.032	0.013	0.006	0.353	353.330	0.353	0.344	0.040
	R2	0.027	0.010	0.004	0.300	299.540	0.300		
	R3	0.034	0.012	0.005	0.378	377.980	0.378		
10	R1	0.041	0.017	0.005	0.453	452.630	0.453	0.434	0.017
	R2	0.038	0.016	0.008	0.419	418.720	0.419		
	R3	0.039	0.016	0.009	0.430	430.180	0.430		
12	R1	0.038	0.016	0.009	0.419	418.580	0.419	0.429	0.039
	R2	0.036	0.015	0.008	0.397	396.830	0.397		
	R3	0.043	0.019	0.006	0.473	473.070	0.473		

vii) *Chlorella vulgaris* UMACC 103

Day	Sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
	R1	0.013	0.003	0.002	0.147	146.590	0.015		
	R2	0.012	0.003	0.001	0.135	135.130	0.014		
0	R3	0.011	0.002	0.001	0.125	124.840	0.013	0.014	0.001
	R1	0.009	0.002	0.002	0.102	101.500	0.102		
	R2	0.008	0.001	0.000	0.091	91.490	0.091		
2	R3	0.007	0.000	0.000	0.081	81.200	0.081	0.091	0.010
	R1	0.034	0.013	0.006	0.377	376.530	0.377		
	R2	0.031	0.011	0.004	0.345	344.630	0.345		
4	R3	0.032	0.012	0.004	0.355	354.920	0.355	0.359	0.016
	R1	0.048	0.016	0.009	0.535	534.580	0.535		
	R2	0.049	0.020	0.010	0.541	540.800	0.541		
6	R3	0.051	0.023	0.014	0.560	559.510	0.560	0.545	0.013
	R1	0.041	0.019	0.012	0.449	449.030	0.449		
	R2	0.053	0.021	0.014	0.585	585.330	0.585		
8	R3	0.057	0.026	0.016	0.625	624.900	0.625	0.553	0.092
	R1	0.063	0.024	0.008	0.698	698.240	0.698		
	R2	0.071	0.031	0.013	0.781	781.170	0.781		
10	R3	0.052	0.019	0.010	0.577	576.910	0.577	0.685	0.103
	R1	0.110	0.044	0.021	1.215	1215.420	1.215		
	R2	0.097	0.039	0.019	1.071	1071.450	1.071		
12	R3	0.120	0.052	0.029	1.320	1319.820	1.320	1.202	0.125

viii) *Chlorella* UMACC 253

Day	Sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
	R1	0.012	0.003	0.000	0.135	135.270	0.014		
	R2	0.014	0.005	0.002	0.156	155.570	0.016		
0	R3	0.012	0.001	0.001	0.138	137.750	0.014	0.015	0.001
	R1	0.008	0.000	0.000	0.093	92.800	0.093		
	R2	0.007	0.001	0.000	0.080	79.890	0.080		
2	R3	0.009	0.000	0.001	0.104	104.260	0.104	0.092	0.012
	R1	0.031	0.009	0.003	0.347	347.390	0.347		
	R2	0.033	0.015	0.007	0.362	362.170	0.362		
4	R3	0.029	0.009	0.004	0.324	324.050	0.324	0.345	0.019
	R1	0.055	0.023	0.011	0.606	606.330	0.606		
	R2	0.046	0.020	0.010	0.506	506.000	0.506		
6	R3	0.022	0.004	0.005	0.249	249.260	0.249	0.454	0.184
	R1	0.046	0.017	0.007	0.510	510.350	0.510		
	R2	0.044	0.016	0.007	0.488	488.460	0.488		
8	R3	0.041	0.011	0.004	0.461	460.630	0.461	0.486	0.025
	R1	0.065	0.027	0.013	0.717	716.810	0.717		
	R2	0.064	0.026	0.008	0.707	707.220	0.707		
10	R3	0.062	0.025	0.011	0.685	684.910	0.685	0.703	0.016
	R1	0.089	0.035	0.015	0.984	984.450	0.984		
	R2	0.089	0.035	0.015	0.984	984.450	0.984		
12	R3	0.093	0.039	0.019	1.025	1025.050	1.025	0.998	0.023

ix) *Chlorella vulgaris* UMACC 245

Day	Sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
	R1	0.018	0.006	0.003	0.201	200.520	0.020		
	R2	0.017	0.006	0.003	0.189	188.920	0.019		
0	R3	0.016	0.006	0.003	0.177	177.320	0.018	0.019	0.001
	R1	0.022	0.003	0.002	0.251	250.990	0.251		
	R2	0.019	0.001	0.000	0.219	219.090	0.219		
2	R3	0.016	0.000	0.000	0.186	185.600	0.186	0.219	0.033
	R1	0.013	0.001	0.000	0.149	149.490	0.149		
	R2	0.016	0.002	0.001	0.183	182.840	0.183		
4	R3	0.013	0.001	0.000	0.149	149.490	0.149	0.161	0.019
	R1	0.035	0.011	0.002	0.391	391.310	0.391		
	R2	0.039	0.012	0.003	0.436	436.260	0.436		
6	R3	0.037	0.009	0.003	0.417	416.990	0.417	0.415	0.023
	R1	0.045	0.010	0.004	0.508	508.340	0.508		
	R2	0.048	0.010	0.004	0.543	543.140	0.543		
8	R3	0.047	0.010	0.004	0.532	531.540	0.532	0.528	0.018
	R1	0.058	0.023	0.010	0.641	641.270	0.641		
	R2	0.064	0.026	0.012	0.707	706.660	0.707		
10	R3	0.062	0.024	0.011	0.686	686.220	0.686	0.678	0.033
	R1	0.058	0.025	0.010	0.639	638.650	0.639		
	R2	0.054	0.027	0.009	0.590	589.770	0.590		
12	R3	0.064	0.028	0.013	0.704	703.900	0.704	0.644	0.057

x) *Chlorella* UMACC 255

Day	Sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
	R1	0.103	0.042	0.022	1.137	1136.700	0.114		
	R2	0.092	0.037	0.019	1.016	1016.070	0.102		
0	R3	0.092	0.037	0.019	1.016	1016.070	0.102	0.106	0.007
	R1	0.011	0.003	0.002	0.123	123.390	0.123		
	R2	0.010	0.002	0.001	0.113	113.240	0.113		
2	R3	0.018	0.002	0.003	0.206	205.760	0.206	0.147	0.051
	R1	0.035	0.012	0.012	0.389	388.600	0.389		
	R2	0.037	0.013	0.009	0.411	410.910	0.411		
4	R3	0.038	0.014	0.014	0.421	420.500	0.421	0.407	0.016
	R1	0.059	0.021	0.007	0.656	655.910	0.656		
	R2	0.054	0.023	0.008	0.595	595.150	0.595		
6	R3	0.053	0.017	0.003	0.592	592.110	0.592	0.614	0.036
	R1	0.068	0.027	0.013	0.752	751.610	0.752		
	R2	0.074	0.032	0.018	0.814	813.960	0.814		
8	R3	0.077	0.036	0.019	0.843	843.380	0.843	0.803	0.047
	R1	0.074	0.029	0.014	0.818	818.450	0.818		
	R2	0.082	0.033	0.018	0.905	905.450	0.905		
10	R3	0.087	0.036	0.018	0.960	959.520	0.960	0.894	0.071
	R1	0.083	0.034	0.019	0.916	915.600	0.916		
	R2	0.084	0.029	0.014	0.934	934.450	0.934		
12	R3	0.089	0.038	0.018	0.980	980.100	0.980	0.943	0.033

d) Specific growth rate of ten microalgae strains

Strains	R1	R2	R3	MEAN	STDEV
Chlorella vulgaris UMACC 001	0.2385	0.262	0.2295	0.2433	0.0168
Scenedesmus obtusus UMACC 100	0.101	0.172	0.125	0.1327	0.0361
Scenedesmus quadricauda UMACC 041	0.2245	0.2025	0.215	0.2140	0.0110
Oocystis UMACC 074	0.153	0.1315	0.1565	0.1470	0.0135
Chlorococcum miniatum UMACC 102	0.127	0.123	0.115	0.1217	0.0061
Chlorella vulgaris UMACC 103	0.1375	0.127	0.137	0.1338	0.0059
Chlorella vulgaris UMACC 245	0.121	0.1265	0.134	0.1272	0.0065
Chlorella UMACC 253	0.127	0.141	0.11	0.1260	0.0155
Chlorella UMACC 255	0.133	0.149	0.1075	0.1298	0.0209
Chlorella UMACC 275	0.2226	0.2295	0.2084	0.2202	0.0108

e) <u>pH of the ten microalgae strains</u>

		Day 0			Day 10		
Species	Sample	pH	Mean	STDEV	pH	Mean	STDEV
Chlorella vulgaris UMACC 001	R1	7.03			6.97		
	R2	7.01			6.90		
	R3	7.03	7.02	0.012	6.97	6.95	0.040
Scenedesmus obtusus UMACC 100	R1	7.11			7.09		
	R2	7.11			7.08		
	R3	7.10	7.11	0.006	7.08	7.08	0.006
Scenedesmus quadricauda UMACC 041	R1	7.19			7.17		
	R2	7.17			7.15		
	R3	7.18	7.18	0.010	7.16	7.16	0.010
Oocystis UMACC 074	R1	6.71			6.69		
	R2	6.71			6.68		
	R3	6.72	6.71	0.006	6.69	6.69	0.006
Chlorococcum miniatum UMACC 102	R1	6.73			6.70		
	R2	6.71			6.68		
	R3	6.71	6.72	0.012	6.69	6.69	0.010
Chlorella UMACC 275	R1	6.89			6.72		
	R2	6.90			6.74		
	R3	6.88	6.89	0.010	6.69	6.72	0.025
Chlorella vulgaris UMACC 103	R1	8.12			8.09		
	R2	8.10			8.07		
	R3	8.13	8.12	0.02	8.10	8.09	0.02
Chlorella UMACC 253	R1	8.19			8.01		
	R2	8.21			8.02		
	R3	8.20	8.20	0.01	8.02	8.02	0.01
Chlorella vulgaris UMACC 245	R1	8.35			8.09		
	R2	8.36			8.07		
	R3	8.35	8.35	0.01	8.11	8.09	0.02
Chlorella UMACC 255	R1	7.94			8.01		
	R2	7.96			8.02		
	R3	7.96	7.95	0.01	8.02	8.02	0.01

APPENDIX B

Carotenoid Content of Preliminary Screening of Ten Microalgae Strains

i) *Chlorella vulgaris* UMACC 001

Day	Sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
	R1	0.046	0.178		
	R2	0.035	0.135		
0	R3	0.048	0.185	0.166	± 0.027
	R1	0.050	0.193		
	R2	0.066	0.255		
2	R3	0.065	0.251	0.233	± 0.035
	R1	0.081	0.313		
	R2	0.096	0.371		
4	R3	0.089	0.344	0.342	± 0.029
	R1	0.088	0.340		
	R2	0.099	0.382		
6	R3	0.101	0.390	0.371	± 0.027
	R1	0.101	0.390		
	R2	0.133	0.513		
8	R3	0.140	0.540	0.481	± 0.080
	R1	0.166	0.641		
	R2	0.181	0.699		
10	R3	0.185	0.714	0.685	± 0.039
	R1	0.303	1.170		
	R2	0.280	1.081		
12	R3	0.212	0.818	1.023	± 0.183

ii) *Chlorella* UMACC 275

Day	Sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
	R1	0.046	0.178		
	R2	0.038	0.147		
0	R3	0.039	0.151	0.158	± 0.017
	R1	0.067	0.259		
	R2	0.050	0.193		
2	R3	0.068	0.262	0.238	± 0.039
	R1	0.054	0.208		
	R2	0.090	0.347		
4	R3	0.059	0.228	0.261	± 0.075
	R1	0.089	0.344		
	R2	0.087	0.336		
6	R3	0.082	0.317	0.332	± 0.014
	R1	0.103	0.398		
	R2	0.098	0.378		
8	R3	0.093	0.359	0.378	± 0.019
	R1	0.168	0.648		
	R2	0.173	0.668		
10	R3	0.183	0.706	0.674	± 0.029
	R1	0.234	0.903		
	R2	0.290	1.119		
12	R3	0.291	1.123	1.049	± 0.126

iii) *Scenedesmus quadricauda* UMACC 041

Day	Sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
	R1	0.025	0.097		
	R2	0.030	0.116		
0	R3	0.026	0.100	0.104	± 0.010
	R1	0.043	0.166		
	R2	0.038	0.147		
2	R3	0.057	0.220	0.178	± 0.038
	R1	0.087	0.336		
	R2	0.087	0.336		
4	R3	0.083	0.320	0.331	± 0.009
	R1	0.067	0.259		
	R2	0.072	0.278		
6	R3	0.068	0.262	0.266	± 0.010
	R1	0.092	0.355		
	R2	0.093	0.359		
8	R3	0.078	0.301	0.338	± 0.032
	R1	0.087	0.336		
	R2	0.098	0.378		
10	R3	0.112	0.432	0.382	± 0.048
	R1	0.101	0.390		
	R2	0.137	0.529		
12	R3	0.108	0.417	0.445	± 0.074

iv) *Oocystis* UMACC 074

Day	Sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
	R1	0.038	0.147		
	R2	0.027	0.104		
0	R3	0.034	0.131	0.127	± 0.021
	R1	0.016	0.062		
	R2	0.006	0.023		
2	R3	0.010	0.039	0.041	± 0.019
	R1	0.051	0.197		
	R2	0.041	0.158		
4	R3	0.039	0.151	0.169	± 0.025
	R1	0.042	0.162		
	R2	0.047	0.181		
6	R3	0.031	0.120	0.154	± 0.032
	R1	0.035	0.135		
	R2	0.033	0.127		
8	R3	0.032	0.124	0.129	± 0.006
	R1	0.037	0.143		
	R2	0.046	0.178		
10	R3	0.046	0.178	0.166	± 0.020
	R1	0.035	0.135		
	R2	0.044	0.170		
12	R3	0.031	0.120	0.142	± 0.026

v) *Scenedesmus obtusus* UMACC 100

Day	Sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
	R1	0.056	0.216		
	R2	0.065	0.251		
0	R3	0.064	0.247	0.238	± 0.019
	R1	0.088	0.340		
	R2	0.085	0.328		
2	R3	0.082	0.317	0.328	± 0.012
	R1	0.069	0.266		
	R2	0.069	0.266		
4	R3	0.077	0.297	0.277	± 0.018
	R1	0.091	0.351		
	R2	0.105	0.405		
6	R3	0.112	0.432	0.396	± 0.041
	R1	0.059	0.228		
	R2	0.071	0.274		
8	R3	0.091	0.351	0.284	± 0.062
	R1	0.088	0.340		
	R2	0.084	0.324		
10	R3	0.091	0.351	0.338	± 0.014
	R1	0.118	0.455		
	R2	0.109	0.421		
12	R3	0.072	0.278	0.385	± 0.094

vi) *Chlorococcum miniatum* UMACC 102

Day	Sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
	R1	0.063	0.243		
	R2	0.058	0.224		
0	R3	0.057	0.220	0.229	± 0.012
	R1	0.015	0.058		
	R2	0.014	0.054		
2	R3	0.021	0.081	0.064	± 0.015
	R1	0.044	0.170		
	R2	0.066	0.255		
4	R3	0.066	0.255	0.226	± 0.049
	R1	0.042	0.162		
	R2	0.068	0.262		
6	R3	0.063	0.243	0.223	± 0.053
	R1	0.058	0.224		
	R2	0.071	0.274		
8	R3	0.065	0.251	0.250	± 0.025
	R1	0.096	0.371		
	R2	0.094	0.363		
10	R3	0.095	0.367	0.367	± 0.004
	R1	0.089	0.344		
	R2	0.093	0.359		
12	R3	0.092	0.355	0.353	± 0.008

vii) *Chlorella vulgaris* UMACC 103

Day	Sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
	R1	0.010	0.004		
	R2	0.012	0.005		
0	R3	0.008	0.003	0.004	± 0.001
	R1	0.012	0.046		
	R2	0.019	0.073		
2	R3	0.014	0.054	0.058	± 0.014
	R1	0.043	0.166		
	R2	0.033	0.127		
4	R3	0.048	0.185	0.160	± 0.029
	R1	0.043	0.166		
	R2	0.037	0.143		
6	R3	0.040	0.154	0.154	± 0.012
	R1	0.043	0.166		
	R2	0.038	0.147		
8	R3	0.056	0.216	0.176	± 0.036
	R1	0.056	0.216		
	R2	0.067	0.259		
10	R3	0.066	0.255	0.243	± 0.023
	R1	0.126	0.486		
	R2	0.113	0.436		
12	R3	0.135	0.521	0.481	± 0.043

viii) *Chlorella* UMACC 253

Day	Sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
0	R1	0.015	0.006	0.006	± 0.001
	R2	0.018	0.007		
	R3	0.016	0.006		
2	R1	0.020	0.077	0.051	± 0.022
	R2	0.010	0.039		
	R3	0.010	0.039		
4	R1	0.067	0.259	0.252	± 0.033
	R2	0.056	0.216		
	R3	0.073	0.282		
6	R1	0.056	0.216	0.194	± 0.028
	R2	0.042	0.162		
	R3	0.053	0.205		
8	R1	0.061	0.235	0.214	± 0.026
	R2	0.057	0.220		
	R3	0.048	0.185		
10	R1	0.082	0.317	0.299	± 0.028
	R2	0.069	0.266		
	R3	0.081	0.313		
12	R1	0.112	0.432	0.435	± 0.004
	R2	0.112	0.432		
	R3	0.114	0.440		

ix) *Chlorella vulgaris* UMACC 245

Day	Sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
	R1	0.029	0.011		
	R2	0.027	0.010		
0	R3	0.027	0.010	0.010	± 0.001
	R1	0.011	0.042		
	R2	0.012	0.046		
2	R3	0.009	0.035	0.041	± 0.006
	R1	0.021	0.081		
	R2	0.020	0.077		
4	R3	0.021	0.081	0.080	± 0.002
	R1	0.030	0.116		
	R2	0.025	0.097		
6	R3	0.031	0.120	0.111	± 0.012
	R1	0.024	0.093		
	R2	0.030	0.116		
8	R3	0.030	0.116	0.108	± 0.013
	R1	0.074	0.286		
	R2	0.104	0.401		
10	R3	0.104	0.401	0.363	± 0.067
	R1	0.077	0.297		
	R2	0.099	0.382		
12	R3	0.098	0.378	0.353	± 0.048

x) *Chlorella* UMACC 255

Day	Sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
	R1	0.131	0.051		
	R2	0.118	0.046		
0	R3	0.118	0.046	0.048	± 0.003
	R1	0.026	0.100		
	R2	0.037	0.143		
2	R3	0.028	0.108	0.117	± 0.023
	R1	0.063	0.243		
	R2	0.066	0.255		
4	R3	0.063	0.243	0.247	± 0.007
	R1	0.076	0.293		
	R2	0.075	0.290		
6	R3	0.081	0.313	0.299	± 0.012
	R1	0.091	0.351		
	R2	0.103	0.398		
8	R3	0.109	0.421	0.390	± 0.035
	R1	0.139	0.537		
	R2	0.095	0.367		
10	R3	0.115	0.444	0.449	± 0.085
	R1	0.129	0.498		
	R2	0.090	0.347		
12	R3	0.098	0.378	0.408	± 0.080

APPENDIX C

Biomass Production of Preliminary Screening of Ten Microalgae Strains on Day 0, Day 4, Day 8 and Day 12 Based on DW

i) *Chlorella vulgaris* UMACC 001

Day	Sample	Pre weight (g) [A]	Post weight (g) [B]	[B] - [A] (g)	dry weight (mg)	biomass (mg/l)	Mean	STDEV
	R1	0.0876	0.0911	0.0035	3.5000	70.00		
	R2	0.0862	0.0894	0.0032	3.2000	64.00		
0	R3	0.0871	0.0908	0.0037	3.7000	74.00	69.33	± 5.033
	R1	0.0873	0.0899	0.0026	2.6000	52.00		
	R2	0.0859	0.0878	0.0019	1.9000	38.00		
4	R3	0.0871	0.0892	0.0021	2.1000	42.00	44.00	± 7.211
	R1	0.0872	0.0911	0.0039	3.9000	78.00		
	R2	0.0861	0.0899	0.0038	3.8000	76.00		
8	R3	0.0856	0.0887	0.0031	3.1000	62.00	72.00	± 8.718
	R1	0.0883	0.1005	0.0122	12.2000	244.00		
	R2	0.0887	0.1009	0.0122	12.2000	244.00		
12	R3	0.0881	0.0998	0.0117	11.7000	234.00	240.67	± 5.774

ii) *Chlorella* UMACC 275

Day	Sample	Pre weight (g) [A]	Post weight (g) [B]	[B] - [A] (g)	dry weight (mg)	biomass (mg/l)	Mean	STDEV
	R1	0.0867	0.0889	0.0022	2.2000	44.00		
	R2	0.0865	0.0891	0.0026	2.6000	52.00		
0	R3	0.0871	0.0893	0.0022	2.2000	44.00	46.67	± 4.619
	R1	0.0874	0.0898	0.0024	2.4000	48.00		
	R2	0.0863	0.0888	0.0025	2.5000	50.00		
4	R3	0.0855	0.0880	0.0025	2.5000	50.00	49.33	± 1.155
	R1	0.0874	0.0916	0.0042	4.2000	84.00		
	R2	0.0868	0.0919	0.0051	5.1000	102.00		
8	R3	0.0874	0.0923	0.0049	4.9000	98.00	94.67	± 9.452
	R1	0.0879	0.0970	0.0091	9.1000	182.00		
	R2	0.0866	0.0986	0.0120	12.0000	240.00		
12	R3	0.0866	0.0941	0.0075	7.5000	150.00	190.67	± 45.622

iii) *Scenedesmus quadricauda* UMACC 041

Day	Sample	Pre weight (g) [A]	Post weight (g) [B]	[B] - [A] (g)	dry weight (mg)	biomass (mg/l)	Mean	STDEV
	R1	0.0874	0.0926	0.0052	5.2000	104.00		
	R2	0.0863	0.0920	0.0057	5.7000	114.00		
0	R3	0.0877	0.0944	0.0067	6.7000	134.00	117.33	± 15.275
	R1	0.0880	0.0903	0.0023	2.3000	46.00		
	R2	0.0869	0.0891	0.0022	2.2000	44.00		
4	R3	0.0858	0.0891	0.0033	3.3000	66.00	52.00	± 12.166
	R1	0.0871	0.0904	0.0033	3.3000	66.00		
	R2	0.0870	0.0901	0.0031	3.1000	62.00		
8	R3	0.0854	0.0889	0.0035	3.5000	70.00	66.00	± 4.000
	R1	0.0875	0.0962	0.0087	8.7000	174.00		
	R2	0.0879	0.0968	0.0089	8.9000	178.00		
12	R3	0.0872	0.0952	0.0080	8.0000	160.00	170.67	± 9.452

iv) *Oocystis* UMACC 074

Day	Sample	Pre weight (g) [A]	Post weight (g) [B]	[B] - [A] (g)	dry weight (mg)	biomass (mg/l)	Mean	STDEV
	R1	0.0878	0.0959	0.0081	8.1000	162.00		
	R2	0.0884	0.0961	0.0077	7.7000	154.00		
0	R3	0.0881	0.0968	0.0087	8.7000	174.00	163.33	± 10.066
	R1	0.0871	0.0902	0.0031	3.1000	62.00		
	R2	0.0880	0.0898	0.0018	1.8000	36.00		
4	R3	0.0873	0.0893	0.0020	2.0000	40.00	46.00	± 14.000
	R1	0.0857	0.0883	0.0026	2.6000	52.00		
	R2	0.0859	0.0894	0.0035	3.5000	70.00		
8	R3	0.0869	0.0912	0.0043	4.3000	86.00	69.33	± 17.010
	R1	0.0883	0.0968	0.0085	8.5000	170.00		
	R2	0.0878	0.0962	0.0084	8.4000	168.00		
12	R3	0.0885	0.0959	0.0074	7.4000	148.00	162.00	± 12.166

v) *Scenedesmus obtusus* UMACC 100

Day	Sample	Pre weight (g) [A]	Post weight (g) [B]	[B] - [A] (g)	dry weight (mg)	biomass (mg/l)	Mean	STDEV
	R1	0.0863	0.0932	0.0069	6.9000	138.00		
	R2	0.0859	0.0929	0.0070	7.0000	140.00		
0	R3	0.0864	0.0921	0.0057	5.7000	114.00	130.67	± 14.468
	R1	0.0872	0.0881	0.0009	0.9000	18.00		
	R2	0.0862	0.0869	0.0007	0.7000	14.00		
4	R3	0.0862	0.0871	0.0009	0.9000	18.00	16.67	± 2.309
	R1	0.0865	0.0892	0.0027	2.7000	54.00		
	R2	0.0873	0.0903	0.0030	3.0000	60.00		
8	R3	0.0854	0.0890	0.0036	3.6000	72.00	62.00	± 9.165
	R1	0.0879	0.0956	0.0077	7.7000	154.00		
	R2	0.0881	0.0944	0.0063	6.3000	126.00		
12	R3	0.0879	0.0949	0.0070	7.0000	140.00	140.00	± 14.000

vi) *Chlorococum miniatum* UMACC 102

Day	Sample	Pre weight (g) [A]	Post weight (g) [B]	[B] - [A] (g)	dry weight (mg)	biomass (mg/l)	Mean	STDEV
	R1	0.0874	0.0985	0.0111	11.1000	222.00		
	R2	0.0881	0.0990	0.0109	10.9000	218.00		
0	R3	0.0891	0.0997	0.0106	10.6000	212.00	217.33	± 5.033
	R1	0.0869	0.0889	0.0020	2.0000	40.00		
	R2	0.0853	0.0868	0.0015	1.5000	30.00		
4	R3	0.0856	0.0879	0.0023	2.3000	46.00	38.67	± 8.083
	R1	0.0880	0.0912	0.0032	3.2000	64.00		
	R2	0.0865	0.0911	0.0046	4.6000	92.00		
8	R3	0.0867	0.0911	0.0044	4.4000	88.00	81.33	± 15.144
	R1	0.0891	0.0971	0.0080	8.0000	160.00		
	R2	0.0887	0.0963	0.0076	7.6000	152.00		
12	R3	0.0886	0.0961	0.0075	7.5000	150.00	154.00	± 5.292

vii) *Chlorella vulgaris* UMACC 103

Day	Sample	Pre weight (g) [A]	Post weight (g) [B]	[B] - [A] (g)	dry weight (mg)	biomass (mg/l)	Mean	STDEV
	R1	0.0884	0.0911	0.0027	2.7000	54.00		
	R2	0.0847	0.0889	0.0042	4.2000	84.00		
0	R3	0.0852	0.0882	0.0030	3.0000	60.00	66.00	± 15.875
	R1	0.0862	0.0876	0.0014	1.4000	28.00		
	R2	0.0862	0.0872	0.0010	1.0000	20.00		
4	R3	0.0865	0.0881	0.0016	1.6000	32.00	26.67	± 6.110
	R1	0.0857	0.0883	0.0026	2.6000	52.00		
	R2	0.0859	0.0894	0.0035	3.5000	70.00		
8	R3	0.0869	0.0912	0.0043	4.3000	86.00	69.33	± 17.010
	R1	0.0883	0.0968	0.0085	8.5000	170.00		
	R2	0.0878	0.0962	0.0084	8.4000	168.00		
12	R3	0.0885	0.0959	0.0074	7.4000	148.00	162.00	± 12.166

viii) *Chlorella* UMACC 253

Day	Sample	Pre weight (g) [A]	Post weight (g) [B]	[B] - [A] (g)	dry weight (mg)	biomass (mg/l)	Mean	STDEV
	R1	0.0871	0.0891	0.0020	2.0000	40.00		
	R2	0.0864	0.0877	0.0013	1.3000	26.00		
0	R3	0.0871	0.0891	0.0020	2.0000	40.00	35.33	± 8.083
	R1	0.086	0.0877	0.0017	1.7000	34.00		
	R2	0.0865	0.0889	0.0024	2.4000	48.00		
4	R3	0.0877	0.0892	0.0015	1.5000	30.00	37.33	± 9.452
	R1	0.088	0.0912	0.0032	3.2000	64.00		
	R2	0.0865	0.0911	0.0046	4.6000	92.00		
8	R3	0.0867	0.0911	0.0044	4.4000	88.00	81.33	± 15.144
	R1	0.0891	0.0971	0.0080	8.0000	160.00		
	R2	0.0887	0.0963	0.0076	7.6000	152.00		
12	R3	0.0886	0.0961	0.0075	7.5000	150.00	154.00	± 5.292

ix) *Chlorella vulgaris* UMACC 245

Day	Sample	Pre weight (g) [A]	Post weight (g) [B]	[B] - [A] (g)	dry weight (mg)	biomass (mg/l)	Mean	STDEV
	R1	0.0872	0.0928	0.0056	5.6000	112.00		
	R2	0.0872	0.0929	0.0057	5.7000	114.00		
0	R3	0.0873	0.0927	0.0054	5.4000	108.00	111.33	± 3.055
	R1	0.0861	0.0878	0.0017	1.7000	34.00		
	R2	0.0863	0.0877	0.0014	1.4000	28.00		
4	R3	0.0877	0.0906	0.0029	2.9000	58.00	40.00	± 15.875
	R1	0.0866	0.0897	0.0031	3.1000	62.00		
	R2	0.0867	0.0896	0.0029	2.9000	58.00		
8	R3	0.0869	0.0908	0.0039	3.9000	78.00	66.00	± 10.583
	R1	0.0861	0.0915	0.0054	5.4000	108.00		
	R2	0.0868	0.0923	0.0055	5.5000	110.00		
12	R3	0.0863	0.0916	0.0053	5.3000	106.00	108.00	± 2.000

x) *Chlorella* UMACC 255

Day	Sample	Pre weight (g) [A]	Post weight (g) [B]	[B] - [A] (g)	dry weight (mg)	biomass (mg/l)	Mean	STDEV
	R1	0.0864	0.0932	0.0068	6.8000	136.00		
	R2	0.0882	0.0934	0.0052	5.2000	104.00		
0	R3	0.0868	0.0921	0.0053	5.3000	106.00	115.33	± 17.926
	R1	0.0884	0.0924	0.0040	4.0000	80.00		
	R2	0.0869	0.0921	0.0052	5.2000	104.00		
4	R3	0.0876	0.0929	0.0053	5.3000	106.00	96.67	± 14.468
	R1	0.0855	0.0908	0.0053	5.3000	106.00		
	R2	0.0841	0.0876	0.0035	3.5000	70.00		
8	R3	0.0843	0.0898	0.0055	5.5000	110.00	95.33	± 22.030
	R1	0.0854	0.0923	0.0069	6.9000	138.00		
	R2	0.0861	0.0932	0.0071	7.1000	142.00		
12	R3	0.0873	0.0955	0.0082	8.2000	164.00	148.00	± 14.000

APPENDIX D

Biochemical Composition of Preliminary Screening of Ten Microalgae Strains on Day0, Day4, Day8 and Day 12

a) Protein Content

i) *Chlorella vulgaris* UMACC 001

Day	Sample	595nm	protein in 100 ug (ug)	protein in 10 ml (ug)	protein in 10 ml (g)	Dry weight (g)	protein (g)/ dry weight(g)	% protein (g)/ dry weight (g)	Mean protein (mg/L)	STDEV
	R1	0.171	21.714	2171.429	0.002171	0.0035	0.6264	62.64		
	R2	0.173	22.032	2203.175	0.002203	0.0035	0.6355	63.55		
0	R3	0.175	22.349	2234.921	0.002235	0.0035	0.6447	64.47	63.55	± 0.916
	R1	0.132	15.524	1552.381	0.001552	0.0022	0.7056	70.56		
	R2	0.135	16.000	1600.000	0.001600	0.0022	0.7273	72.73		
4	R3	0.124	14.254	1425.397	0.001425	0.0022	0.6479	64.79	69.36	± 4.103
	R1	0.181	23.302	2330.159	0.002330	0.0036	0.6473	64.73		
	R2	0.182	23.460	2346.032	0.002346	0.0036	0.6517	65.17		
8	R3	0.175	22.349	2234.921	0.002235	0.0036	0.6208	62.08	63.99	± 1.669
	R1	0.322	45.683	4568.254	0.004568	0.0120	0.3796	37.96		
	R2	0.364	52.349	5234.921	0.005235	0.0120	0.4350	43.50		
12	R3	0.341	48.698	4869.841	0.004870	0.0120	0.4047	40.47	40.65	± 2.774

ii) *Chlorella* UMACC 275

Day	Sample	595nm	protein in 100 ug (ug)	protein in 10 ml (ug)	protein in 10 ml (g)	Dry weight (g)	protein (g)/ dry weight(g)	% protein (g)/ dry weight (g)	Mean protein (mg/L)	STDEV
	R1	0.093	9.333	933.333	0.000933	0.0023	0.4058	40.58		
	R2	0.103	10.921	1092.063	0.001092	0.0023	0.4748	47.48		
0	R3	0.108	11.714	1171.429	0.001171	0.0023	0.5093	50.93	46.33	± 5.271
	R1	0.107	11.556	1155.556	0.001156	0.0025	0.4622	46.22		
	R2	0.090	8.857	885.714	0.000886	0.0025	0.3543	35.43		
4	R3	0.098	10.127	1012.698	0.001013	0.0025	0.4051	40.51	40.72	± 5.400
	R1	0.162	20.286	2028.571	0.002029	0.0047	0.4316	43.16		
	R2	0.178	22.825	2282.540	0.002283	0.0047	0.4856	48.56		
8	R3	0.180	23.143	2314.286	0.002314	0.0047	0.4924	49.24	46.99	± 3.332
	R1	0.251	34.413	3441.270	0.003441	0.0095	0.3622	36.22		
	R2	0.265	36.635	3663.492	0.003663	0.0095	0.3856	38.56		
12	R3	0.278	38.698	3869.841	0.00387	0.0095	0.4074	40.74	38.51	± 2.256

iii) *Scenedesmus quadricauda* UMACC 041

Day	Sample	595nm	protein in 100 ug (ug)	protein in 10 ml (ug)	protein in 10 ml (g)	Dry weight (g)	protein (g)/ dry weight(g)	% protein (g)/ dry weight (g)	Mean protein (mg/L)	STDEV
	R1	0.230	31.079	3107.937	0.003108	0.0059	0.5298	52.98		
	R2	0.218	29.175	2917.460	0.002917	0.0059	0.4973	49.73		
0	R3	0.204	26.952	2695.238	0.002695	0.0059	0.4594	45.94	49.55	± 3.521
	R1	0.139	16.635	1663.492	0.001663	0.0026	0.6398	63.98		
	R2	0.140	16.794	1679.365	0.001679	0.0026	0.6459	64.59		
4	R3	0.150	18.381	1838.095	0.001838	0.0026	0.7070	70.70	66.42	± 3.714
	R1	0.153	18.857	1885.714	0.001886	0.0033	0.5714	57.14		
	R2	0.146	17.746	1774.603	0.001775	0.0033	0.5378	53.78		
8	R3	0.149	18.222	1822.222	0.001822	0.0033	0.5522	55.22	55.38	± 1.689
	R1	0.123	14.095	1409.524	0.001410	0.0085	0.1652	16.52		
	R2	0.095	9.651	965.079	0.000965	0.0085	0.1131	11.31		
12	R3	0.106	11.397	1139.683	0.001140	0.0085	0.1336	13.36	13.73	± 2.624

iv) *Oocystis* UMACC 074

Day	Sample	595nm	protein in 100 ug (ug)	protein in 10 ml (ug)	protein in 10 ml (g)	Dry weight (g)	protein (g)/ dry weight(g)	% protein (g)/ dry weight (g)	Mean protein (mg/L)	STDEV
	R1	0.146	17.746	1774.603	0.001775	0.0082	0.21730	21.73		
	R2	0.154	19.016	1901.587	0.001902	0.0082	0.23285	23.28		
0	R3	0.146	17.746	1774.603	0.001775	0.0082	0.21730	21.73	22.25	± 0.898
	R1	0.139	16.635	1663.492	0.001663	0.0023	0.72326	72.33		
	R2	0.136	16.159	1615.873	0.001616	0.0023	0.70255	70.26		
4	R3	0.140	16.794	1679.365	0.001679	0.0023	0.73016	73.02	71.87	± 1.437
	R1	0.100	10.444	1044.444	0.001044	0.0027	0.38211	38.21		
	R2	0.105	11.238	1123.810	0.001124	0.0027	0.41115	41.11		
8	R3	0.094	9.492	949.206	0.000949	0.0027	0.34727	34.73	38.02	± 3.198
	R1	0.093	9.333	933.333	0.000933	0.0059	0.15909	15.91		
	R2	0.079	7.111	711.111	0.000711	0.0059	0.12121	12.12		
12	R3	0.086	8.222	822.222	0.000822	0.0059	0.14015	14.02	14.02	± 1.894

v) *Scenedesmus obtusus* UMACC 100

Day	Sample	595nm	protein in 100 ug (ug)	protein in 10 ml (ug)	protein in 10 ml (g)	Dry weight (g)	protein (g)/ dry weight(g)	% protein (g)/ dry weight (g)	Mean protein (mg/L)	STDEV
	R1	0.181	23.302	2330.159	0.002330	0.0065	0.35666	35.67		
	R2	0.189	24.571	2457.143	0.002457	0.0065	0.37609	37.61		
0	R3	0.176	22.508	2250.794	0.002251	0.0065	0.34451	34.45	35.91	± 1.593
	R1	0.087	8.381	838.095	0.000838	0.0008	1.00571	100.57		
	R2	0.078	6.952	695.238	0.000695	0.0008	0.83429	83.43		
4	R3	0.076	6.635	663.492	0.000663	0.0008	0.79619	79.62	87.87	± 11.161
	R1	0.138	16.476	1647.619	0.001648	0.0031	0.53149	53.15		
	R2	0.104	11.079	1107.937	0.001108	0.0031	0.35740	35.74		
8	R3	0.143	17.270	1726.984	0.001727	0.0031	0.55709	55.71	48.20	± 10.866
	R1	0.124	14.254	1425.397	0.001425	0.0070	0.20363	20.36		
	R2	0.156	19.333	1933.333	0.001933	0.0070	0.27619	27.62		
12	R3	0.131	15.365	1536.508	0.001537	0.0070	0.21950	21.95	23.31	± 3.815

vi) *Chlorococum miniatum* UMACC 102

Day	Sample	595nm	protein in 100 ug (ug)	protein in 10 ml (ug)	protein in 10 ml (g)	Dry weight (g)	protein (g)/ dry weight(g)	% protein (g)/ dry weight (g)	Mean protein (mg/L)	STDEV
	R1	0.206	27.270	2726.984	0.002727	0.0109	0.25095	25.09		
	R2	0.185	23.937	2393.651	0.002394	0.0109	0.22027	22.03		
0	R3	0.189	24.571	2457.143	0.002457	0.0109	0.22612	22.61	23.24	± 1.629
	R1	0.139	16.635	1663.492	0.001663	0.0019	0.86043	86.04		
	R2	0.133	15.683	1568.254	0.001568	0.0019	0.81117	81.12		
4	R3	0.141	16.952	1695.238	0.001695	0.0019	0.87685	87.68	84.95	± 3.418
	R1	0.143	17.270	1726.984	0.001727	0.0036	0.47972	47.97		
	R2	0.132	15.524	1552.381	0.001552	0.0036	0.43122	43.12		
8	R3	0.130	15.206	1520.635	0.001521	0.0036	0.42240	42.24	44.44	±3.086
	R1	0.080	7.270	726.984	0.000727	0.0061	0.11853	11.85		
	R2	0.064	4.730	473.016	0.000473	0.0061	0.07712	7.71		
12	R3	0.100	10.444	1044.444	0.001044	0.0061	0.17029	17.03	12.20	± 4.668

vii) *Chlorella vulgaris* UMACC 103

Day	Sample	595nm	protein in 100 µg (ug)	protein in 10 ml (µg)	protein in 10 ml (g)	Dry weight (g)	protein (g)/ dry weight(g)	% protein (g)/ dry weight (g)	Mean protein (mg/L)	STDEV
	R1	0.172	21.873	2187.302	0.002187	0.0033	0.6628	66.28		
	R2	0.167	21.079	2107.937	0.002108	0.0033	0.6388	63.88		
0	R3	0.163	20.444	2044.444	0.002044	0.0033	0.6195	61.95	64.04	±2.169
	R1	0.096	9.810	980.952	0.000981	0.0013	0.7357	73.57		
	R2	0.093	9.333	933.333	0.000933	0.0013	0.7000	70.00		
4	R3	0.094	9.492	949.206	0.000949	0.0013	0.7119	71.19	71.59	±1.818
	R1	0.148	18.063	1806.349	0.001806	0.0035	0.5211	52.11		
	R2	0.148	18.063	1806.349	0.001806	0.0035	0.5211	52.11		
8	R3	0.157	19.492	1949.206	0.001949	0.0035	0.5623	56.23	53.48	±2.379
	R1	0.135	16.000	1600.000	0.001600	0.0081	0.1975	19.75		
	R2	0.131	15.365	1536.508	0.001537	0.0081	0.1897	18.97		
12	R3	0.119	13.460	1346.032	0.001346	0.0081	0.1662	16.62	18.45	±1.632

viii) *Chlorella* UMACC 253

Day	Sample	595nm	protein in 100 ug (ug)	protein in 10 ml (ug)	protein in 10 ml (g)	Dry weight (g)	protein (g)/ dry weight(g)	% protein (g)/ dry weight (g)	Mean protein (mg/L)	STDEV
	R1	0.086	8.222	822.222	0.000822	0.0018	0.4654	46.54		
	R2	0.089	8.698	869.841	0.000870	0.0018	0.4924	49.24		
0	R3	0.092	9.175	917.460	0.000917	0.0018	0.5193	51.93	49.24	± 2.695
	R1	0.124	14.254	1425.397	0.001425	0.0019	0.7636	76.36		
	R2	0.121	13.778	1377.778	0.001378	0.0019	0.7381	73.81		
4	R3	0.117	13.143	1314.286	0.001314	0.0019	0.7041	70.41	73.53	± 2.986
	R1	0.154	19.016	1901.587	0.001902	0.0041	0.4676	46.76		
	R2	0.143	17.270	1726.984	0.001727	0.0041	0.4247	42.47		
8	R3	0.148	18.063	1806.349	0.001806	0.0041	0.4442	44.42	44.55	± 2.150
	R1	0.100	10.444	1044.444	0.001044	0.0077	0.1356	13.56		
	R2	0.097	9.968	996.825	0.000997	0.0077	0.1295	12.95		
12	R3	0.128	14.889	1488.889	0.001489	0.0077	0.1934	19.34	15.28	± 3.525

ix) *Chlorella vulgaris* UMACC 245

Day	Sample	595nm	protein in 100 ug (ug)	protein in 10 ml (ug)	protein in 10 ml (g)	Dry weight (g)	protein (g)/ dry weight(g)	% protein (g)/ dry weight (g)	Mean protein (mg/L)	STDEV
	R1	0.171	21.714	2171.429	0.002171	0.0056	0.3901	39.01		
	R2	0.166	20.921	2092.063	0.002092	0.0056	0.3758	37.58		
0	R3	0.165	20.762	2076.190	0.002076	0.0056	0.3730	37.30	37.96	± 0.917
	R1	0.103	10.921	1092.063	0.001092	0.0020	0.5460	54.60		
	R2	0.102	10.762	1076.190	0.001076	0.0020	0.5381	53.81		
4	R3	0.101	10.603	1060.317	0.001060	0.0020	0.5302	53.02	53.81	± 0.794
	R1	0.188	24.413	2441.270	0.002441	0.0033	0.7398	73.98		
	R2	0.184	23.778	2377.778	0.002378	0.0033	0.7205	72.05		
8	R3	0.183	23.619	2361.905	0.002362	0.0033	0.7157	71.57	72.53	± 1.273
	R1	0.061	4.254	425.397	0.000425	0.0054	0.0788	7.88		
	R2	0.069	5.524	552.381	0.000552	0.0054	0.1023	10.23		
12	R3	0.079	7.111	711.111	0.000711	0.0054	0.1317	13.17	10.43	± 2.651

x) *Chlorella* UMACC 255

Day	Sample	595nm	protein in 100 ug (ug)	protein in 10 ml (ug)	protein in 10 ml (g)	Dry weight (g)	protein (g)/ dry weight(g)	% protein (g)/ dry weight (g)	Mean protein (mg/L)	STDEV
	R1	0.166	20.921	2092.063	0.002092	0.0058	0.3628	36.28		
	R2	0.188	24.413	2441.270	0.002441	0.0058	0.4233	42.33		
0	R3	0.164	20.603	2060.317	0.002060	0.0058	0.3573	35.73	38.11	± 3.665
	R1	0.162	20.286	2028.571	0.002029	0.0048	0.4197	41.97		
	R2	0.169	21.397	2139.683	0.002140	0.0048	0.4427	44.27		
4	R3	0.165	20.762	2076.190	0.002076	0.0048	0.4296	42.96	43.07	± 1.153
	R1	0.162	20.286	2028.571	0.002029	0.0048	0.4256	42.56		
	R2	0.177	22.667	2266.667	0.002267	0.0048	0.4755	47.55		
8	R3	0.177	22.667	2266.667	0.002267	0.0048	0.4755	47.55	45.89	± 2.884
	R1	0.096	9.810	980.952	0.000981	0.0074	0.1326	13.26		
	R2	0.098	10.127	1012.698	0.001013	0.0074	0.1369	13.69		
12	R3	0.113	12.508	1250.794	0.001251	0.0074	0.1690	16.90	14.61	± 1.993

b) Carbohydrate Content

i) *Chlorella vulgaris* UMACC 001

Day	Sample	OD 485nm	carbohydrate in 500 ug (ug)	carbohydrate in 10 ml (ug)	carbohydrate in 10 ml (g)	Dry weight (g)	carbohydrate (g)/dry weight(g)	% carbohydrate (g)/dry weight (g)	Mean carbohydrate (mg/L)	STDEV
	R1	0.465	5.0944	101.8872	0.000102	0.0035	0.0294	2.94		
	R2	0.523	5.7234	114.4685	0.000114	0.0035	0.0330	3.30		
0	R3	0.551	6.0271	120.5423	0.000121	0.0035	0.0348	3.48	3.24	± 0.274
	R1	0.210	2.3286	46.5727	0.000047	0.0022	0.0212	2.12		
	R2	0.176	1.9599	39.1974	0.000039	0.0022	0.0178	1.78		
4	R3	0.183	2.0358	40.7158	0.000041	0.0022	0.0185	1.85	1.92	± 0.177
	R1	0.191	2.1226	42.4512	0.000042	0.0036	0.0118	1.18		
	R2	0.195	2.1659	43.3189	0.000043	0.0036	0.0120	1.20		
8	R3	0.201	2.2310	44.6204	0.000045	0.0036	0.0124	1.24	1.21	± 0.030
	R1	0.270	2.9794	59.5879	0.000060	0.0120	0.0050	0.50		
	R2	0.307	3.3807	67.6139	0.000068	0.0120	0.0056	0.56		
12	R3	0.322	3.5434	70.8677	0.000071	0.0120	0.0059	0.59	0.55	± 0.048

ii) *Chlorella* UMACC 275

Day	Sample	OD 485nm	carbohydrate in 500 ug (ug)	carbohydrate in 10 ml (ug)	carbohydrate in 10 ml (g)	Dry weight (g)	carbohydrate (g)/dry weight(g)	% carbohydrate (g)/dry weight (g)	Mean carbohydrate (mg/L)	STDEV
	R1	0.289	3.185466	63.70933	0.000064	0.0023	0.0277	2.77		
	R2	0.278	3.066161	61.32321	0.000061	0.0023	0.0267	2.67		
0	R3	0.277	3.055315	61.10629	0.000061	0.0023	0.0266	2.66	2.70	± 0.063
	R1	0.223	2.469631	49.39262	0.000049	0.0025	0.0198	1.98		
	R2	0.292	3.218004	64.36009	0.000064	0.0025	0.0257	2.57		
4	R3	0.293	3.22885	64.57701	0.000065	0.0025	0.0258	2.58	2.38	± 0.348
	R1	0.220	2.437093	48.74187	0.000049	0.0047	0.0104	1.04		
	R2	0.236	2.610629	52.21258	0.000052	0.0047	0.0111	1.11		
8	R3	0.232	2.567245	51.3449	0.000051	0.0047	0.0109	1.09	1.08	± 0.038
	R1	0.496	5.430586	108.6117	0.000109	0.0095	0.0114	1.14		
	R2	0.490	5.36551	107.3102	0.000107	0.0095	0.0113	1.13		
12	R3	0.414	4.541215	90.8243	0.000091	0.0095	0.0096	0.96	1.08	± 0.104

iii) *Scenedesmus quadricauda* UMACC 041

Day	Sample	OD 485nm	carbohydrate in 500 ug (ug)	carbohydrate in 10 ml (ug)	carbohydrate in 10 ml (g)	Dry weight (g)	carbohydrate (g)/dry weight(g)	% carbohydrate (g)/dry weight (g)	Mean carbohydrate (mg/L)	STDEV
	R1	0.365	4.0098	80.1952	0.000080	0.0059	0.0137	1.37		
	R2	0.356	3.9121	78.2430	0.000078	0.0059	0.0133	1.33		
0	R3	0.310	3.4132	68.2646	0.000068	0.0059	0.0116	1.16	1.29	± 0.109
	R1	0.196	2.1768	43.5358	0.000044	0.0026	0.0167	1.67		
	R2	0.157	1.7538	35.0759	0.000035	0.0026	0.0135	1.35		
4	R3	0.173	1.9273	38.5466	0.000039	0.0026	0.0148	1.48	1.50	± 0.164
	R1	0.194	2.1551	43.1020	0.000043	0.0033	0.0131	1.31		
	R2	0.170	1.8948	37.8959	0.000038	0.0033	0.0115	1.15		
8	R3	0.218	2.4154	48.3080	0.000048	0.0033	0.0146	1.46	1.31	± 0.158
	R1	0.240	2.6540	53.0803	0.000053	0.0085	0.0062	0.62		
	R2	0.224	2.4805	49.6095	0.000050	0.0085	0.0058	0.58		
12	R3	0.216	2.3937	47.8742	0.000048	0.0085	0.0056	0.56	0.59	± 0.031

iv) *Oocystis* UMACC 074

Day	Sample	OD 485nm	carbohydrate in 500 ug (ug)	carbohydrate in 10 ml (ug)	carbohydrate in 10 ml (g)	Dry weight (g)	carbohydrate (g)/dry weight(g)	% carbohydrate (g)/dry weight (g)	Mean carbohydrate (mg/L)	STDEV
	R1	0.485	5.3113	106.2256	0.000106	0.0082	0.0130	1.30		
	R2	0.568	6.2115	124.2299	0.000124	0.0082	0.0152	1.52		
0	R3	0.557	6.0922	121.8438	0.000122	0.0082	0.0149	1.49	1.44	± 0.120
	R1	0.165	1.8406	36.8113	0.000037	0.0023	0.0160	1.60		
	R2	0.105	1.1898	23.7961	0.000024	0.0023	0.0103	1.03		
4	R3	0.134	1.5043	30.0868	0.000030	0.0023	0.0131	1.31	1.31	± 0.283
	R1	0.158	1.7646	35.2928	0.000035	0.0027	0.0129	1.29		
	R2	0.181	2.0141	40.2820	0.000040	0.0027	0.0147	1.47		
8	R3	0.187	2.0792	41.5835	0.000042	0.0027	0.0152	1.52	1.43	± 0.121
	R1	0.146	1.6345	32.6898	0.000033	0.0059	0.0056	0.56		
	R2	0.187	2.0792	41.5835	0.000042	0.0059	0.0071	0.71		
12	R3	0.176	1.9599	39.1974	0.000039	0.0059	0.0067	0.67	0.64	± 0.078

v) *Scenedesmus obtusus* UMACC 100

Day	Sample	OD 485nm	carbohydrate in 500 ug (ug)	carbohydrate in 10 ml (ug)	carbohydrate in 10 ml (g)	Dry weight (g)	carbohydrate (g)/dry weight(g)	% carbohydrate (g)/dry weight (g)	Mean carbohydrate (mg/L)	STDEV
	R1	0.412	4.5195	90.3905	0.000090	0.0065	0.0138	1.38		
	R2	0.630	6.8839	137.6790	0.000138	0.0065	0.0211	2.11		
0	R3	0.503	5.5065	110.1302	0.000110	0.0065	0.0169	1.69	1.73	± 0.364
	R1	0.067	0.7777	15.5531	0.000016	0.0008	0.0187	1.87		
	R2	0.087	0.9946	19.8915	0.000020	0.0008	0.0239	2.39		
4	R3	0.065	0.7560	15.1193	0.000015	0.0008	0.0181	1.81	2.02	± 0.317
	R1	0.177	1.9707	39.4143	0.000039	0.0031	0.0127	1.27		
	R2	0.156	1.7430	34.8590	0.000035	0.0031	0.0112	1.12		
8	R3	0.159	1.7755	35.5098	0.000036	0.0031	0.0115	1.15	1.18	± 0.079
	R1	0.184	2.0466	40.9328	0.000041	0.0070	0.0058	0.58		
	R2	0.178	1.9816	39.6312	0.000040	0.0070	0.0057	0.57		
12	R3	0.167	1.8623	37.2451	0.000037	0.0070	0.0053	0.53	0.56	± 0.027

vi) *Chlorococum miniatum* UMACC 102

Day	Sample	OD 485nm	carbohydrate in 500 ug (ug)	carbohydrate in 10 ml (ug)	carbohydrate in 10 ml (g)	Dry weight (g)	carbohydrate (g)/dry weight(g)	% carbohydrate (g)/dry weight (g)	Mean carbohydrate (mg/L)	STDEV
	R1	0.624	6.8189	136.3774	0.000136	0.0109	0.0126	1.26		
	R2	0.607	6.6345	132.6898	0.000133	0.0109	0.0122	1.22		
0	R3	0.707	7.7191	154.3818	0.000154	0.0109	0.0142	1.42	1.30	± 0.107
	R1	0.136	1.5260	30.5206	0.000031	0.0019	0.0158	1.58		
	R2	0.116	1.3091	26.1822	0.000026	0.0019	0.0135	1.35		
4	R3	0.136	1.5260	30.5206	0.000031	0.0019	0.0158	1.58	1.50	± 0.130
	R1	0.193	2.1443	42.8850	0.000043	0.0036	0.0119	1.19		
	R2	0.246	2.7191	54.3818	0.000054	0.0036	0.0151	1.51		
8	R3	0.223	2.4696	49.3926	0.000049	0.0036	0.0137	1.37	1.36	± 0.160
	R1	0.189	2.1009	42.0174	0.000042	0.0061	0.0069	0.69		
	R2	0.213	2.3612	47.2234	0.000047	0.0061	0.0077	0.77		
12	R3	0.221	2.4479	48.9588	0.000049	0.0061	0.0080	0.80	0.75	± 0.059

vii) *Chlorella vulgaris* UMACC 103

Day	Sample	OD 485nm	carbohydrate in 500 ug (ug)	carbohydrate in 10 ml (ug)	carbohydrate in 10 ml (g)	Dry weight (g)	carbohydrate (g)/dry weight(g)	% carbohydrate (g)/dry weight (g)	Mean carbohydrate (mg/L)	STDEV
	R1	0.345	3.7928	75.8568	0.000076	0.0033	0.022987	2.30		
	R2	0.353	3.8796	77.5922	0.000078	0.0033	0.023513	2.35		
0	R3	0.311	3.4241	68.4816	0.000068	0.0033	0.020752	2.08	2.24	± 0.147
	R1	0.156	1.7430	34.8590	0.000035	0.0013	0.026144	2.61		
	R2	0.152	1.6996	33.9913	0.000034	0.0013	0.025493	2.55		
4	R3	0.131	1.4718	29.4360	0.000029	0.0013	0.022077	2.21	2.46	± 0.218
	R1	0.154	1.7213	34.4252	0.000034	0.0035	0.009930	0.99		
	R2	0.164	1.8297	36.5944	0.000037	0.0035	0.010556	1.06		
8	R3	0.175	1.9490	38.9805	0.000039	0.0035	0.011244	1.12	1.06	± 0.066
	R1	0.288	3.1746	63.4924	0.000063	0.0081	0.007839	0.78		
	R2	0.255	2.8167	56.3341	0.000056	0.0081	0.006955	0.70		
12	R3	0.252	2.7842	55.6833	0.000056	0.0081	0.006874	0.69	0.72	± 0.053

viii) *Chlorella* UMACC 253

Day	Sample	OD 485nm	carbohydrate in 500 ug (ug)	carbohydrate in 10 ml (ug)	carbohydrate in 10 ml (g)	Dry weight (g)	carbohydrate (g)/dry weight(g)	% carbohydrate (g)/dry weight (g)	Mean carbohydrate (mg/L)	STDEV
	R1	0.242	2.6757	53.5141	0.000054	0.0018	0.030291	3.03		
	R2	0.221	2.4479	48.9588	0.000049	0.0018	0.027713	2.77		
0	R3	0.223	2.4696	49.3926	0.000049	0.0018	0.027958	2.80	2.87	± 0.142
	R1	0.146	1.6345	32.6898	0.000033	0.0019	0.017512	1.75		
	R2	0.143	1.6020	32.0390	0.000032	0.0019	0.017164	1.72		
4	R3	0.148	1.6562	33.1236	0.000033	0.0019	0.017745	1.77	1.75	± 0.029
	R1	0.158	1.7646	35.2928	0.000035	0.0041	0.008679	0.87		
	R2	0.145	1.6236	32.4729	0.000032	0.0041	0.007985	0.80		
8	R3	0.179	1.9924	39.8482	0.000040	0.0041	0.009799	0.98	0.88	± 0.092
	R1	0.249	2.7516	55.0325	0.000055	0.0077	0.007147	0.71		
	R2	0.244	2.6974	53.9479	0.000054	0.0077	0.007006	0.70		
12	R3	0.273	3.0119	60.2386	0.000060	0.0077	0.007823	0.78	0.73	± 0.044

ix) *Chlorella vulgaris* UMACC 245

Day	Sample	OD 485nm	carbohydrate in 500 ug (ug)	carbohydrate in 10 ml (ug)	carbohydrate in 10 ml (g)	Dry weight (g)	carbohydrate (g)/dry weight(g)	% carbohydrate (g)/dry weight (g)	Mean carbohydrate (mg/L)	STDEV
	R1	0.262	2.8926	57.8525	0.000058	0.0056	0.010393	1.04		
	R2	0.258	2.8492	56.9848	0.000057	0.0056	0.010237	1.02		
0	R3	0.238	2.6323	52.6464	0.000053	0.0056	0.009457	0.95	1.00	± 0.050
	R1	0.092	1.0488	20.9761	0.000021	0.0020	0.010488	1.05		
	R2	0.093	1.0597	21.1931	0.000021	0.0020	0.010597	1.06		
4	R3	0.096	1.0922	21.8438	0.000022	0.0020	0.010922	1.09	1.07	± 0.023
	R1	0.151	1.6887	33.7744	0.000034	0.0033	0.010235	1.02		
	R2	0.169	1.8839	37.6790	0.000038	0.0033	0.011418	1.14		
8	R3	0.150	1.6779	33.5575	0.000034	0.0033	0.010169	1.02	1.06	± 0.070
	R1	0.132	1.4826	29.6529	0.000030	0.0054	0.005491	0.55		
	R2	0.146	1.6345	32.6898	0.000033	0.0054	0.006054	0.61		
12	R3	0.151	1.6887	33.7744	0.000034	0.0054	0.006255	0.63	0.59	± 0.040

x) *Chlorella* UMACC 255

Day	Sample	OD 485nm	carbohydrate in 500 ug (ug)	carbohydrate in 10 ml (ug)	carbohydrate in 10 ml (g)	Dry weight (g)	carbohydrate (g)/dry weight(g)	% carbohydrate (g)/dry weight (g)	Mean carbohydrate (mg/L)	STDEV
	R1	0.312	3.4349	68.6985	0.000069	0.0058	0.011913	1.19		
	R2	0.308	3.3915	67.8308	0.000068	0.0058	0.011763	1.18		
0	R3	0.308	3.3915	67.8308	0.000068	0.0058	0.011763	1.18	1.18	± 0.009
	R1	0.135	1.5152	30.3037	0.000030	0.0048	0.006270	0.63		
	R2	0.134	1.5043	30.0868	0.000030	0.0048	0.006225	0.62		
4	R3	0.133	1.4935	29.8698	0.000030	0.0048	0.006180	0.62	0.62	± 0.004
	R1	0.192	2.1334	42.6681	0.000043	0.0048	0.008951	0.90		
	R2	0.176	1.9599	39.1974	0.000039	0.0048	0.008223	0.82		
8	R3	0.169	1.8839	37.6790	0.000038	0.0048	0.007905	0.79	0.84	± 0.054
	R1	0.189	2.1009	42.0174	0.000042	0.0074	0.005678	0.57		
	R2	0.178	1.9816	39.6312	0.000040	0.0074	0.005356	0.54		
12	R3	0.152	1.6996	33.9913	0.000034	0.0074	0.004593	0.46	0.52	± 0.056

c) Lipid Content

i) *Chlorella vulgaris* UMACC 001

Day	Sample	blank glass vial(g)	blank glass vial + lipid (g)	lipid(g)	dry weight (g)	lipid(g)/dry weight(g)	% lipid/dry weight	mean	STDEV
	R1	3.1474	3.1480	0.0006	0.0035	0.1731	17.31		
	R2	3.1762	3.1769	0.0007	0.0035	0.2019	20.19		
0	R3	3.1425	3.1432	0.0007	0.0035	0.2019	20.19	19.23	± 1.665
	R1	3.1933	3.1938	0.0005	0.0022	0.2273	22.73		
	R2	3.2252	3.2257	0.0005	0.0022	0.2273	22.73		
4	R3	3.2230	3.2236	0.0006	0.0022	0.2727	27.27	24.24	± 2.624
	R1	3.1904	3.1916	0.0012	0.0036	0.3333	33.33		
	R2	3.2061	3.2071	0.0010	0.0036	0.2778	27.78		
8	R3	3.1750	3.1762	0.0012	0.0036	0.3333	33.33	31.48	± 3.208
	R1	3.2323	3.2338	0.0015	0.0120	0.1247	12.47		
	R2	3.5245	3.5258	0.0013	0.0120	0.1080	10.80		
12	R3	3.1736	3.1748	0.0012	0.0120	0.0997	9.97	11.08	± 1.269

ii) *Chlorella* UMACC 275

Day	Sample	blank glass vial(g)	blank glass vial + lipid (g)	lipid(g)	dry weight (g)	lipid(g)/dry weight(g)	% lipid/dry weight	mean	STDEV
	R1	3.2043	3.2049	0.0006	0.0023	0.260869565	26.09		
	R2	3.1783	3.1788	0.0005	0.0023	0.217391304	21.74		
0	R3	3.2218	3.2223	0.0005	0.0023	0.217391304	21.74	23.19	± 2.510
	R1	3.1868	3.1879	0.0011	0.0025	0.4400	44.00		
	R2	3.1300	3.1309	0.0009	0.0025	0.3600	36.00		
4	R3	3.1370	3.1379	0.0009	0.0025	0.3600	36.00	38.67	± 4.619
	R1	3.1297	3.1306	0.0009	0.0047	0.1915	19.15		
	R2	3.1906	3.1917	0.0011	0.0047	0.2340	23.40		
8	R3	3.1635	3.1645	0.001	0.0047	0.2128	21.28	21.28	± 2.128
	R1	3.1772	3.1787	0.0015	0.0095	0.157894737	15.79		
	R2	3.2035	3.2050	0.0015	0.0095	0.157894737	15.79		
12	R3	3.2491	3.2511	0.002	0.0095	0.210526316	21.05	17.54	± 3.039

iii) *Scenedesmus quadricauda* UMACC 041

Day	Sample	blank glass vial(g)	blank glass vial + lipid (g)	lipid(g)	dry weight (g)	lipid(g)/dry weight(g)	% lipid/dry weight	mean	STDEV
	R1	3.1354	3.1365	0.0011	0.0059	0.1875	18.75		
	R2	3.1565	3.1572	0.0007	0.0059	0.1193	11.93		
0	R3	3.1431	3.1439	0.0008	0.0059	0.1364	13.64	14.77	± 3.548
	R1	3.1713	3.1717	0.0004	0.0026	0.1538	15.38		
	R2	3.2080	3.2086	0.0006	0.0026	0.2308	23.08		
4	R3	3.1638	3.1642	0.0004	0.0026	0.1538	15.38	17.95	± 4.441
	R1	3.1350	3.1356	0.0006	0.0033	0.1818	18.18		
	R2	3.1926	3.1932	0.0006	0.0033	0.1818	18.18		
8	R3	3.1872	3.1880	0.0008	0.0033	0.2424	24.24	20.20	± 3.499
	R1	3.2346	3.2353	0.0007	0.0085	0.0820	8.20		
	R2	3.2422	3.2429	0.0007	0.0085	0.0820	8.20		
12	R3	3.2100	3.2108	0.0008	0.0085	0.0937	9.37	8.59	± 0.677

iv) *Oocystis* UMACC 074

Day	Sample	blank glass vial(g)	blank glass vial + lipid (g)	lipid(g)	dry weight (g)	lipid(g)/dry weight(g)	% lipid/dry weight	mean	STDEV
	R1	3.1818	3.1828	0.0010	0.0082	0.1224	12.24		
	R2	3.1787	3.1801	0.0014	0.0082	0.1714	17.14		
0	R3	3.1823	3.1838	0.0015	0.0082	0.1837	18.37	15.92	± 3.240
	R1	3.2472	3.2476	0.0004	0.0023	0.1739	17.39		
	R2	3.2242	3.2245	0.0003	0.0023	0.1304	13.04		
4	R3	3.1390	3.1396	0.0006	0.0023	0.2609	26.09	18.84	± 6.641
	R1	3.1904	3.1912	0.0008	0.0027	0.2927	29.27		
	R2	3.2061	3.2066	0.0005	0.0027	0.1829	18.29		
8	R3	3.1750	3.1757	0.0007	0.0027	0.2561	25.61	24.39	± 5.589
	R1	3.2323	3.2331	0.0008	0.0059	0.1364	13.64		
	R2	3.5245	3.5255	0.0010	0.0059	0.1705	17.05		
12	R3	3.1736	3.1746	0.0010	0.0059	0.1705	17.05	15.91	± 1.968

v) *Scenedesmus obtusus* UMACC 100

Day	Sample	blank glass vial(g)	blank glass vial + lipid (g)	lipid(g)	dry weight (g)	lipid(g)/dry weight(g)	% lipid/dry weight	mean	STDEV
	R1	3.2770	3.2781	0.0011	0.0065	0.1684	16.84		
	R2	3.1746	3.1757	0.0011	0.0065	0.1684	16.84		
0	R3	3.1995	3.2005	0.0010	0.0065	0.1531	15.31	16.33	± 0.884
	R1	3.1677	3.1678	0.0001	0.0008	0.1200	12.00		
	R2	3.1399	3.1401	0.0002	0.0008	0.2400	24.00		
4	R3	3.2130	3.2132	0.0002	0.0008	0.2400	24.00	20.00	± 6.928
	R1	3.1835	3.1840	0.0005	0.0031	0.1613	16.13		
	R2	3.1908	3.1914	0.0006	0.0031	0.1935	19.35		
8	R3	3.2009	3.2014	0.0005	0.0031	0.1613	16.13	17.20	± 1.862
	R1	3.1884	3.1891	0.0007	0.0070	0.1000	10.00		
	R2	3.2334	3.2340	0.0006	0.0070	0.0857	8.57		
12	R3	3.1535	3.1538	0.0003	0.0070	0.0429	4.29	7.62	± 2.974

vi) *Chlorococum miniatum* UMACC 102

Day	Sample	blank glass vial(g)	blank glass vial + lipid (g)	lipid(g)	dry weight (g)	lipid(g)/dry weight(g)	% lipid/dry weight	mean	STDEV
	R1	3.1853	3.1859	0.0006	0.0109	0.0552	5.52		
	R2	3.1560	3.1568	0.0008	0.0109	0.0736	7.36		
0	R3	3.1678	3.1682	0.0004	0.0109	0.0368	3.68	5.52	± 1.840
	R1	3.1920	3.1924	0.0004	0.0019	0.2069	20.69		
	R2	3.1777	3.1780	0.0003	0.0019	0.1552	15.52		
4	R3	3.1730	3.1732	0.0002	0.0019	0.1034	10.34	15.52	± 5.172
	R1	3.1935	3.1940	0.0005	0.0036	0.1389	13.89		
	R2	3.1908	3.1913	0.0005	0.0036	0.1389	13.89		
8	R3	3.2118	3.2122	0.0004	0.0036	0.1111	11.11	12.96	± 1.604
	R1	3.1884	3.1891	0.0007	0.0061	0.1141	11.41		
	R2	3.2334	3.2340	0.0006	0.0061	0.0978	9.78		
12	R3	3.1535	3.1538	0.0003	0.0061	0.0489	4.89	8.70	± 3.394

vii) *Chlorella vulgaris* UMACC 103

Day	Sample	blank glass vial(g)	blank glass vial + lipid (g)	lipid(g)	dry weight (g)	lipid(g)/dry weight(g)	% lipid/dry weight	mean	STDEV
	R1	3.2012	3.2020	0.0008	0.0033	0.242	24.2424		
	R2	3.1376	3.1387	0.0011	0.0033	0.333	33.3333		
0	R3	3.2267	3.2278	0.0011	0.0033	0.333	33.3333	30.30	± 5.249
	R1	3.1876	3.1879	0.0003	0.0013	0.225	22.5000		
	R2	3.2019	3.2022	0.0003	0.0013	0.225	22.5000		
4	R3	3.2039	3.2041	0.0002	0.0013	0.150	15.0000	20.00	± 4.330
	R1	3.1975	3.1982	0.0007	0.0035	0.202	20.1923		
	R2	3.2106	3.2115	0.0009	0.0035	0.260	25.9615		
8	R3	3.1406	3.1415	0.0009	0.0035	0.260	25.9615	24.04	± 3.331
	R1	3.1381	3.1389	0.0008	0.0081	0.099	9.8765		
	R2	3.2292	3.2299	0.0007	0.0081	0.086	8.6420		
12	R3	3.2137	3.2148	0.0011	0.0081	0.136	13.5802	10.70	± 2.570

viii) *Chlorella* UMACC 253

Day	Sample	blank glass vial(g)	blank glass vial + lipid (g)	lipid(g)	dry weight (g)	lipid(g)/dry weight(g)	% lipid/dry weight	mean	STDEV
	R1	3.2288	3.2294	0.0006	0.0018	0.340	33.9623		
	R2	3.1906	3.1913	0.0007	0.0018	0.396	39.6226		
0	R3	3.2035	3.2041	0.0006	0.0018	0.340	33.9623	35.85	± 3.268
	R1	3.1896	3.1899	0.0003	0.0019	0.161	16.0714		
	R2	3.1806	3.181	0.0004	0.0019	0.214	21.4286		
4	R3	3.2393	3.2398	0.0005	0.0019	0.268	26.7857	21.43	± 5.357
	R1	3.2157	3.2166	0.0009	0.0041	0.221	22.1311		
	R2	3.1472	3.1480	0.0008	0.0041	0.197	19.6721		
8	R3	3.2288	3.2297	0.0009	0.0041	0.221	22.1311	21.31	± 1.420
	R1	3.2364	3.2373	0.0009	0.0077	0.117	11.6883		
	R2	3.1311	3.1318	0.0007	0.0077	0.091	9.0909		
12	R3	3.2310	3.2324	0.0014	0.0077	0.182	18.1818	12.99	± 4.683

ix) *Chlorella vulgaris* UMACC 245

Day	Sample	blank glass vial(g)	blank glass vial + lipid (g)	lipid(g)	dry weight (g)	lipid(g)/dry weight(g)	% lipid/dry weight	mean	STDEV
	R1	3.1975	3.1982	0.0007	0.0056	0.126	12.5749		
	R2	3.1886	3.1892	0.0006	0.0056	0.108	10.7784		
0	R3	3.1632	3.1638	0.0006	0.0056	0.108	10.7784	11.38	± 1.037
	R1	3.1398	3.1404	0.0006	0.0020	0.300	30.0000		
	R2	3.1464	3.1469	0.0005	0.0020	0.250	25.0000		
4	R3	3.1532	3.1536	0.0004	0.0020	0.200	20.0000	25.00	± 5.000
	R1	3.1350	3.1356	0.0006	0.0033	0.182	18.1818		
	R2	3.1926	3.1932	0.0006	0.0033	0.182	18.1818		
8	R3	3.1872	3.1880	0.0008	0.0033	0.242	24.2424	20.20	± 3.499
	R1	3.2346	3.2353	0.0007	0.0054	0.130	12.9630		
	R2	3.2422	3.2429	0.0007	0.0054	0.130	12.9630		
12	R3	3.2100	3.2108	0.0008	0.0054	0.148	14.8148	13.58	± 1.069

x) *Chlorella* UMACC 255

Day	Sample	blank glass vial(g)	blank glass vial + lipid (g)	lipid(g)	dry weight (g)	lipid(g)/dry weight(g)	% lipid/dry weight	mean	STDEV
	R1	3.2098	3.2115	0.0017	0.0058	0.295	29.4798		
	R2	3.2457	3.2471	0.0014	0.0058	0.243	24.2775		
0	R3	3.2675	3.2684	0.0009	0.0058	0.156	15.6069	23.12	± 7.008
	R1	3.1511	3.1521	0.0010	0.0048	0.207	20.6897		
	R2	3.2425	3.2438	0.0013	0.0048	0.269	26.8966		
4	R3	3.1586	3.1596	0.0010	0.0048	0.207	20.6897	22.76	± 3.584
	R1	3.1975	3.1982	0.0007	0.0048	0.147	14.6853		
	R2	3.2106	3.2115	0.0009	0.0048	0.189	18.8811		
8	R3	3.1406	3.1415	0.0009	0.0048	0.189	18.8811	17.48	± 2.422
	R1	3.1381	3.1389	0.0008	0.0074	0.108	10.8108		
	R2	3.2292	3.2299	0.0007	0.0074	0.095	9.4595		
12	R3	3.2137	3.2148	0.0011	0.0074	0.149	14.8649	11.71	± 2.813

APPENDIX E

Distribution of Saturated, Monounsaturated and Polyunsaturated Fatty Acids of Preliminary Screening of Ten Microalgae Strains on Day 0, Day 4, Day 8 and Day 12

a) *Chlorella vulgaris* UMACC 001

i) Day 0

Fatty acid	Day 0				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	1.65	1.63	1.71	1.66	± 0.042
16:0	22.23	21.99	23.64	22.62	± 0.891
18:0	3.01	4.24	1.97	3.07	± 1.136
Sum	26.89	27.86	27.32	27.36	
Monounsaturated					
16:1	3.95	1.86	3.7	3.17	± 1.141
18:1	2.00	0.83	3.66	2.16	± 1.422
Sum	5.95	2.69	7.36	5.33	
Polyunsaturated					
16:2	0.38	3.86	11.11	5.12	± 5.474
16:3	12.35	12.16	5.77	10.09	± 3.745
18:2	11.81	11.44	11.31	11.52	± 0.259
18:3	42.62	41.99	37.13	40.58	± 3.004
Sum	67.16	69.45	65.32	67.31	2.069
TOTAL	100	100	100	100	

ii) Day 4

Fatty acid	Day 4				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	2.69	2.62	2.65	2.65	± 0.035
16:0	39.37	39.43	38.98	39.26	± 0.244
18:0	5.49	5.85	5.92	5.75	± 0.231
Sum	47.55	47.9	47.55	47.67	
Monounsaturated					
16:1	0.00	4.35	4.39	2.91	± 2.523
18:1	0.00	0.00	0.00	0.00	± 0.000
Sum	0	4.35	4.39	2.91	
Polyunsaturated					
16:2	3.74	0.00	0.76	1.50	± 1.977
16:3	9.11	9.07	7.54	8.57	± 0.895
18:2	12.26	11.92	15.33	13.17	± 1.878
18:3	27.34	26.76	24.43	26.18	± 1.540
Sum	52.45	47.75	48.06	49.42	2.629
TOTAL	100	100	100	100	

iii) Day 8

Fatty acid	Day 8				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	1.99	3.03	3.02	2.68	± 0.598
16:0	33.46	34.37	34.43	34.09	± 0.544
18:0	5.55	5.61	5.59	5.58	± 0.031
Sum	41.00	43.01	43.04	42.35	
Monounsaturated					
16:1	5.90	6.03	5.94	5.96	± 0.067
18:1	0.91	1.21	1.12	1.08	± 0.154
Sum	6.81	7.24	7.06	7.04	
Polyunsaturated					
16:2	4.79	4.73	4.77	4.76	± 0.031
16:3	7.65	7.04	7.54	7.41	± 0.325
18:2	15.61	14.54	14.44	14.86	± 0.649
18:3	24.14	23.44	23.15	23.58	± 0.509
Sum	52.19	49.75	49.9	50.61	1.367
TOTAL	100	100	100	100	

iv) Day 12

Fatty acid	Day 12				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	1.91	1.87	1.93	1.90	± 0.031
16:0	26.42	25.61	26.06	26.03	± 0.406
18:0	5.21	5.88	5.54	5.54	± 0.335
Sum	33.54	33.36	33.53	33.48	
Monounsaturated					
16:1	7.22	0.34	3.32	3.63	± 3.450
18:1	0.00	1.21	0.98	0.73	± 0.643
Sum	7.22	1.55	4.3	4.36	
Polyunsaturated					
16:2	0.26	7.27	5.45	4.33	± 3.638
16:3	11.11	10.95	11.03	11.03	± 0.080
18:2	19.15	16.10	19.33	18.19	± 1.815
18:3	28.72	30.77	26.36	28.62	± 2.207
Sum	59.24	65.09	62.17	62.17	2.925
TOTAL	100	100	100	100	

b) *Chlorella* UMACC 275

i) Day 0

Fatty acid	Day 0				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	3.44	3.32	3.31	3.36	± 0.072
16:0	43.23	42.68	41.76	42.56	± 0.743
18:0	4.22	3.75	4.04	4.00	± 0.237
Sum	50.89	49.75	49.11	49.91667	
Monounsaturated					
16:1	7.67	6.11	5.67	6.48	± 1.051
18:1	1.98	1.15	1.43	1.52	± 0.422
Sum	9.65	7.26	7.1	8.00	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	± 0.000
16:3	4.32	5.76	5.96	5.35	± 0.895
18:2	15.89	14.68	15.41	15.33	± 0.609
18:3	19.25	22.55	22.42	21.41	± 1.869
Sum	39.46	42.99	43.79	42.08	2.304
TOTAL	100	100	100	100	

ii) Day 4

Fatty acid	Day 4				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	3.03	3.49	3.34	3.29	± 0.235
16:0	40.04	41.03	41.23	40.77	± 0.637
18:0	5.02	4.37	4.89	4.76	± 0.344
Sum	48.09	48.89	49.46	48.81	
Monounsaturated					
16:1	9.56	12.23	11.44	11.08	± 1.372
18:1	2.38	1.18	1.76	1.77	± 0.600
Sum	11.94	13.41	13.2	12.85	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	± 0.000
16:3	6.73	4.87	6.25	5.95	± 0.966
18:2	21.78	25.05	24.46	23.76	± 1.743
18:3	11.46	7.78	6.63	8.62	± 2.523
Sum	39.97	37.7	37.34	38.34	1.426
TOTAL	100	100	100	100	

iii) Day 8

Fatty acid	Day 8				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	2.35	2.45	2.43	2.41	0.053
16:0	38.78	37.79	38.97	38.51	0.634
18:0	4.98	4.01	4.56	4.52	0.486
Sum	46.11	44.25	45.96	45.44	
Monounsaturated					
16:1	7.78	7.62	7.08	7.49	0.367
18:1	1.95	0.98	1.04	1.32	0.543538
Sum	9.73	8.60	8.12	8.82	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	0.000
16:3	6.37	7.04	7.42	6.94	0.532
18:2	20.67	22.34	23.56	22.19	1.451
18:3	17.12	17.77	14.94	16.61	1.482
Sum	44.16	47.15	45.92	45.74	1.503
TOTAL	100	100	100	100	

iv) Day 12

Fatty acid	Day 12				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	2.21	2.97	2.88	2.69	± 0.415
16:0	36.54	35.66	34.41	35.54	± 1.070
18:0	3.93	3.54	4.03	3.83	± 0.259
Sum	42.68	42.17	41.32	42.06	
Monounsaturated					
16:1	6.87	6.7	5.65	6.40	± 0.656
18:1	1.54	0.91	0.89	1.11	± 0.370
Sum	8.41	7.59	6.54	7.51	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	± 0.000
16:3	5.77	6.04	5.82	5.88	± 0.144
18:2	21.56	25.51	24.43	23.83	± 2.041
18:3	21.58	18.69	21.89	20.72	± 1.765
Sum	48.91	50.24	52.14	50.43	1.623
TOTAL	100	100	100	100	

c) *Scenedesmus quadricauda* UMACC 041

i) Day 0

Fatty acid	Day 0				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	0.65	0.97	0.74	0.79	±0.165
16:0	21.58	23.95	22.73	22.75	±1.185
18:0	7.78	8.18	7.57	7.84	±0.310
Sum	30.01	33.1	31.04	31.38	
Monounsaturated					
16:1	2.83	3.07	2.86	2.92	±0.131
18:1	0.00	0.00	0.00	0.00	±0.000
Sum	2.83	3.07	2.86	2.92	
Polyunsaturated					
16:2	0.99	0.87	0.00	0.62	±0.540
16:3	16.23	15.68	17.11	16.34	±0.721
18:2	11.31	10.89	10.86	11.02	±0.252
18:3	38.63	36.39	38.13	37.72	±1.176
Sum	67.16	63.83	66.1	65.70	1.701
TOTAL	100	100	100	100	

ii) Day 4

Fatty acid	Day 4				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	2.15	3.10	3.04	2.76	±0.532
16:0	32.30	35.95	34.44	34.23	±1.834
18:0	7.74	9.02	7.73	8.16	±0.742
Sum	42.19	48.07	45.21	45.16	
Monounsaturated					
16:1	2.69	2.48	2.59	2.59	±0.105
18:1	1.62	1.45	1.67	1.58	±0.115
Sum	4.31	3.93	4.26	4.17	
Polyunsaturated					
16:2	2.02	1.65	1.87	1.85	±0.186
16:3	10.16	9.43	10.03	9.87	±0.389
18:2	13.32	12.88	12.53	12.91	±0.396
18:3	28.00	24.04	26.10	26.05	±1.981
Sum	53.5	48	50.53	50.68	2.753
TOTAL	100	100	100	100	

iii) Day 8

Fatty acid	Day 8				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	2.04	3.32	2.70	2.69	±0.640
16:0	29.46	39.87	31.12	33.48	±5.593
18:0	6.40	6.11	7.16	6.56	±0.542
Sum	37.9	49.3	40.98	42.73	
Monounsaturated					
16:1	4.93	0.00	5.53	3.49	±3.034
18:1	3.46	0.00	4.29	2.58	±2.276
Sum	8.39	0	9.82	6.07	
Polyunsaturated					
16:2	3.33	3.95	3.66	3.65	±0.310
16:3	10.34	14.14	11.17	11.88	±1.998
18:2	18.31	15.41	9.82	14.51	±4.315
18:3	21.73	17.20	24.55	21.16	±3.708
Sum	53.71	50.7	49.2	51.20	2.297
TOTAL	100	100	100	100	

iv) Day 12

Fatty acid	Day 12				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	1.33	1.69	2.04	1.69	±0.355
16:0	27.47	38.01	31.95	32.48	±5.290
18:0	5.84	11.07	8.69	8.53	±2.619
Sum	34.64	50.77	42.68	42.70	
Monounsaturated					
16:1	3.90	4.7	4.55	4.37	±0.411
18:1	3.15	1.04	2.72	2.30	±1.115
Sum	7.05	5.7	7.27	6.67	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	±0.000
16:3	12.15	14.01	17.68	14.61	±2.814
18:2	19.26	27.09	29.4	25.25	±5.315
18:3	26.90	2.43	2.97	10.77	±13.974
Sum	58.31	43.53	50.05	50.63	7.407
TOTAL	100	100	100	100	

d) *Oocystis* UMACC 074

i) Day 0

Fatty acid	Day 0				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	2.20	2.14	2.45	2.26	±0.164
16:0	31.15	35.42	33.08	33.22	±2.138
18:0	9.20	10.19	11.19	10.19	±0.995
Sum	42.55	47.75	46.72	45.67	
Monounsaturated					
16:1	0.00	0.00	0.00	0.00	±0.000
18:1	0.00	0.00	0.00	0.00	±0.000
Sum	0	0	0	0.00	
Polyunsaturated					
16:2	2.80	2.04	2.16	2.33	±0.409
16:3	8.00	7.78	7.89	7.89	±0.110
18:2	18.45	14.32	15.77	16.18	±2.095
18:3	28.20	28.11	27.46	27.92	±0.404
Sum	57.45	52.25	53.28	54.33	2.753
TOTAL	100	100	100	100	

ii) Day 4

Fatty acid	Day 4				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	3.24	3.53	3.87	3.55	±0.315
16:0	52.82	48.17	49.87	50.29	±2.353
18:0	18.61	14.52	16.45	16.53	±2.046
Sum	74.67	66.22	70.19	70.36	
Monounsaturated					
16:1	3.83	6.24	3.62	4.56	±1.456
18:1	0.00	0.00	0.00	0.00	±0.000
Sum	3.83	6.24	3.62	4.56	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	±0.000
16:3	1.12	7.73	6.73	5.19	±3.563
18:2	20.38	19.81	19.46	19.88	±0.464
18:3	0.00	0.00	0.00	0.00	±0.000
Sum	21.5	27.54	26.19	25.08	3.170
TOTAL	100	100	100	100	

iii) Day 8

Fatty acid	Day 8				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	1.79	1.67	1.54	1.67	±0.125
16:0	26.44	28.87	29.87	28.39	±1.764
18:0	10.00	8.83	8.56	9.13	±0.765
Sum	38.23	39.37	39.97	39.19	
Monounsaturated					
16:1	2.54	3.32	3.67	3.18	±0.578
18:1	0.00	0.00	0.00	0.00	±0.000
Sum	2.54	3.32	3.67	3.18	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	±0.000
16:3	7.22	8.32	8.84	8.13	±0.827
18:2	20.36	19.93	19.77	20.02	±0.305
18:3	31.65	29.06	27.75	29.49	±1.985
Sum	59.23	57.31	56.36	57.63	1.462
TOTAL	100	100	100	100	

iv) Day 12

Fatty acid	Day 12				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	2.20	2.22	2.21	2.21	±0.010
16:0	30.15	29.32	31.76	30.41	±1.241
18:0	10.07	9.44	8.99	9.50	±0.542
Sum	42.42	40.98	42.96	42.12	
Monounsaturated					
16:1	0.00	0.00	0.00	0.00	±0.000
18:1	0.00	0.00	0.00	0.00	±0.000
Sum	0	0	0	0.00	
Polyunsaturated					
16:2	2.97	3.03	3.12	3.04	±0.075
16:3	2.33	3.11	2.79	2.74	±0.392
18:2	20.76	18.94	17.67	19.12	±1.553
18:3	31.52	33.94	33.46	32.97	±1.281
Sum	57.58	59.02	57.04	57.88	±1.024
TOTAL	100	100	100	100	

e) *Scenedesmus obtusus* UMACC 100

i) Day 0

Fatty acid	Day 0				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	1.52	1.76	1.36	1.55	±0.201
16:0	20.05	24.68	14.33	19.69	±5.185
18:0	4.52	4.66	2.37	3.85	±1.284
Sum	26.09	31.1	18.06	25.08	
Monounsaturated					
16:1	2.37	3.78	1.54	2.56	±1.132
18:1	1.95	0.78	0.18	0.97	±0.900
Sum	4.32	4.56	1.72	3.53	
Polyunsaturated					
16:2	0.55	0.00	0.60	0.38	±0.333
16:3	14.16	11.77	18.00	14.64	±3.143
18:2	2.19	11.33	3.98	5.83	±4.844
18:3	52.69	41.24	57.64	50.52	±8.412
Sum	69.59	64.34	80.22	71.38	8.090
TOTAL	100	100	100	100	

ii) Day 4

Fatty acid	Day 4				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	5.24	4.00	4.20	4.48	±0.666
16:0	48.31	60.15	51.06	53.17	±6.196
18:0	13.47	10.31	11.58	11.79	±1.590
Sum	67.02	74.46	66.84	69.44	
Monounsaturated					
16:1	3.83	6.00	1.79	3.87	±2.105
18:1	1.18	0.00	3.10	1.43	±1.565
Sum	5.01	6	4.89	5.30	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	±0.000
16:3	16.46	15.69	16.83	16.33	±0.582
18:2	9.02	3.85	8.48	7.12	±2.842
18:3	2.49	0.00	2.96	1.82	±1.591
Sum	27.97	19.54	28.27	25.26	4.956
TOTAL	100	100	100	100	

iii) Day 8

Fatty acid	Day 8				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	2.66	3.78	3.43	3.29	±0.573
16:0	33.30	45.78	38.54	39.21	±6.267
18:0	4.90	11.60	7.67	8.06	±3.367
Sum	40.86	61.16	49.64	50.55	
Monounsaturated					
16:1	6.42	8.75	6.54	7.24	±1.312
18:1	2.23	3.16	2.98	2.79	±0.493
Sum	8.65	11.91	9.52	10.03	
Polyunsaturated					
16:2	1.96	0.00	0.00	0.65	±1.132
16:3	9.09	12.41	11.97	11.16	±1.803
18:2	8.76	12.53	9.54	10.28	±1.990
18:3	30.68	1.99	19.33	17.33	±14.449
Sum	50.49	26.93	40.84	39.42	11.844
TOTAL	100	100	100	100	

iv) Day 12

Fatty acid	Day 12				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	2.31	2.07	2.59	2.32	±0.260
16:0	46.63	28.03	49.07	41.24	±11.508
18:0	7.89	5.49	3.80	5.73	±2.055
Sum	56.83	35.59	55.46	49.29	
Monounsaturated					
16:1	4.98	1.75	5.24	3.99	±1.944
18:1	4.86	1.69	4.40	3.65	±1.713
Sum	9.84	3.44	9.64	7.64	
Polyunsaturated					
16:2	0.00	1.14	1.21	0.78	±0.679
16:3	15.60	12.07	16.21	14.63	±2.235
18:2	15.12	12.34	15.13	14.20	±1.608
18:3	2.61	35.42	2.35	13.46	±19.018
Sum	33.33	60.97	34.9	43.07	15.525
TOTAL	100	100	100	100	

f) *Chlorococcum miniatum* UMACC 102

i) Day 0

Fatty acid	Day 0				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	1.20	1.23	0.85	1.09	±0.211
16:0	29.69	29.17	21.13	26.66	±4.799
18:0	17.63	21.06	15.41	18.03	±2.847
Sum	48.52	51.46	37.39	45.79	
Monounsaturated					
16:1	2.19	3.92	2.87	2.99	±0.872
18:1	0.29	0.00	0.34	0.21	±0.184
Sum	2.48	3.92	3.21	3.20	
Polyunsaturated					
16:2	0.57	0.00	0.00	0.19	±0.329
16:3	13.15	12.20	9.06	11.47	±2.140
18:2	35.28	32.42	50.34	39.35	±9.627
18:3	0.00	0.00	0.00	0.00	±0.000
Sum	49	44.62	59.4	51.01	7.592
TOTAL	100	100	100	100	

ii) Day 4

Fatty acid	Day 4				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	3.01	3.18	3.43	3.21	±0.211
16:0	44.38	48.44	47.34	46.72	±2.100
18:0	17.47	9.08	11.79	12.78	±4.282
Sum	64.86	60.7	62.56	62.71	
Monounsaturated					
16:1	2.76	3.05	2.98	2.93	±0.151
18:1	0.00	0.00	0.00	0.00	±0.000
Sum	2.76	3.05	2.98	2.93	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	±0.000
16:3	9.46	10.42	10.32	10.07	±0.528
18:2	22.92	25.83	24.14	24.30	±1.461
18:3	0.00	0.00	0.00	0.00	±0.000
Sum	32.38	36.25	34.46	34.36	1.937
TOTAL	100	100	100	100	

iii) Day 8

Fatty acid	Day 8				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	2.90	0.89	1.23	1.67	±1.076
16:0	44.30	24.61	24.85	31.25	±11.299
18:0	17.78	15.83	16.77	16.79	±0.975
Sum	64.98	41.33	42.85	49.72	
Monounsaturated					
16:1	2.74	6.12	5.71	4.86	±1.845
18:1	0.00	0.00	0.00	0.00	±0.00
Sum	2.74	6.12	5.71	4.86	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	±0.000
16:3	9.59	9.03	8.69	9.10	±0.454
18:2	22.69	22.60	21.75	22.35	±0.519
18:3	0.00	20.92	21.00	13.97	±12.101
Sum	32.28	52.55	51.44	45.42	11.396
TOTAL	100	100	100	100	

iv) Day 12

Fatty acid	Day 12				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	1.21	4.16	3.33	2.90	±1.521
16:0	25.29	50.58	45.54	40.47	±13.386
18:0	11.21	12.57	11.44	11.74	±0.728
Sum	37.71	67.31	60.31	55.11	
Monounsaturated					
16:1	0.35	5.1	3.12	2.85	±2.381
18:1	0.00	0.00	0.00	0.00	±0.000
Sum	0.35	5.09	3.12	2.85	
Polyunsaturated					
16:2	6.73	0.00	0.00	2.24	±3.886
16:3	10.06	9.87	12.79	10.91	±1.634
18:2	23.58	17.73	23.78	21.70	±3.437
18:3	21.57	0.00	0.00	7.19	±12.453
Sum	61.94	27.6	36.57	42.04	17.811
TOTAL	100	100	100	100	

g) *Chlorella vulgaris* UMACC 103

i) Day 0

Fatty acid	Day 0				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	3.52	3.14	2.98	3.21	±0.277
16:0	45.64	44.43	47.76	45.94	±1.686
18:0	9.58	10.19	11.23	10.33	±0.834
Sum	58.74	57.76	61.97	59.49	
Monounsaturated					
16:1	3.84	2.54	3.67	3.35	±0.707
18:1	0.00	0.00	0.00	0.00	±0.000
Sum	3.84	2.54	3.67	3.35	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	±0.000
16:3	8.83	5.76	7.89	7.49	±1.573
18:2	5.90	4.78	5.89	5.52	±0.644
18:3	22.69	29.16	20.58	24.14	±4.471
Sum	37.42	39.7	34.36	37.16	2.679
TOTAL	100	100	100	100	

ii) Day 4

Fatty acid	Day 4				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	4.07	1.49	3.87	3.14	±1.435
16:0	55.60	30.37	43.23	43.07	±12.616
18:0	7.54	10.47	7.83	8.61	±1.614
Sum	67.21	42.33	54.93	54.82	
Monounsaturated					
16:1	0.00	5.73	3.21	2.98	±2.872
18:1	0.00	0.00	0.00	0.00	±0.000
Sum	0.00	5.73	3.21	2.98	
Polyunsaturated					
16:2	0.00	5.73	0.00	1.91	±3.308
16:3	6.31	10.39	6.73	7.81	±2.244
18:2	10.39	13.12	14.56	12.69	±2.118
18:3	16.09	22.70	20.57	19.79	±3.374
Sum	32.79	51.94	41.86	42.20	9.579
TOTAL	100	100	100	100	

iii) Day 8

Fatty acid	Day 8				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	1.56	1.07	1.43	1.35	±0.254
16:0	32.07	31.98	34.44	32.83	±1.395
18:0	11.25	7.01	8.56	8.94	±2.145
Sum	44.88	40.06	44.43	43.12	
Monounsaturated					
16:1	6.01	6.71	6.54	6.42	±0.365
18:1	1.06	0.39	1.03	0.83	±0.378
Sum	7.07	7.1	7.57	7.25	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	±0.000
16:3	10.92	12.04	13.43	12.13	±1.257
18:2	13.76	16.02	14.56	14.78	±1.146
18:3	23.37	24.78	20.01	22.72	±2.451
Sum	48.05	52.84	48	49.63	2.780
TOTAL	100	100	100	100	

iv) Day 12

Fatty acid	Day 12				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	1.24	0.97	1.21	1.14	±0.148
16:0	30.99	29.32	32.74	31.02	±1.710
18:0	3.53	7.62	6.43	5.86	±2.104
Sum	35.76	37.91	40.38	38.02	
Monounsaturated					
16:1	8.15	3.6	4.67	5.47	±2.383
18:1	0.57	0.00	0.48	0.35	±0.306
Sum	8.72	3.59	5.15	5.82	
Polyunsaturated					
16:2	0.00	6.61	0.00	2.20	±3.816
16:3	12.92	12.65	12.79	12.79	±0.135
18:2	17.57	15.02	14.56	15.72	±1.621
18:3	25.03	24.22	27.12	25.46	±1.496
Sum	55.52	58.5	54.47	56.16	2.091
TOTAL	100	100	100	100	

h) *Chlorella* UMACC 253

i) Day 0

Fatty acid	Day 0				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	3.15	2.64	3.98	3.26	±0.676
16:0	43.44	45.67	46.78	45.30	±1.701
18:0	10.91	11.34	11.67	11.31	±0.381
Sum	57.50	59.65	62.43	59.86	
Monounsaturated					
16:1	4.21	5.78	3.78	4.59	±1.053
18:1	0.00	0.00	0.00	0.00	±0.000
Sum	4.21	5.78	3.78	4.59	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	±0.000
16:3	8.99	9.76	9.65	9.47	±0.416
18:2	7.76	6.54	7.41	7.24	±0.628
18:3	21.54	18.27	16.73	18.85	±2.456
Sum	38.29	34.57	33.79	35.55	2.405
TOTAL	100.00	100.00	100.00	100.00	

ii) Day 4

Fatty acid	Day 4				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	4.30	4.32	4.90	4.51	±0.341
16:0	54.97	52.33	43.49	50.26	±6.013
18:0	7.45	8.90	11.72	9.36	±2.171
Sum	66.72	65.55	60.11	64.13	
Monounsaturated					
16:1	4.53	5.54	5.83	5.30	±0.682
18:1	0.00	0.00	0.00	0.00	±0.000
Sum	4.53	5.54	5.83	5.30	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	±0.000
16:3	3.81	4.32	4.74	4.29	±0.466
18:2	12.69	13.00	15.34	13.68	±1.449
18:3	12.25	11.59	13.98	12.61	±1.234
Sum	28.75	28.91	34.06	30.57	3.021
TOTAL	100.00	100.00	100.00	100.00	

iii) Day 8

Fatty acid	Day 8				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	2.61	2.96	2.29	2.62	±0.335
16:0	23.15	19.51	29.61	24.09	±5.115
18:0	11.54	10.79	11.72	11.35	±0.493
Sum	37.30	33.26	43.62	38.06	
Monounsaturated					
16:1	11.35	13.26	10.52	11.71	±1.405
18:1	0.38	0.00	1.02	0.47	±0.515
Sum	11.73	13.26	11.54	12.18	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	±0.000
16:3	8.14	10.03	6.73	8.30	±1.656
18:2	28.02	24.33	22.55	24.97	±2.790
18:3	14.81	19.12	15.56	16.50	±2.303
Sum	50.97	53.48	44.84	49.76	4.445
TOTAL	100.00	100.00	100.00	100.00	

iv) Day 12

Fatty acid	Day 12				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	1.27	0.90	0.94	1.04	±0.203
16:0	35.73	22.29	29.42	29.15	±6.724
18:0	8.82	3.60	3.52	5.31	±3.037
Sum	45.82	26.79	33.88	35.50	
Monounsaturated					
16:1	10.37	2.02	3.73	5.37	±4.411
18:1	0.00	10.91	7.11	6.01	±5.538
Sum	10.37	12.93	10.84	11.38	
Polyunsaturated					
16:2	0.00	11.05	10.69	7.25	±6.278
16:3	5.82	6.63	6.40	6.28	±0.417
18:2	23.79	26.23	23.52	24.51	±1.493
18:3	14.20	16.37	14.67	15.08	±1.142
Sum	43.81	60.28	55.28	53.12	8.444
TOTAL	100.00	100.00	100.00	100.00	

i) *Chlorella vulgaris* UMACC 245

i) Day 0

Fatty acid	Day 0				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	1.43	1.21	1.29	1.31	±0.111
16:0	29.72	23.00	25.67	26.13	±3.384
18:0	22.81	21.89	22.84	22.51	±0.540
Sum	53.96	46.10	49.80	49.95	
Monounsaturated					
16:1	4.72	3.89	5.33	4.65	±0.723
18:1	0.27	0.69	1.09	0.68	±0.410
Sum	4.99	4.58	6.42	5.33	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	±0.000
16:3	4.37	5.32	4.66	4.78	±0.487
18:2	26.48	24.54	24.71	25.24	±1.074
18:3	10.20	19.46	14.41	14.69	±4.636
Sum	41.05	49.32	43.78	44.72	4.214
TOTAL	100.00	100.00	100.00	100.00	

ii) Day 4

Fatty acid	Day 4				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	2.21	2.11	1.89	2.07	±0.164
16:0	31.45	33.49	35.00	33.31	±1.782
18:0	19.87	18.87	19.19	19.31	±0.511
Sum	53.53	54.47	56.08	54.69	
Monounsaturated					
16:1	4.43	3.56	4.44	4.14	±0.505
18:1	0.67	0.78	1.04	0.83	±0.190
Sum	5.10	4.34	5.48	4.97	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	±0.000
16:3	5.67	4.46	4.11	4.75	±0.819
18:2	26.06	23.43	21.51	23.67	±2.284
18:3	9.64	13.30	12.82	11.92	±1.989
Sum	41.37	41.19	38.44	40.33	1.642
TOTAL	100.00	100.00	100.00	100.00	

iii) Day 8

Fatty acid	Day 8				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	0.88	0.93	1.01	0.94	±0.066
16:0	22.81	21.43	23.55	22.60	±1.076
18:0	10.56	12.32	10.35	11.08	±1.082
Sum	34.25	34.68	34.91	34.61	
Monounsaturated					
16:1	1.47	1.31	1.78	1.52	±0.239
18:1	0.00	0.00	0.00	0.00	±0.000
Sum	1.47	1.31	1.78	1.52	
Polyunsaturated					
16:2	8.22	8.73	7.66	8.20	±0.535
16:3	8.22	7.71	8.13	8.02	±0.272
18:2	26.33	23.59	23.57	24.50	±1.588
18:3	21.51	23.98	23.95	23.15	±1.417
Sum	64.28	64.01	63.31	63.87	0.501
TOTAL	100.00	100.00	100.00	100.00	

iv) Day 12

Fatty acid	Day 12				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	1.09	1.91	1.87	1.62	±0.462
16:0	2.72	2.14	2.32	2.39	±0.297
18:0	5.34	4.88	5.39	5.20	±0.281
Sum	9.15	8.93	9.58	9.22	
Monounsaturated					
16:1	0.00	0.00	0.00	0.00	±0.000
18:1	6.65	6.38	6.73	6.59	±0.183
Sum	6.65	6.38	6.73	6.59	
Polyunsaturated					
16:2	12.58	14.28	13.31	13.39	±0.853
16:3	13.90	14.20	11.89	13.33	±1.256
18:2	23.46	23.79	24.54	23.93	±0.553
18:3	34.26	32.42	33.95	33.54	±0.985
Sum	84.20	84.69	83.69	84.19	0.500
TOTAL	100.00	100.00	100.00	100.00	

j) *Chlorella* UMACC 255

i) Day 0

Fatty acid	Day 0				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	1.57	1.76	1.49	1.61	±0.139
16:0	22.72	21.68	23.84	22.75	±1.080
18:0	0.50	0.63	0.73	0.62	±0.115
Sum	24.79	24.07	26.06	24.97	
Monounsaturated					
16:1	1.21	2.55	2.23	2.00	±0.700
18:1	0.00	0.00	0.00	0.00	±0.000
Sum	1.21	2.55	2.23	2.00	
Polyunsaturated					
16:2	13.37	13.43	12.89	13.23	±0.296
16:3	13.03	14.67	14.11	13.94	±0.834
18:2	18.54	17.27	18.87	18.23	±0.845
18:3	29.06	28.01	25.84	27.64	±1.642
Sum	74.00	73.38	71.71	73.03	1.184
TOTAL	100.00	100.00	100.00	100.00	

ii) Day 4

Fatty acid	Day 4				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	5.89	4.43	4.61	4.98	±0.796
16:0	62.74	58.76	55.83	59.11	±3.468
18:0	0.00	2.31	2.81	1.71	±1.499
Sum	68.63	65.50	63.25	65.79	
Monounsaturated					
16:1	5.98	3.75	4.23	4.65	±1.174
18:1	0.00	0.00	0.00	0.00	±0.000
Sum	5.98	3.75	4.23	4.65	
Polyunsaturated					
16:2	0.00	0.00	0.00	0.00	±0.000
16:3	5.11	5.67	4.77	5.18	±0.454
18:2	5.81	4.89	4.81	5.17	±0.556
18:3	14.47	20.19	22.94	19.20	±4.321
Sum	25.39	30.75	32.52	29.55	3.713
TOTAL	100.00	100.00	100.00	100.00	

iii) Day 8

Fatty acid	Day 8				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	0.93	1.34	0.98	1.08	±0.224
16:0	23.58	21.89	22.29	22.59	±0.883
18:0	5.22	5.55	4.77	5.18	±0.392
Sum	29.73	28.78	28.04	28.85	
Monounsaturated					
16:1	1.08	2.71	1.23	1.67	±0.901
18:1	22.27	19.98	21.76	21.34	±1.202
Sum	23.35	22.69	22.99	23.01	
Polyunsaturated					
16:2	6.76	5.32	6.14	6.07	±0.722
16:3	7.01	7.57	7.24	7.27	±0.281
18:2	12.40	13.20	14.18	13.26	±0.892
18:3	20.75	22.44	21.41	21.53	±0.852
Sum	46.92	48.53	48.97	48.14	1.079
TOTAL	100.00	100.00	100.00	100.00	

iv) Day 12

Fatty acid	Day 12				
	R1	R2	R3	Mean	STDEV
Saturated					
14:0	1.11	1.43	1.23	1.26	±0.162
16:0	22.13	21.68	21.11	21.64	±0.511
18:0	3.96	4.31	4.26	4.18	±0.189
Sum	27.20	27.42	26.60	27.07	
Monounsaturated					
16:1	3.02	3.51	4.07	3.53	±0.525
18:1	6.76	5.85	6.00	6.20	±0.488
Sum	9.78	9.36	10.07	9.74	
Polyunsaturated					
16:2	8.83	7.68	7.59	8.03	±0.691
16:3	9.93	7.55	8.57	8.68	±1.194
18:2	19.40	17.21	14.18	16.93	±2.621
18:3	24.86	30.78	32.99	29.54	±4.204
Sum	63.02	63.22	63.33	63.19	0.157
TOTAL	100.00	100.00	100.00	100.00	

APPENDIX F

Calibration of CO_2 Levels

a) pH and DIC at different flow rates in BBM medium (5% CO_2)

i) 10ml CO_2/min

Time(min)	pH	DIC	Mean	STDEV
0	6.80	0.0000018		
	6.82	0.0000018		
	6.80	0.0000018	0.0000018	0.000000004
10	6.56	0.0000017		
	6.63	0.0000017		
	6.66	0.0000017	0.0000017	0.000000029
20	6.45	0.0000016		
	6.51	0.0000017		
	6.62	0.0000017	0.0000017	0.000000058
30	6.39	0.0000016		
	6.46	0.0000016		
	6.51	0.0000017	0.0000016	0.000000050
40	6.33	0.0000015		
	6.40	0.0000016		
	6.45	0.0000016	0.0000016	0.000000057
50	6.29	0.0000015		
	6.31	0.0000015		
	6.41	0.0000016	0.0000015	0.000000066
60	6.28	0.0000014		
	6.26	0.0000014		
	6.39	0.0000016	0.0000015	0.000000076

ii) 20ml CO_2/min

Time(min)	pH	DIC	Mean	STDEV
0	6.82	0.0000018		
	6.81	0.0000018		
	6.83	0.0000018	0.0000018	0.0000000035
10	6.71	0.0000018		
	6.77	0.0000018		
	6.76	0.0000018	0.0000018	0.0000000135
20	6.65	0.0000017		
	6.69	0.0000018		
	6.71	0.0000018	0.0000018	0.0000000148
30	6.52	0.0000017		
	6.62	0.0000017		
	6.67	0.0000018	0.0000017	0.0000000453
40	6.40	0.0000016		
	6.58	0.0000017		
	6.66	0.0000017	0.0000017	0.0000000934
50	6.26	0.0000014		
	6.52	0.0000017		
	6.56	0.0000017	0.0000016	0.0000001512
60	6.28	0.0000014		
	6.32	0.0000015		
	6.39	0.0000016	0.0000015	0.0000000592

iii) 30ml CO_2/min

Time(min)	pH	DIC	Mean	STDEV
0	6.80	0.0000018		
	6.80	0.0000018		
	6.78	0.0000018	0.0000018	0.000000004
10	6.54	0.0000017		
	6.58	0.0000017		
	6.55	0.0000017	0.0000017	0.000000013
20	6.25	0.0000014		
	6.36	0.0000015		
	6.36	0.0000015	0.0000015	0.000000073
30	6.05	0.0000011		
	6.16	0.0000013		
	6.19	0.0000013	0.0000012	0.000000131
40	5.98	0.0000009		
	6.01	0.0000010		
	6.09	0.0000012	0.0000010	0.000000120
50	5.95	0.0000008		
	5.91	0.0000007		
	5.99	0.0000009	0.0000008	0.000000104
60	5.89	0.0000007		
	5.98	0.0000009		
	5.88	0.0000006	0.0000007	0.000000149

iv) 40ml CO_2/min

Time(min)	pH	DIC	Mean	STDEV
0	6.83	0.0000018		
	6.80	0.0000018		
	6.82	0.0000018	0.0000018	0.00000001
10	6.78	0.0000018		
	6.64	0.0000017		
	6.75	0.0000018	0.0000018	0.00000003
20	6.72	0.0000018		
	6.63	0.0000017		
	6.71	0.0000018	0.0000018	0.00000002
30	6.69	0.0000018		
	6.61	0.0000017		
	6.69	0.0000018	0.0000017	0.00000002
40	6.66	0.0000017		
	6.59	0.0000017		
	6.68	0.0000018	0.0000017	0.00000003
50	6.63	0.0000017		
	6.58	0.0000017		
	6.64	0.0000017	0.0000017	0.00000002
60	6.49	0.0000016		
	6.55	0.0000017		
	6.62	0.0000017	0.0000017	0.00000004

v) 50ml CO_2/min

Time(min)	pH	DIC	Mean	STDEV
0	6.81	0.0000018		
	6.81	0.0000018		
	6.82	0.0000018	0.0000018	0.0000000
10	6.77	0.0000018		
	6.76	0.0000018		
	6.70	0.0000018	0.0000018	0.0000000
20	6.70	0.0000018		
	6.70	0.0000018		
	6.63	0.0000017	0.0000018	0.0000000
30	6.63	0.0000017		
	6.62	0.0000017		
	6.57	0.0000017	0.0000017	0.0000000
40	6.58	0.0000017		
	6.57	0.0000017		
	6.54	0.0000017	0.0000017	0.0000000
50	6.50	0.0000016		
	6.53	0.0000017		
	6.53	0.0000017	0.0000017	0.0000000
60	6.54	0.0000017		
	6.43	0.0000016		
	6.44	0.0000016	0.0000016	0.0000000

b) pH and DIC at different flow rates in BBM medium(10% CO_2)

i) 10ml CO_2/min

time	pH	DIC	mean	stdev
0	6.85	0.0000018	0.0000018	0.0000000
	6.81	0.0000018		
	6.80	0.0000018		
10	6.58	0.0000017	0.0000017	0.0000000
	6.62	0.0000017		
	6.68	0.0000018		
20	6.45	0.0000016	0.0000017	0.0000000
	6.51	0.0000017		
	6.59	0.0000017		
30	6.42	0.0000016	0.0000016	0.0000001
	6.42	0.0000016		
	6.55	0.0000017		
40	6.35	0.0000015	0.0000016	0.0000000
	6.40	0.0000016		
	6.41	0.0000016		
50	6.32	0.0000015	0.0000015	0.0000000
	6.31	0.0000015		
	6.39	0.0000016		
60	6.28	0.0000014	0.0000015	0.0000001
	6.26	0.0000014		
	6.39	0.0000016		

ii) 20ml CO_2/min

time	pH	DIC	mean	stdev
	6.83	0.0000018	0.0000018	0.0000000
	6.82	0.0000018		
0	6.83	0.0000018		
	6.77	0.0000018	0.0000018	0.0000000
	6.71	0.0000018		
10	6.74	0.0000018		
	6.63	0.0000017	0.0000018	0.0000000
	6.69	0.0000018		
20	6.71	0.0000018		
	6.62	0.0000017	0.0000017	0.0000000
	6.59	0.0000017		
30	6.72	0.0000018		
	6.52	0.0000017	0.0000017	0.0000000
	6.58	0.0000017		
40	6.66	0.0000017		
	6.38	0.0000015	0.0000016	0.0000001
	6.45	0.0000016		
50	6.53	0.0000017		
	6.31	0.0000015	0.0000015	0.0000000
	6.32	0.0000015		
60	6.39	0.0000016		

iii) 30ml CO_2/min

time	pH		mean	stdev
0	6.80	0.0000018	0.0000018	0.0000000
	6.80	0.0000018		
	6.83	0.0000018		
10	6.53	0.0000017	0.0000017	0.0000000
	6.57	0.0000017		
	6.58	0.0000017		
20	6.23	0.0000014	0.0000015	0.0000001
	6.32	0.0000015		
	6.34	0.0000015		
30	6.03	0.0000010	0.0000012	0.0000001
	6.14	0.0000012		
	6.18	0.0000013		
40	5.97	0.0000009	0.0000009	0.0000000
	5.98	0.0000009		
	5.98	0.0000009		
50	5.96	0.0000009	0.0000008	0.0000001
	5.89	0.0000007		
	5.94	0.0000008		
60	5.89	0.0000007	0.0000007	0.0000001
	5.98	0.0000009		
	5.88	0.0000006		

iv) 40ml CO_2/min

time	pH		mean	stdev
0	6.82	0.0000018	0.0000018	0.0000000
	6.80	0.0000018		
	6.82	0.0000018		
10	6.81	0.0000018	0.0000018	0.0000000
	6.74	0.0000018		
	6.77	0.0000018		
20	6.74	0.0000018	0.0000018	0.0000000
	6.65	0.0000017		
	6.69	0.0000018		
30	6.73	0.0000018	0.0000018	0.0000000
	6.63	0.0000017		
	6.71	0.0000018		
40	6.67	0.0000018	0.0000017	0.0000000
	6.61	0.0000017		
	6.72	0.0000018		
50	6.64	0.0000017	0.0000017	0.0000000
	6.61	0.0000017		
	6.65	0.0000017		
60	6.51	0.0000017	0.0000017	0.0000000
	6.55	0.0000017		
	6.61	0.0000017		

v) 50ml CO_2/min

time	pH		mean	stdev
0	6.81	0.00000181	0.00000181	0.00000000
	6.81	0.00000181		
	6.82	0.00000181		
10	6.78	0.00000180	0.00000179	0.00000002
	6.79	0.00000180		
	6.70	0.00000177		
20	6.72	0.00000178	0.00000177	0.00000001
	6.73	0.00000178		
	6.67	0.00000175		
30	6.65	0.00000174	0.00000174	0.00000000
	6.65	0.00000174		
	6.66	0.00000175		
40	6.61	0.00000172	0.00000170	0.00000003
	6.62	0.00000173		
	6.52	0.00000166		
50	6.50	0.00000165	0.00000166	0.00000001
	6.52	0.00000166		
	6.53	0.00000167		
60	6.54	0.00000168	0.00000162	0.00000005
	6.43	0.00000159		
	6.44	0.00000160		

APPENDIX G

<u>The Growth Trends of Selected Microalgae Grown at Elevated Levels of Carbon Dioxide</u>

a) OD_{620nm}

i) *Chlorella vulgaris* UMACC 001

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	0.023			0.023			0.023			0.023		
	R2	0.023			0.023			0.023			0.023		
0	R3	0.023	0.023	± 0.000	0.023	0.023	± 0.000	0.023	0.023	± 0.000	0.023	0.023	± 0.000
	R1	0.025			0.021			0.023			0.031		
	R2	0.026			0.025			0.024			0.026		
1	R3	0.024	0.025	± 0.001	0.024	0.023	± 0.002	0.027	0.025	± 0.002	0.024	0.027	± 0.004
	R1	0.029			0.042			0.046			0.042		
	R2	0.031			0.037			0.045			0.039		
2	R3	0.031	0.030	± 0.001	0.033	0.037	± 0.005	0.047	0.046	± 0.001	0.044	0.042	± 0.003
	R1	0.036			0.053			0.078			0.071		
	R2	0.039			0.048			0.080			0.067		
3	R3	0.037	0.037	± 0.002	0.068	0.056	± 0.010	0.078	0.079	± 0.001	0.064	0.067	± 0.004
	R1	0.048			0.077			0.108			0.087		
	R2	0.047			0.069			0.111			0.075		
4	R3	0.049	0.048	± 0.001	0.089	0.078	± 0.010	0.103	0.107	± 0.004	0.079	0.080	± 0.006
	R1	0.061			0.116			0.175			0.123		
	R2	0.063			0.090			0.184			0.113		
5	R3	0.062	0.062	± 0.001	0.139	0.115	± 0.025	0.174	0.178	± 0.006	0.115	0.117	± 0.005

Optical Density (OD_{620nm}) *Chlorella vulgaris* UMACC 001 (continue)

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	0.081			0.181			0.283			0.252		
	R2	0.078			0.129			0.273			0.255		
6	R3	0.081	0.080	± 0.002	0.192	0.167	± 0.034	0.254	0.270	± 0.015	0.262	0.256	± 0.005
	R1	0.089			0.222			0.354			0.305		
	R2	0.088			0.180			0.367			0.312		
7	R3	0.088	0.088	± 0.001	0.227	0.210	± 0.026	0.388	0.370	± 0.017	0.337	0.318	± 0.017
	R1	0.112			0.351			0.499			0.441		
	R2	0.119			0.289			0.468			0.416		
8	R3	0.109	0.113	± 0.005	0.324	0.321	± 0.031	0.435	0.467	± 0.032	0.418	0.425	± 0.014
	R1	0.125			0.417			0.553			0.510		
	R2	0.118			0.370			0.576			0.555		
9	R3	0.129	0.124	± 0.006	0.437	0.408	± 0.034	0.552	0.560	± 0.014	0.599	0.555	± 0.045
	R1	0.151			0.536			0.669			0.611		
	R2	0.129			0.474			0.685			0.683		
10	R3	0.134	0.138	± 0.012	0.482	0.497	± 0.034	0.678	0.677	± 0.008	0.650	0.648	± 0.036

ii) *Chlorella* UMACC 275

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	0.024			0.024			0.024			0.023		
	R2	0.024			0.024			0.024			0.023		
0	R3	0.024	0.024	±0.000	0.024	0.024	±0.000	0.024	0.024	±0.000	0.023	0.023	±0.000
	R1	0.024			0.027			0.027			0.025		
	R2	0.025			0.026			0.026			0.026		
1	R3	0.025	0.025	±0.001	0.025	0.026	±0.001	0.025	0.026	±0.001	0.027	0.026	±0.001
	R1	0.025			0.031			0.046			0.041		
	R2	0.026			0.029			0.038			0.039		
2	R3	0.024	0.025	±0.001	0.033	0.031	±0.002	0.037	0.040	±0.005	0.044	0.041	±0.003
	R1	0.025			0.045			0.052			0.065		
	R2	0.025			0.042			0.048			0.071		
3	R3	0.026	0.025	±0.001	0.041	0.043	±0.002	0.058	0.053	±0.005	0.059	0.065	±0.006
	R1	0.027			0.067			0.073			0.089		
	R2	0.024			0.071			0.067			0.091		
4	R3	0.025	0.025	±0.002	0.056	0.065	±0.008	0.077	0.072	±0.005	0.084	0.088	±0.004
	R1	0.027			0.071			0.089			0.115		
	R2	0.028			0.079			0.096			0.123		
5	R3	0.026	0.027	±0.001	0.071	0.074	±0.005	0.091	0.092	±0.004	0.119	0.119	±0.004

Optical Density *Chlorella* UMACC 275 (continue)

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	0.031			0.082			0.103			0.132		
	R2	0.032			0.088			0.112			0.144		
6	R3	0.034	0.032	±0.002	0.085	0.085	±0.003	0.105	0.107	±0.005	0.139	0.138	±0.006
	R1	0.033			0.089			0.167			0.172		
	R2	0.032			0.092			0.145			0.209		
7	R3	0.035	0.033	±0.002	0.098	0.093	±0.005	0.154	0.155	±0.011	0.221	0.201	±0.026
	R1	0.036			0.130			0.214			0.229		
	R2	0.034			0.091			0.198			0.256		
8	R3	0.035	0.035	±0.001	0.094	0.105	±0.022	0.224	0.212	±0.013	0.276	0.254	±0.024
	R1	0.039			0.119			0.245			0.276		
	R2	0.041			0.134			0.253			0.283		
9	R3	0.038	0.039	±0.002	0.108	0.120	±0.013	0.277	0.258	±0.017	0.294	0.284	±0.009
	R1	0.039			0.127			0.309			0.305		
	R2	0.041			0.146			0.295			0.346		
10	R3	0.039	0.040	±0.001	0.111	0.128	±0.018	0.286	0.297	±0.012	0.339	0.330	±0.022

iii) *Scenedesmus quadricauda* UMACC 041

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	0.025			0.025			0.025			0.029		
	R2	0.025			0.025			0.025			0.029		
0	R3	0.025	0.025	±0.000	0.025	0.025	±0.000	0.025	0.025	±0.000	0.029	0.029	±0.000
	R1	0.025			0.030			0.025			0.031		
	R2	0.026			0.025			0.034			0.034		
1	R3	0.026	0.026	±0.001	0.029	0.028	±0.003	0.029	0.029	±0.005	0.035	0.033	±0.002
	R1	0.031			0.041			0.054			0.052		
	R2	0.031			0.050			0.064			0.052		
2	R3	0.029	0.030	±0.001	0.050	0.047	±0.005	0.064	0.061	±0.006	0.063	0.056	±0.006
	R1	0.034			0.068			0.077			0.069		
	R2	0.033			0.064			0.088			0.068		
3	R3	0.031	0.033	±0.002	0.064	0.065	±0.002	0.091	0.085	±0.007	0.076	0.071	±0.004
	R1	0.038			0.081			0.103			0.093		
	R2	0.037			0.087			0.082			0.089		
4	R3	0.039	0.038	±0.001	0.085	0.084	±0.003	0.093	0.093	±0.011	0.091	0.091	±0.002
	R1	0.066			0.115			0.123			0.147		
	R2	0.058			0.123			0.141			0.147		
5	R3	0.064	0.063	±0.004	0.105	0.114	±0.009	0.161	0.142	±0.019	0.159	0.151	±0.007

Optical Density *Scenedesmus quadricauda* UMACC 041 (continue)

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	0.067			0.154			0.165			0.193		
	R2	0.061			0.136			0.236			0.196		
6	R3	0.068	0.065	±0.004	0.136	0.142	±0.010	0.206	0.202	±0.036	0.199	0.196	±0.003
	R1	0.080			0.173			0.271			0.190		
	R2	0.064			0.182			0.256			0.241		
7	R3	0.073	0.072	±0.008	0.159	0.171	±0.012	0.279	0.269	±0.012	0.277	0.236	±0.044
	R1	0.083			0.235			0.275			0.307		
	R2	0.072			0.222			0.323			0.315		
8	R3	0.075	0.077	±0.006	0.196	0.218	±0.020	0.368	0.322	±0.047	0.324	0.315	±0.009
	R1	0.085			0.265			0.353			0.374		
	R2	0.075			0.273			0.378			0.368		
9	R3	0.079	0.080	±0.005	0.253	0.264	±0.010	0.436	0.389	±0.043	0.389	0.377	±0.011
	R1	0.092			0.322			0.501			0.411		
	R2	0.083			0.358			0.621			0.591		
10	R3	0.089	0.088	±0.005	0.289	0.323	±0.035	0.589	0.570	±0.062	0.522	0.508	±0.091

iv) *Oocystis* UMACC 074

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	0.021			0.021			0.021			0.021		
	R2	0.021			0.021			0.021			0.021		
0	R3	0.021	0.021	±0.000	0.021	0.021	±0.000	0.021	0.021	±0.000	0.021	0.021	±0.000
	R1	0.023			0.023			0.026			0.025		
	R2	0.021			0.023			0.026			0.026		
1	R3	0.022	0.022	±0.001	0.024	0.023	±0.001	0.029	0.027	±0.002	0.026	0.026	±0.001
	R1	0.034			0.041			0.052			0.046		
	R2	0.035			0.032			0.050			0.048		
2	R3	0.036	0.035	±0.001	0.043	0.039	±0.006	0.057	0.053	±0.004	0.044	0.046	±0.002
	R1	0.041			0.051			0.061			0.056		
	R2	0.048			0.052			0.062			0.058		
3	R3	0.045	0.045	±0.004	0.054	0.052	±0.002	0.071	0.065	±0.006	0.055	0.056	±0.002
	R1	0.048			0.060			0.096			0.081		
	R2	0.052			0.062			0.092			0.088		
4	R3	0.048	0.049	±0.002	0.077	0.066	±0.009	0.114	0.101	±0.012	0.089	0.086	±0.004
	R1	0.057			0.078			0.119			0.096		
	R2	0.055			0.077			0.123			0.099		
5	R3	0.052	0.055	±0.003	0.084	0.080	±0.004	0.128	0.123	±0.005	0.106	0.100	±0.005

Optical Density *Oocystis* UMACC 074 (continue)

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	0.061			0.087			0.131			0.123		
	R2	0.058			0.091			0.141			0.117		
6	R3	0.057	0.059	±0.002	0.094	0.091	±0.004	0.139	0.137	±0.005	0.129	0.123	±0.006
	R1	0.065			0.109			0.155			0.142		
	R2	0.062			0.113			0.168			0.139		
7	R3	0.061	0.063	±0.002	0.118	0.113	±0.005	0.149	0.157	±0.010	0.153	0.145	±0.007
	R1	0.071			0.156			0.189			0.168		
	R2	0.068			0.131			0.221			0.171		
8	R3	0.069	0.069	±0.002	0.162	0.150	±0.016	0.177	0.196	±0.023	0.183	0.174	±0.008
	R1	0.078			0.182			0.223			0.208		
	R2	0.071			0.159			0.267			0.219		
9	R3	0.077	0.075	±0.004	0.177	0.173	±0.012	0.237	0.242	±0.022	0.232	0.220	±0.012
	R1	0.084			0.209			0.251			0.234		
	R2	0.074			0.165			0.317			0.253		
10	R3	0.082	0.080	±0.005	0.214	0.196	±0.027	0.289	0.286	±0.033	0.267	0.251	±0.017

b) Cell count

i) *Chlorella vulgaris* UMACC 001

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	18			18			18			18		
	R2	18			18			18			18		
0	R3	18	18	±0.000	18	18	±0.000	18	18	±0.000	17	18	±.577
	R1	16			23			21			19		
	R2	19			23			23			22		
1	R3	20	18	±2.082	20	22	±1.732	21	22	±1.155	21	21	±1.528
	R1	18			26			40			29		
	R2	18			18			37			34		
2	R3	19	18	±0.577	25	23	±4.359	34	37	±3.000	35	33	±3.215
	R1	19			46			72			51		
	R2	26			35			78			68		
3	R3	23	23	±3.512	43	41	±5.686	69	73	±4.583	66	62	±9.292
	R1	42			53			101			78		
	R2	45			50			96			87		
4	R3	43	43	±1.528	63	55	±6.807	99	99	±2.517	82	82	±4.509
	R1	64			86			179			119		
	R2	69			86			165			123		
5	R3	63	65	±3.215	101	91	±8.660	138	161	±20.841	145	129	±14.000

Cell count *Chlorella vulgaris* UMACC 001(continue)

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	79			164			278			348		
	R2	77			147			335			310		
6	R3	75	77	±2.000	178	163	±15.524	354	322	±39.552	264	307	±42.063
	R1	95			184			365			464		
	R2	96			183			453			392		
7	R3	95	95	±0.577	244	204	±34.933	476	431	±58.586	368	408	±49.960
	R1	150			272			558			564		
	R2	150			320			571			475		
8	R3	148	149	±1.155	290	294	±24.249	567	565	±6.658	481	507	±49.743
	R1	143			445			643			612		
	R2	159			436			612			552		
9	R3	157	153	±8.718	459	447	±11.590	664	640	±26.160	536	567	±40.067
	R1	163			520			669			667		
	R2	163			530			645			640		
10	R3	169	165	±3.464	562	537	±21.939	712	675	±33.946	680	662	±20.404

ii) *Chlorella* UMACC 275

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	16			16			16			17		
	R2	14			14			14			13		
0	R3	12	14	±2.000	12	14	±2.000	12	14	±2.000	14	15	±2.082
	R1	16			18			24			26		
	R2	15			21			25			28		
1	R3	13	15	±1.528	23	21	±2.517	22	24	±1.528	31	28	±2.517
	R1	17			24			33			36		
	R2	15			25			29			38		
2	R3	16	16	±1.000	22	24	±1.528	34	32	±2.646	44	39	±4.163
	R1	18			31			41			53		
	R2	15			29			46			59		
3	R3	16	16	±1.528	33	31	±2.000	39	42	±3.606	54	55	±3.215
	R1	19			34			66			128		
	R2	17			35			71			124		
4	R3	19	18	±1.155	37	35	±1.528	89	75	±12.097	109	120	±10.017
	R1	21			59			89			134		
	R2	23			61			78			144		
5	R3	19	21	±2.000	65	62	±3.055	91	86	±7.000	154	144	±10.000

Cell count *Chlorella* UMACC 275 (continue)

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	24			74			105			184		
	R2	23			77			117			177		
6	R3	25	24	±1.000	81	77	±3.512	121	114	±8.327	167	176	±8.544
	R1	27			84			134			210		
	R2	29			89			143			201		
7	R3	31	29	±2.000	93	89	±4.509	159	145	±12.662	198	203	±6.245
	R1	32			101			178			224		
	R2	29			110			189			237		
8	R3	33	31	±2.082	129	113	±14.295	184	184	±5.508	248	236	±12.014
	R1	34			137			213			256		
	R2	36			147			224			261		
9	R3	39	36	±2.517	151	145	±7.211	237	225	±12.014	271	263	±7.638
	R1	42			156			245			301		
	R2	38			161			268			321		
10	R3	44	41	±3.055	178	165	±11.533	259	257	±11.590	331	318	±15.275

iii) *Scenedesmus quadricauda* UMACC 041

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	11			11			11			11		
	R2	12			12			12			12		
0	R3	13	12	±1.000	13	12	±1.000	13	12	±1.000	13	12	±1.000
	R1	11			11			11			11		
	R2	12			12			12			12		
1	R3	13	12	±1.000	13	12	±1.000	13	12	±1.000	13	12	±1.000
	R1	12			11			16			13		
	R2	11			12			14			17		
2	R3	12	12	±0.577	14	12	±1.528	16	15	±1.155	16	15	±2.082
	R1	17			17			19			22		
	R2	16			23			22			23		
3	R3	15	16	±1.000	22	21	±3.215	25	22	±3.000	29	25	±3.786
	R1	17			21			28			25		
	R2	18			25			27			29		
4	R3	16	17	±1.000	24	23	±2.082	30	28	±1.528	31	28	±3.055
	R1	17			35			38			39		
	R2	20			34			40			34		
5	R3	18	18	±1.528	31	33	±2.082	39	39	±1.000	39	37	±2.887

Cell count *Scenedesmus quadricauda* UMACC 041(continue)

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	25			49			47			51		
	R2	24			36			61			54		
6	R3	23	24	±1.000	32	39	±8.888	54	54	±7.000	58	54	±3.512
	R1	30			52			77			63		
	R2	30			67			75			64		
7	R3	31	30	±0.577	49	56	±9.644	80	77	±2.517	73	67	±5.508
	R1	39			72			88			80		
	R2	38			77			94			79		
8	R3	37	38	±1.000	62	70	±7.638	94	92	±3.464	83	81	±2.082
	R1	42			84			108			99		
	R2	44			81			121			108		
9	R3	43	43	±1.000	76	80	±4.041	123	117	±8.145	111	106	±6.245
	R1	49			93			112			132		
	R2	51			103			132			125		
10	R3	48	49	±1.528	93	96	±5.774	150	131	±19.009	129	129	±3.512

iv) *Oocystis* UMACC 074

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	10			10			10			10		
	R2	10			10			10			10		
0	R3	10	10	±0.000	10	10	±0.000	10	10	±0.000	10	10	±0.000
	R1	11			13			11			11		
	R2	10			14			20			14		
1	R3	10	10	±0.577	11	13	±1.528	19	17	±4.933	18	14	±3.512
	R1	13			23			28			22		
	R2	12			17			27			25		
2	R3	11	12	±1.000	19	20	±3.055	28	28	±0.577	23	23	±1.528
	R1	21			25			31			26		
	R2	17			24			30			26		
3	R3	19	19	±2.000	25	25	±0.577	30	30	±0.577	28	27	±1.155
	R1	25			32			33			28		
	R2	22			25			35			31		
4	R3	26	24	±2.082	25	27	±4.041	36	35	±1.528	29	29	±1.528
	R1	27			36			43			34		
	R2	25			29			47			36		
5	R3	29	27	±2.000	32	32	±3.512	48	46	±2.646	39	36	±2.517

Cell count *Oocystis* UMACC 074 (continue)

Day	Sample	Control (no aeration)	Mean	STDEV	0.04% CO_2	Mean	STDEV	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
	R1	31			39			53			47		
	R2	29			33			55			49		
6	R3	32	31	±1.528	37	36	±3.055	58	55	±2.517	51	49	±2.000
	R1	32			46			64			55		
	R2	31			42			63			59		
7	R3	33	32	±1.000	44	44	±2.000	68	65	±2.646	63	59	±4.000
	R1	34			53			75			68		
	R2	33			49			77			71		
8	R3	35	34	±1.000	47	50	±3.055	82	78	±3.606	73	71	±2.517
	R1	36			66			89			78		
	R2	35			54			92			81		
9	R3	37	36	±1.000	51	57	±7.937	97	93	±4.041	86	82	±4.041
	R1	38			70			97			98		
	R2	38			59			111			89		
10	R3	37	38	±0.577	55	61	±7.767	113	107	±8.718	101	96	±6.245

c) Chlorophyll –a content

i) *Chlorella vulgaris* UMACC 001

Day	treatment	sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
0	control (no aeration)	R1	0.151	0.059	0.029	1.67025	1670.25	0.167	0.147667	0.033486
		R2	0.098	0.035	0.014	1.08899	1088.99	0.109		
		R3	0.151	0.059	0.029	1.67025	1670.25	0.167		
	0.04% CO_2	R1	0.151	0.059	0.029	1.67025	1670.25	0.167	0.147667	0.033486
		R2	0.098	0.035	0.014	1.08899	1088.99	0.109		
		R3	0.151	0.059	0.029	1.67025	1670.25	0.167		
	5% CO_2	R1	0.151	0.059	0.029	1.67025	1670.25	0.167	0.147667	0.033486
		R2	0.098	0.035	0.014	1.08899	1088.99	0.109		
		R3	0.151	0.059	0.029	1.67025	1670.25	0.167		
	10% CO_2	R1	0.119	0.050	0.025	1.31140	1311.40	0.131	0.123333	0.01159
		R2	0.117	0.051	0.031	1.28605	1286.05	0.129		
		R3	0.099	0.036	0.014	1.09928	1099.28	0.110		
2	control (no aeration)	R1	0.015	0.006	0.003	0.16572	165.72	0.166	0.169103	0.007025
		R2	0.015	0.007	0.003	0.16441	164.41	0.164		
		R3	0.016	0.006	0.004	0.17718	177.18	0.177		
	0.04% CO_2	R1	0.017	0.006	0.002	0.18906	189.06	0.189	0.20699	0.021796
		R2	0.021	0.009	0.004	0.23125	231.25	0.231		
		R3	0.018	0.006	0.002	0.20066	200.66	0.201		
	5% CO_2	R1	0.028	0.016	0.009	0.30258	302.58	0.303	0.31312	0.022258
		R2	0.027	0.011	0.005	0.29809	298.09	0.298		
		R3	0.031	0.015	0.009	0.33869	338.69	0.339		
	10%CO_2	R1	0.039	0.015	0.007	0.43177	431.77	0.432	0.391077	0.035716
		R2	0.033	0.013	0.006	0.36493	364.93	0.365		
		R3	0.034	0.013	0.006	0.37653	376.53	0.377		

Chlorophyll –a content *Chlorella vulgaris* UMACC 001(continue)

Day	treatment	sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
4	control (no aeration)	R1	0.019	0.005	0.000	0.21385	213.85	0.214	0.22941	0.014671
		R2	0.022	0.009	0.003	0.24299	242.99	0.243		
		R3	0.021	0.009	0.003	0.23139	231.39	0.231		
	0.04% CO_2	R1	0.055	0.021	0.012	0.60881	608.81	0.609	0.607983	0.014727
		R2	0.053	0.016	0.007	0.59286	592.86	0.593		
		R3	0.056	0.020	0.008	0.62228	622.28	0.622		
	5% CO_2	R1	0.062	0.024	0.011	0.68622	686.22	0.686	0.71628	0.032223
		R2	0.064	0.022	0.009	0.71232	712.32	0.712		
		R3	0.068	0.028	0.013	0.7503	750.3	0.750		
	10% CO_2	R1	0.058	0.021	0.008	0.64417	644.17	0.644	0.642627	0.019621
		R2	0.059	0.017	0.005	0.66143	661.43	0.661		
		R3	0.056	0.020	0.008	0.62228	622.28	0.622		
6	control (no aeration)	R1	0.042	0.010	0.002	0.47382	473.82	0.474	0.48542	0.0116
		R2	0.044	0.010	0.002	0.49702	497.02	0.497		
		R3	0.043	0.01	0.002	0.48542	485.42	0.485		
	0.04% CO_2	R1	0.079	0.028	0.013	0.8779	877.9	0.878	0.912123	0.056026
		R2	0.079	0.025	0.014	0.88169	881.69	0.882		
		R3	0.088	0.032	0.015	0.97678	976.78	0.977		
	5% CO_2	R1	0.129	0.047	0.020	1.43203	1432.03	1.432	1.457227	0.026567
		R2	0.136	0.066	0.044	1.48498	1484.98	1.485		
		R3	0.131	0.047	0.024	1.45467	1454.67	1.455		
	10% CO_2	R1	0.112	0.037	0.018	1.24821	1248.21	1.248	1.303637	0.048821
		R2	0.121	0.046	0.022	1.34026	1340.26	1.340		
		R3	0.119	0.042	0.021	1.32244	1322.44	1.322		

Chlorophyll –a content *Chlorella vulgaris* UMACC 001(continue)

Day	treatment	sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
8	control (no aeration)	R1	0.054	0.018	0.009	0.60156	601.56	0.602	0.638293	0.040245
		R2	0.057	0.021	0.012	0.63201	632.01	0.632		
		R3	0.061	0.019	0.010	0.68131	681.31	0.681		
	0.04% CO_2	R1	0.103	0.042	0.019	1.13712	1137.12	1.137	1.2522	0.208545
		R2	0.102	0.041	0.021	1.12655	1126.55	1.127		
		R3	0.135	0.053	0.026	1.49293	1492.93	1.493		
	5% CO_2	R1	0.149	0.059	0.029	1.64705	1647.05	1.647	2.61499	1.000649
		R2	0.331	0.140	0.077	3.64542	3645.42	3.645		
		R3	0.231	0.092	0.047	2.5525	2552.5	2.553		
	10% CO_2	R1	0.199	0.087	0.053	2.18701	2187.01	2.187	2.24515	0.168976
		R2	0.222	0.100	0.062	2.43552	2435.52	2.436		
		R3	0.192	0.082	0.049	2.11292	2112.92	2.113		
10	control (no aeration)	R1	0.080	0.030	0.013	0.88688	886.88	0.887	0.861607	0.052809
		R2	0.072	0.025	0.011	0.80091	800.91	0.801		
		R3	0.081	0.031	0.014	0.89703	897.03	0.897		
	0.04% CO_2	R1	0.360	0.148	0.077	3.97134	3971.34	3.971	3.535313	0.863106
		R2	0.230	0.092	0.045	2.54118	2541.18	2.541		
		R3	0.371	0.152	0.079	4.09342	4093.42	4.093		
	5% CO_2	R1	0.371	0.154	0.076	4.09122	4091.22	4.091	4.358533	0.325122
		R2	0.428	0.177	0.089	4.72047	4720.47	4.720		
		R3	0.387	0.163	0.084	4.26391	4263.91	4.264		
	10% CO_2	R1	0.313	0.151	0.080	3.42179	3421.79	3.422	4.00581	0.694136
		R2	0.434	0.189	0.097	4.77323	4773.23	4.773		
		R3	0.347	0.147	0.073	3.82241	3822.41	3.822		

ii) *Chlorella* UMACC 275

Day	treatment	sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
		R1	0.006	0.003	0.002	0.06539	65.39	0.06539		
		R2	0.009	0.004	0.002	0.09888	98.88	0.09888		
	Control (no aeration)	R3	0.007	0.003	0.002	0.07699	76.99	0.07699	0.080	±0.017
		R1	0.006	0.003	0.002	0.06539	65.39	0.06539		
		R2	0.009	0.004	0.002	0.09888	98.88	0.09888		
	0.04% CO_2	R3	0.007	0.003	0.002	0.07699	76.99	0.07699	0.080	±0.017
		R1	0.006	0.003	0.002	0.06539	65.39	0.06539		
		R2	0.009	0.004	0.002	0.09888	98.88	0.09888		
	5% CO_2	R3	0.007	0.003	0.002	0.07699	76.99	0.07699	0.080	±0.017
		R1	0.006	0.003	0.002	0.06539	65.39	0.06539		
		R2	0.009	0.004	0.002	0.09888	98.88	0.09888		
0	10% CO_2	R3	0.007	0.003	0.002	0.07699	76.99	0.07699	0.080	±0.017
		R1	0.009	0.003	0.002	0.10019	100.19	0.10019		
		R2	0.010	0.004	0.002	0.11048	110.48	0.11048		
	Control (no aeration)	R3	0.009	0.003	0.002	0.10019	100.19	0.10019	0.104	±0.006
		R1	0.019	0.007	0.004	0.21067	210.67	0.21067		
		R2	0.023	0.008	0.006	0.25548	255.48	0.25548		
	0.04% CO_2	R3	0.025	0.009	0.006	0.27737	277.37	0.27737	0.248	±0.034
		R1	0.030	0.012	0.008	0.33116	331.16	0.33116		
		R2	0.025	0.012	0.008	0.27316	273.16	0.27316		
	5% CO_2	R3	0.024	0.010	0.007	0.26432	264.32	0.26432	0.290	±0.036
		R1	0.035	0.014	0.009	0.38640	386.40	0.38640		
		R2	0.033	0.012	0.008	0.36596	365.96	0.36596		
2	10% CO_2	R3	0.038	0.017	0.009	0.41727	417.27	0.41727	0.390	±0.026

Chlorophyll –a content *Chlorella* UMACC 275 (continue)

Day	treatment	sample	665nm	645nm	630nm	Ca	Chla (mgm-3)	Chla (mg/L)	Mean	STDEV
4	control (no aeration)	R1	0.012	0.009	0.009	0.12615	126.15	0.126	0.123	0.006
		R2	0.011	0.008	0.009	0.11586	115.86	0.116		
		R3	0.012	0.009	0.008	0.12629	126.29	0.126		
	0.04% CO_2	R1	0.043	0.022	0.017	0.46760	467.60	0.468	0.453	0.036
		R2	0.044	0.022	0.017	0.47920	479.20	0.479		
		R3	0.038	0.021	0.016	0.41105	411.05	0.411		
	5% CO_2	R1	0.038	0.019	0.018	0.41339	413.39	0.413	0.425	0.028
		R2	0.042	0.021	0.019	0.45703	457.03	0.457		
		R3	0.037	0.017	0.008	0.40581	405.81	0.406		
	10% CO_2	R1	0.045	0.022	0.015	0.49108	491.08	0.491	0.529	0.047
		R2	0.047	0.022	0.016	0.51414	514.14	0.514		
		R3	0.053	0.024	0.019	0.58070	580.70	0.581		
6	control (no aeration)	R1	0.014	0.011	0.009	0.14673	146.73	0.147	0.146	0.011
		R2	0.013	0.011	0.009	0.13513	135.13	0.135		
		R3	0.015	0.012	0.008	0.15716	157.16	0.157		
	0.04% CO_2	R1	0.065	0.025	0.026	0.71761	717.61	0.718	0.795	0.098
		R2	0.069	0.026	0.026	0.7627	762.7	0.763		
		R3	0.082	0.032	0.029	0.90522	905.22	0.905		
	5% CO2	R1	0.083	0.029	0.021	0.92187	921.87	0.922	0.949	0.026
		R2	0.086	0.032	0.024	0.95232	952.32	0.952		
		R3	0.088	0.033	0.024	0.97421	974.21	0.974		
	10% CO2	R1	0.09	0.034	0.031	0.99512	995.12	0.995	1.017	0.022
		R2	0.092	0.036	0.033	1.01542	1015.42	1.015		
		R3	0.094	0.036	0.029	1.03918	1039.18	1.039		

Chlorophyll –a content *Chlorella* UMACC 275 (continue)

Day	treatment	sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
8	control (no aeration)	R1	0.037	0.009	0.005	0.41671	416.71	0.417	0.395	0.022
		R2	0.035	0.007	0.004	0.39627	396.27	0.396		
		R3	0.033	0.007	0.005	0.37293	372.93	0.373		
	0.04% CO_2	R1	0.105	0.039	0.019	1.16425	1164.25	1.164	1.042	0.221
		R2	0.071	0.026	0.021	0.7866	786.6	0.787		
		R3	0.106	0.039	0.019	1.17585	1175.85	1.176		
	5% CO_2	R1	0.183	0.073	0.039	2.02171	2021.71	2.022	2.048	0.099
		R2	0.195	0.075	0.038	2.15843	2158.43	2.158		
		R3	0.178	0.072	0.037	1.9653	1965.3	1.965		
	10% CO_2	R1	0.174	0.059	0.054	1.93355	1933.55	1.934	1.896	0.117
		R2	0.157	0.042	0.011	1.76464	1764.64	1.765		
		R3	0.178	0.055	0.020	1.98995	1989.95	1.990		
10	control (no aeration)	R1	0.045	0.010	0.005	0.5082	508.2	0.508	0.533	0.024
		R2	0.047	0.008	0.004	0.53416	534.16	0.534		
		R3	0.049	0.009	0.005	0.55591	555.91	0.556		
	0.04% CO_2	R1	0.131	0.052	0.027	1.4477	1447.7	1.448	1.810	0.411
		R2	0.204	0.079	0.041	2.25717	2257.17	2.257		
		R3	0.156	0.061	0.032	1.72521	1725.21	1.725		
	5% CO_2	R1	0.312	0.128	0.057	3.44354	3443.54	3.444	3.052	0.784
		R2	0.194	0.073	0.039	2.14931	2149.31	2.149		
		R3	0.323	0.134	0.063	3.56244	3562.44	3.562		
	10% CO_2	R1	0.280	0.111	0.057	3.09461	3094.61	3.095	3.603	0.473
		R2	0.365	0.147	0.076	4.03079	4030.79	4.031		
		R3	0.334	0.138	0.064	3.68466	3684.66	3.685		

iii) *Scenedesmus quadricauda* UMACC 041

Day	treatment	sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
0	Control (no aeration)	R1	0.043	0.012	0.003	0.48266	482.66	0.0483		
		R2	0.049	0.015	0.005	0.54805	548.05	0.0548		
		R3	0.054	0.017	0.006	0.60329	603.29	0.0603	0.0545	±0.0060
	0.04% CO_2	R1	0.043	0.012	0.003	0.48266	482.66	0.0483		
		R2	0.049	0.015	0.005	0.54805	548.05	0.0548		
		R3	0.054	0.017	0.006	0.60329	603.29	0.0603	0.0545	±0.0060
	5% CO_2	R1	0.043	0.012	0.003	0.48266	482.66	0.0483		
		R2	0.049	0.015	0.005	0.54805	548.05	0.0548		
		R3	0.054	0.017	0.006	0.60329	603.29	0.0603	0.0545	±0.0060
	10% CO_2	R1	0.043	0.012	0.003	0.48266	482.66	0.0483		
		R2	0.049	0.015	0.005	0.54805	548.05	0.0548		
		R3	0.054	0.017	0.006	0.60329	603.29	0.0603	0.0545	±0.0060
2	Control (no aeration)	R1	0.014	0.001	0.000	0.16109	161.09	0.1611		
		R2	0.013	0.001	0.000	0.14949	149.49	0.1495		
		R3	0.015	0.002	0.001	0.17124	171.24	0.1712	0.1606	±0.0109
	0.04% CO_2	R1	0.026	0.009	0.004	0.28925	289.25	0.2893		
		R2	0.028	0.012	0.009	0.30782	307.82	0.3078		
		R3	0.027	0.011	0.009	0.29753	297.53	0.2975	0.2982	±0.0093
	5% CO_2	R1	0.029	0.010	0.004	0.32274	322.74	0.32274		
		R2	0.028	0.012	0.004	0.30852	308.52	0.30852		
		R3	0.026	0.009	0.003	0.28939	289.39	0.28939	0.3069	±0.0167
	10% CO_2	R1	0.036	0.009	0.009	0.40455	404.55	0.40455		
		R2	0.038	0.012	0.007	0.4241	424.1	0.4241		
		R3	0.034	0.011	0.009	0.37873	378.73	0.37873	0.4025	±0.0228

Chlorophyll –a content *Scenedesmus quadricauda* UMACC 041(continue)

Day	treatment	sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
4	control (no aeration)	R1	0.021	0.008	0.002	0.2328	232.84	0.2328	0.2504	0.0242
		R2	0.022	0.011	0.003	0.2404	240.37	0.2404		
		R3	0.025	0.009	0.002	0.2779	277.93	0.2779		
	0.04% CO_2	R1	0.058	0.025	0.021	0.6371	637.11	0.6371	0.6212	0.0147
		R2	0.056	0.022	0.018	0.6183	618.26	0.6183		
		R3	0.055	0.021	0.016	0.6083	608.25	0.6083		
	5% CO_2	R1	0.098	0.039	0.025	1.08221	1082.21	1.0822	1.0741	0.0188
		R2	0.095	0.035	0.026	1.05251	1052.51	1.0525		
		R3	0.098	0.035	0.025	1.08745	1087.45	1.0875		
	10% CO_2	R1	0.078	0.027	0.027	0.86565	865.65	0.8657	0.8730	0.0337
		R2	0.082	0.029	0.025	0.90971	909.71	0.9097		
		R3	0.076	0.027	0.019	0.84357	843.57	0.8436		
6	control (no aeration)	R1	0.043	0.021	0.015	0.46919	469.19	0.4692	0.4984	0.0297
		R2	0.048	0.020	0.014	0.52864	528.64	0.5286		
		R3	0.045	0.017	0.016	0.49749	497.49	0.4975		
	0.04% CO_2	R1	0.092	0.038	0.021	1.01448	1014.48	1.0145	0.9499	0.0559
		R2	0.082	0.025	0.012	0.91677	916.77	0.9168		
		R3	0.083	0.032	0.017	0.9185	918.5	0.9185		
	5% CO_2	R1	0.159	0.065	0.035	1.75435	1754.35	1.7544	1.7450	0.0594
		R2	0.163	0.066	0.037	1.79916	1799.16	1.7992		
		R3	0.152	0.059	0.032	1.68143	1681.43	1.6814		
	10% CO_2	R1	0.132	0.047	0.021	1.46669	1466.69	1.4667	1.4800	0.0313
		R2	0.136	0.045	0.021	1.51571	1515.71	1.5157		
		R3	0.131	0.045	0.022	1.45757	1457.57	1.4576		

Chlorophyll –a content *Scenedesmus quadricauda* UMACC 041(continue)

Day	treatment	sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
8	Control (no aeration)	R1	0.063	0.024	0.011	0.69782	697.82	0.69782		
		R2	0.056	0.022	0.011	0.61924	619.24	0.61924		
		R3	0.061	0.023	0.011	0.67593	675.93	0.67593	0.6643	±0.0406
	0.04% CO_2	R1	0.090	0.030	0.011	1.00316	1003.16	1.00316		
		R2	0.099	0.033	0.012	1.10349	1103.49	1.10349		
		R3	0.119	0.041	0.016	1.32445	1324.45	1.32445	1.1437	±0.1644
	5% CO_2	R1	0.215	0.085	0.044	2.37649	2376.49	2.37649		
		R2	0.240	0.098	0.052	2.64834	2648.34	2.64834		
		R3	0.222	0.089	0.047	2.45203	2452.03	2.45203	2.4923	±0.1403
	10% CO_2	R1	0.189	0.079	0.045	2.08261	2082.61	2.08261		
		R2	0.184	0.072	0.037	2.0349	2034.9	2.0349		
		R3	0.231	0.091	0.047	2.55381	2553.81	2.55381	2.2238	±0.2868
10	Control (no aeration)	R1	0.065	0.019	0.005	0.72841	728.41	0.72841		
		R2	0.068	0.021	0.008	0.76017	760.17	0.76017		
		R3	0.069	0.022	0.008	0.77046	770.46	0.77046	0.7530	±0.0219
	0.04% CO_2	R1	0.187	0.074	0.038	2.06694	2066.94	2.06694		
		R2	0.139	0.054	0.028	1.53774	1537.74	1.53774		
		R3	0.125	0.049	0.025	1.38231	1382.31	1.38231	1.6623	±0.3589
	5% CO_2	R1	0.250	0.100	0.054	2.76144	2761.44	2.76144		
		R2	0.312	0.126	0.068	3.44462	3444.62	3.44462		
		R3	0.235	0.096	0.055	2.59254	2592.54	2.59254	2.9329	±0.4512
	10% CO_2	R1	0.276	0.103	0.049	3.05981	3059.81	3.05981		
		R2	0.204	0.074	0.035	2.26456	2264.56	2.26456		
		R3	0.164	0.057	0.025	1.82423	1824.23	1.82423	2.3829	±0.6262

iv) *Oocystis* UMACC 074

Day	treatment	sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
0	control (no aeration)	R1	0.005	0.002	0.001	0.055	55.240	0.055	0.044	0.012
		R2	0.003	0.002	0.001	0.032	32.040	0.032		
		R3	0.004	0.002	0.001	0.044	43.640	0.044		
	0.04% CO_2	R1	0.005	0.002	0.001	0.055	55.240	0.055	0.044	0.012
		R2	0.003	0.002	0.001	0.032	32.040	0.032		
		R3	0.004	0.002	0.001	0.044	43.640	0.044		
	5% CO_2	R1	0.005	0.002	0.001	0.055	55.240	0.055	0.044	0.012
		R2	0.003	0.002	0.001	0.032	32.040	0.032		
		R3	0.004	0.002	0.001	0.044	43.640	0.044		
	10% CO_2	R1	0.005	0.002	0.001	0.055	55.240	0.055	0.048	0.007
		R2	0.004	0.002	0.001	0.044	43.640	0.044		
		R3	0.004	0.002	0.001	0.044	43.640	0.044		
2	control (no aeration)	R1	0.006	0.001	0.000	0.068	68.290	0.068	0.072	0.006
		R2	0.007	0.002	0.001	0.078	78.440	0.078		
		R3	0.006	0.001	0.000	0.068	68.290	0.068		
	0.04% CO_2	R1	0.019	0.008	0.005	0.209	209.220	0.209	0.190	0.020
		R2	0.015	0.003	0.001	0.170	169.930	0.170		
		R3	0.017	0.005	0.001	0.191	190.510	0.191		
	5% CO_2	R1	0.018	0.004	0.001	0.203	203.420	0.203	0.227	0.049
		R2	0.017	0.003	0.001	0.193	193.130	0.193		
		R3	0.025	0.005	0.001	0.283	283.310	0.283		
	10%CO_2	R1	0.015	0.002	0.001	0.171	171.240	0.171	0.189	0.023
		R2	0.016	0.003	0.001	0.182	181.530	0.182		
		R3	0.019	0.004	0.001	0.215	215.020	0.215		

Chlorophyll –a content *Oocystis* UMACC 074 (continue)

Day	treatment	sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
4	control (no aeration)	R1	0.009	0.002	0.002	0.102	101.500	0.102	0.105	0.005
		R2	0.010	0.004	0.002	0.110	110.480	0.110		
		R3	0.009	0.002	0.001	0.102	101.640	0.102		
	0.04% CO_2	R1	0.036	0.015	0.008	0.397	396.830	0.397	0.328	0.078
		R2	0.031	0.011	0.008	0.344	344.070	0.344		
		R3	0.022	0.008	0.004	0.244	244.160	0.244		
	5% CO_2	R1	0.042	0.021	0.013	0.458	457.870	0.458	0.432	0.039
		R2	0.035	0.014	0.008	0.387	386.540	0.387		
		R3	0.041	0.018	0.007	0.451	451.040	0.451		
	10% CO_2	R1	0.035	0.014	0.008	0.387	386.540	0.387	0.353	0.033
		R2	0.032	0.013	0.006	0.353	353.330	0.353		
		R3	0.029	0.012	0.008	0.320	319.560	0.320		
6	control (no aeration)	R1	0.031	0.010	0.004	0.346	345.940	0.346	0.336	0.010
		R2	0.030	0.009	0.004	0.336	335.650	0.336		
		R3	0.029	0.008	0.003	0.326	325.500	0.326		
	0.04% CO_2	R1	0.046	0.019	0.006	0.508	507.870	0.508	0.500	0.032
		R2	0.048	0.021	0.009	0.528	528.030	0.528		
		R3	0.042	0.016	0.007	0.465	465.260	0.465		
	5% CO_2	R1	0.089	0.036	0.019	0.983	982.580	0.983	1.029	0.049
		R2	0.098	0.041	0.024	1.080	1079.730	1.080		
		R3	0.093	0.039	0.021	1.025	1024.770	1.025		
	10% CO_2	R1	0.089	0.035	0.015	0.984	984.450	0.984	0.998	0.023
		R2	0.089	0.035	0.015	0.984	984.450	0.984		
		R3	0.093	0.039	0.019	1.025	1025.050	1.025		

Chlorophyll –a content *Oocystis* UMACC 074 (continue)

Day	treatment	sample	665nm	645nm	630nm	Ca	Chla (mgm^{-3})	Chla (mg/L)	Mean	STDEV
8	control (no aeration)	R1	0.028	0.011	0.005	0.310	309.690	0.310	0.336	0.036
		R2	0.029	0.011	0.004	0.321	321.430	0.321		
		R3	0.034	0.013	0.008	0.376	376.250	0.376		
	0.04% CO_2	R1	0.078	0.033	0.015	0.859	859.470	0.859	0.805	0.050
		R2	0.072	0.028	0.013	0.797	796.700	0.797		
		R3	0.068	0.021	0.011	0.760	759.750	0.760		
	5% CO_2	R1	0.114	0.048	0.029	1.255	1255.460	1.255	1.292	0.055
		R2	0.123	0.051	0.033	1.355	1355.370	1.355		
		R3	0.115	0.049	0.031	1.265	1265.470	1.265		
	10% CO_2	R1	0.093	0.043	0.022	1.019	1019.390	1.019	1.208	0.189
		R2	0.109	0.041	0.025	1.207	1207.190	1.207		
		R3	0.127	0.054	0.037	1.397	1397.280	1.397		
10	control (no aeration)	R1	0.034	0.014	0.008	0.375	374.940	0.375	0.384	0.034
		R2	0.032	0.012	0.008	0.354	354.360	0.354		
		R3	0.038	0.014	0.009	0.421	421.200	0.421		
	0.04% CO_2	R1	0.094	0.035	0.020	1.042	1041.750	1.042	1.017	0.121
		R2	0.102	0.043	0.027	1.123	1123.090	1.123		
		R3	0.080	0.031	0.017	0.885	885.010	0.885		
	5% CO_2	R1	0.160	0.061	0.034	1.771	1771.330	1.771	1.800	0.256
		R2	0.187	0.072	0.041	2.069	2069.140	2.069		
		R3	0.141	0.055	0.030	1.559	1559.350	1.559		
	10% CO_2	R1	0.163	0.062	0.035	1.805	1804.680	1.805	1.882	0.083
		R2	0.178	0.068	0.039	1.970	1970.260	1.970		
		R3	0.169	0.065	0.036	1.870	1870.210	1.870		

d) Specific growth rates of selected microalgae at elevated levels of CO_2

i) *Chlorella vulgaris* UMACC 001

Treatment	RI	R2	R3	Mean	STDEV
Control (no aeration)	0.1300	0.1270	0.1270	0.1280	±0.0017
0.04% CO_2	0.2100	0.1810	0.2105	0.2005	±0.0169
5% CO_2	0.3730	0.3865	0.3525	0.3707	±0.0171
10% CO_2	0.3020	0.3395	0.3500	0.3305	±0.0252

ii) *Chlorella* UMACC 275

Treatment	RI	R2	R3	Mean	STDEV
Control (no aeration)	0.1350	0.1305	0.1080	0.1245	±0.0145
0.04% CO_2	0.1250	0.1420	0.2470	0.1713	±0.0661
5% CO_2	0.2545	0.2475	0.2840	0.2620	±0.0194
10% CO_2	0.3607	0.3127	0.3522	0.3419	±0.0256

iii) *Scenedesmus quadricauda* UMACC 041

Treatment	R1	R2	R3	Mean	STDEV
Control (no aeration)	0.1182	0.1441	0.1098	0.1240	±0.0179
0.04% CO_2	0.1887	0.1493	0.1551	0.1644	±0.0213
5% CO_2	0.3361	0.3733	0.2969	0.3354	±0.0382
10% CO_2	0.3005	0.3030	0.3070	0.3035	±0.0033

iv) *Oocystis* UMACC 074

Treatment	RI	R2	R3	Mean	STDEV
Control (no aeration)	0.1222	0.1126	0.1120	0.1156	±0.0057
0.04% CO_2	0.1755	0.1345	0.1475	0.1525	±0.0210
5% CO_2	0.2625	0.3465	0.2870	0.2987	±0.0432
10% CO_2	0.2985	0.3155	0.3525	0.3222	±0.0276

e) pH of culture medium

i) *Chlorella vulgaris* UMACC 001

Day	Sample	pH ($5\%CO_2$)	Mean	STDEV	pH ($10\%CO_2$)	Mean	STDEV
	R1	6.89			6.98		
	R2	6.90			6.94		
0	R3	6.87	6.89	±0.02	6.88	6.93	±0.05
1	R1	6.92			7.01		
	R2	6.91			6.88		
	R3	6.94	6.92	±0.02	7.13	7.01	±0.13
2	R1	6.89			7.02		
	R2	6.97			6.98		
	R3	7.01	6.96	±0.06	7.23	7.08	±0.13
3	R1	6.47			7.03		
	R2	7.13			6.74		
	R3	7.02	6.87	±0.35	6.88	6.88	±0.15
4	R1	7.32			6.87		
	R2	7.14			6.89		
	R3	7.21	7.22	±0.09	6.91	6.89	±0.02
5	R1	7.21			6.93		
	R2	7.45			7.04		
	R3	7.47	7.38	±0.14	7.11	7.03	±0.09
6	R1	7.21			7.23		
	R2	7.09			7.04		
	R3	7.01	7.10	±0.10	7.12	7.13	±0.10
7	R1	6.88			7.23		
	R2	7.55			7.31		
	R3	7.63	7.35	±0.41	7.29	7.28	±0.04
8	R1	7.21			7.45		
	R2	7.55			7.19		
	R3	7.63	7.46	±0.22	7.33	7.32	±0.13
9	R1	7.41			7.38		
	R2	7.99			6.86		
	R3	8.07	7.82	±0.36	6.89	7.04	±0.29
	R1	7.42			6.97		
	R2	7.67			6.87		
10	R3	7.43	7.51	±0.14	6.78	6.87	±0.10

ii) *Chlorella* UMACC 275

Day	Sample	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
0	R1	6.87	6.87	±0.01	6.89	6.86	±0.02
	R2	6.88			6.85		
	R3	6.86			6.85		
1	R1	6.89	6.91	±0.02	6.95	6.95	±0.02
	R2	6.93			6.93		
	R3	6.92			6.97		
2	R1	6.93	6.95	±0.02	7.02	7.08	±0.05
	R2	6.95			7.12		
	R3	6.97			7.09		
3	R1	6.84	6.86	±0.03	6.93	6.86	±0.09
	R2	6.85			6.88		
	R3	6.89			6.76		
4	R1	7.12	7.15	±0.07	6.87	6.90	±0.03
	R2	7.11			6.89		
	R3	7.23			6.93		
5	R1	7.22	7.28	±0.05	7.04	7.08	±0.04
	R2	7.32			7.08		
	R3	7.29			7.11		
6	R1	6.89	6.92	±0.04	7.25	7.28	±0.03
	R2	6.92			7.31		
	R3	6.96			7.29		
7	R1	7.07	7.12	±0.04	7.06	7.02	±0.05
	R2	7.13			7.03		
	R3	7.15			6.96		
8	R1	7.22	7.26	±0.05	7.14	7.09	±0.05
	R2	7.31			7.08		
	R3	7.26			7.04		
9	R1	7.33	7.32	±0.06	7.21	7.26	±0.05
	R2	7.38			7.25		
	R3	7.26			7.31		
10	R1	7.41	7.40	±0.06	7.33	7.43	±0.09
	R2	7.34			7.46		
	R3	7.46			7.51		

iii) *Scenedesmus quadricauda* UMACC 041

Day	Sample	pH (5% CO_2)	Mean	STDEV	pH (10% CO_2)	Mean	STDEV
0	R1	6.78	6.83	±0.050	6.89	6.92	±0.025
	R2	6.84			6.94		
	R3	6.88			6.92		
1	R1	6.98	6.81	±0.236	6.79	6.80	±0.036
	R2	6.54			6.77		
	R3	6.91			6.84		
2	R1	6.34	6.66	±0.315	7.13	7.01	±0.120
	R2	6.66			7.02		
	R3	6.97			6.89		
3	R1	6.56	6.74	±0.168	6.92	7.00	±0.104
	R2	6.78			6.97		
	R3	6.89			7.12		
4	R1	7.02	7.05	±0.049	7.11	7.18	±0.064
	R2	7.11			7.23		
	R3	7.03			7.21		
5	R1	6.74	6.71	±0.187	7.18	7.06	±0.161
	R2	6.88			7.13		
	R3	6.51			6.88		
6	R1	6.45	6.34	±0.110	6.93	7.02	±0.110
	R2	6.34			6.98		
	R3	6.23			7.14		
7	R1	7.04	7.13	±0.096	7.11	7.14	±0.030
	R2	7.11			7.14		
	R3	7.23			7.17		
8	R1	7.04	7.13	±0.095	6.97	6.95	±0.053
	R2	7.12			6.99		
	R3	7.23			6.89		
9	R1	7.31	7.35	±0.087	7.10	7.15	±0.046
	R2	7.29			7.16		
	R3	7.45			7.19		
10	R1	7.19	7.30	±0.098	7.21	7.17	±0.051
	R2	7.33			7.18		
	R3	7.38			7.11		

iv) *Oocystis* UMACC 074

Day	Sample	5% CO_2	Mean	STDEV	10% CO_2	Mean	STDEV
0	R1	6.93	6.94	±0.05	6.94	6.95	±0.02
	R2	6.89			6.95		
	R3	6.99			6.97		
1	R1	6.93	6.94	±0.02	6.85	6.86	±0.03
	R2	6.94			6.89		
	R3	6.96			6.83		
2	R1	7.02	7.05	±0.04	7.02	7.08	±0.13
	R2	7.05			6.98		
	R3	7.09			7.23		
3	R1	7.11	7.09	±0.06	7.03	6.88	±0.15
	R2	7.13			6.74		
	R3	7.02			6.88		
4	R1	6.89	6.93	±0.04	6.87	6.89	±0.02
	R2	6.92			6.89		
	R3	6.97			6.91		
5	R1	7.12	7.15	±0.07	6.93	7.03	±0.09
	R2	7.11			7.04		
	R3	7.23			7.11		
6	R1	7.21	7.17	±0.04	7.23	7.13	±0.10
	R2	7.18			7.04		
	R3	7.13			7.12		
7	R1	6.88	6.93	±0.05	7.32	7.28	±0.04
	R2	6.93			7.28		
	R3	6.98			7.24		
8	R1	7.14	7.19	±0.05	6.88	6.93	±0.05
	R2	7.18			6.93		
	R3	7.24			6.98		
9	R1	7.55	7.67	±0.12	7.14	7.19	±0.05
	R2	7.78			7.18		
	R3	7.69			7.24		
10	R1	7.48	7.48	±0.06	7.23	7.23	±0.03
	R2	7.54			7.26		
	R3	7.43			7.21		

e) Amount of Dissolved Carbon Dioxide at 5% CO_2 and 10% CO_2 i) *Chlorella vulgaris* UMACC 001

Day	Sample	pH (5% CO_2)	Amount of dissolved CO2 (mg/l)	Mean	STDEV	pH (10% CO_2)	Amount of dissolved CO_2 (mg/l)	Mean	STDEV
	R1	6.89	0.00000007			6.98	0.00000009		
	R2	6.90	0.00000007			6.94	0.00000008		
0	R3	6.87	0.00000006	0.00000007	±0.000000005	6.88	0.00000007	0.00000008	±0.000000014
	R1	6.92	0.00000008			7.01	0.00000010		
	R2	6.91	0.00000008			6.88	0.00000007		
1	R3	6.94	0.00000008	0.00000008	±0.000000004	7.13	0.00000012	0.00000010	±0.000000029
	R1	6.89	0.00000007			7.02	0.00000010		
	R2	6.97	0.00000009			6.98	0.00000009		
2	R3	7.01	0.00000010	0.00000009	±0.000000016	7.23	0.00000014	0.00000011	±0.000000024
	R1	6.47	0.00000014			7.03	0.00000011		
	R2	7.13	0.00000012			6.74	0.00000002		
3	R3	7.02	0.00000010	0.00000012	±0.000000019	6.88	0.00000007	0.00000006	±0.000000044
	R1	7.32	0.00000015			6.87	0.00000006		
	R2	7.14	0.00000013			6.89	0.00000007		
4	R3	7.21	0.00000014	0.00000014	±0.000000012	6.91	0.00000008	0.00000007	±0.000000006
	R1	7.21	0.00000014			6.93	0.00000008		
	R2	7.45	0.00000016			7.04	0.00000011		
5	R3	7.47	0.00000016	0.00000015	±0.000000016	7.11	0.00000012	0.00000010	±0.000000020
	R1	7.21	0.00000014			7.23	0.00000014		
	R2	7.09	0.00000012			7.04	0.00000011		
6	R3	7.01	0.00000010	0.00000012	±0.000000018	7.12	0.00000012	0.00000012	±0.000000016
	R1	6.88	0.00000007			7.23	0.00000014		
	R2	7.55	0.00000017			7.31	0.00000015		
7	R3	7.63	0.00000018	0.00000014	±0.000000061	7.29	0.00000015	0.00000015	±0.000000005
	R1	7.21	0.00000014			7.45	0.00000016		
	R2	7.55	0.00000017			7.19	0.00000013		
8	R3	7.63	0.00000018	0.00000016	±0.000000021	7.33	0.00000015	0.00000015	±0.000000015
	R1	7.41	0.00000016			7.38	0.00000016		
	R2	7.99	0.00000019			6.86	0.00000006		
9	R3	8.07	0.00000019	0.00000018	±0.000000017	6.89	0.00000007	0.00000010	±0.000000053
	R1	7.42	0.00000016			6.97	0.00000009		
	R2	7.67	0.00000018			6.87	0.00000006		
10	R3	7.43	0.00000016	0.00000017	±0.000000009	6.78	0.00000003	0.00000006	±0.000000029

ii) *Chlorella* UMACC 275

Day	Sample	pH (5% CO_2)	Amount of dissolved CO_2 (mg/l)	Mean	STDEV	pH (10% CO_2)	Amount of dissolved CO_2 (mg/l)	Mean	STDEV
	R1	6.87	0.00000006			6.89	0.00000007		
	R2	6.88	0.00000007			6.85	0.00000006		
0	R3	6.86	0.00000006	0.00000006	±0.00000000	6.85	0.00000006	0.00000006	±0.00000001
	R1	6.89	0.00000007			6.95	0.00000009		
	R2	6.93	0.00000008			6.93	0.00000008		
1	R3	6.92	0.00000008	0.00000008	±0.00000001	6.97	0.00000009	0.00000009	±0.00000001
	R1	6.93	0.00000008			7.02	0.00000010		
	R2	6.95	0.00000009			7.12	0.00000012		
2	R3	6.97	0.00000009	0.00000009	±0.00000001	7.09	0.00000012	0.00000011	±0.00000001
	R1	6.84	0.00000014			6.93	0.00000008		
	R2	6.85	0.00000006			6.88	0.00000007		
3	R3	6.89	0.00000007	0.00000009	±0.00000004	6.76	0.00000002	0.00000006	±0.00000003
	R1	7.12	0.00000012			6.87	0.00000006		
	R2	7.11	0.00000012			6.89	0.00000007		
4	R3	7.23	0.00000014	0.00000013	±0.00000001	6.93	0.00000008	0.00000007	±0.00000001
	R1	7.22	0.00000014			7.04	0.00000011		
	R2	7.32	0.00000015			7.08	0.00000012		
5	R3	7.29	0.00000015	0.00000015	±0.00000001	7.11	0.00000012	0.00000011	±0.00000001
	R1	6.89	0.00000007			7.25	0.00000014		
	R2	6.92	0.00000008			7.31	0.00000015		
6	R3	6.96	0.00000009	0.00000008	±0.00000001	7.29	0.00000015	0.00000015	±0.00000000
	R1	7.07	0.00000011			7.06	0.00000011		
	R2	7.13	0.00000012			7.03	0.00000011		
7	R3	7.15	0.00000013	0.00000012	±0.00000001	6.96	0.00000009	0.00000010	±0.00000001
	R1	7.22	0.00000014			7.14	0.00000013		
	R2	7.31	0.00000015			7.08	0.00000012		
8	R3	7.26	0.00000014	0.00000014	±0.00000001	7.04	0.00000011	0.00000012	±0.00000001
	R1	7.33	0.00000015			7.21	0.00000014		
	R2	7.38	0.00000016			7.25	0.00000014		
9	R3	7.26	0.00000014	0.00000015	±0.00000001	7.31	0.00000015	0.00000014	±0.00000001
	R1	7.41	0.00000016			7.33	0.00000015		
	R2	7.34	0.00000015			7.46	0.00000016		
10	R3	7.46	0.00000016	0.00000016	±0.00000001	7.51	0.00000017	0.00000016	±0.00000001

iii) *Scenedesmus quadricauda* UMACC 041

day	Sample	pH (5% CO_2)	Amount of dissolved CO_2 (mg/l)	Mean	STDEV	pH (10% CO_2)	Amount of dissolved CO_2 (mg/l)	Mean	STDEV
	R1	6.78	0.00000003			6.89	0.00000007		
	R2	6.84	0.00000005			6.94	0.00000008		
0	R3	6.88	0.00000007	0.00000005	±0.00000002	6.92	0.00000008	0.00000008	±0.00000001
	R1	6.98	0.00000009			6.79	0.00000004		
	R2	6.78	0.00000003			6.77	0.00000003		
1	R3	6.91	0.00000008	0.00000007	±0.00000003	6.84	0.00000005	0.00000004	±0.00000001
	R1	6.73	0.00000001			7.13	0.00000012		
	R2	6.79	0.00000004			7.02	0.00000010		
2	R3	6.80	0.00000004	0.00000003	±0.00000002	6.89	0.00000007	0.00000010	±0.00000003
	R1	6.83	0.00000005			6.92	0.00000008		
	R2	6.78	0.00000003			6.97	0.00000009		
3	R3	6.89	0.00000007	0.00000005	±0.00000002	7.12	0.00000012	0.00000010	±0.00000002
	R1	7.02	0.00000010			7.11	0.00000012		
	R2	7.11	0.00000012			7.23	0.00000014		
4	R3	7.03	0.00000011	0.00000011	±0.00000001	7.21	0.00000014	0.00000013	±0.00000001
	R1	6.74	0.00000002			7.18	0.00000013		
	R2	6.88	0.00000007			7.13	0.00000012		
5	R3	6.79	0.00000004	0.00000004	±0.00000003	6.88	0.00000007	0.00000011	±0.00000004
	R1	6.81	0.00000004			6.93	0.00000008		
	R2	6.83	0.00000005			6.98	0.00000009		
6	R3	6.78	0.00000003	0.00000004	±0.00000001	7.14	0.00000013	0.00000010	±0.00000002
	R1	7.04	0.00000011			7.11	0.00000012		
	R2	7.11	0.00000012			7.14	0.00000013		
7	R3	7.23	0.00000014	0.00000012	±0.00000002	7.17	0.00000013	0.00000013	±0.00000001
	R1	7.04	0.00000011			6.97	0.00000009		
	R2	7.12	0.00000012			6.99	0.00000010		
8	R3	7.23	0.00000014	0.00000012	±0.00000002	6.89	0.00000007	0.00000009	±0.00000001
	R1	7.31	0.00000015			7.10	0.00000012		
	R2	7.29	0.00000015			7.16	0.00000013		
9	R3	7.45	0.00000016	0.00000015	±0.00000001	7.19	0.00000013	0.00000013	±0.00000001
	R1	7.19	0.00000013			7.21	0.00000014		
	R2	7.33	0.00000015			7.18	0.00000013		
10	R3	7.38	0.00000016	0.00000015	±0.00000001	7.11	0.00000012	0.00000013	±0.00000001

iv) *Oocystis* UMACC 074

Day	Sample	pH (5% CO_2)	Amount of dissolved CO_2 (mg/l)	Mean	STDEV	pH (10% CO_2)	Amount of dissolved CO_2 (mg/l)	Mean	STDEV
	R1	6.93	0.00000008			6.94	0.00000008		
	R2	6.89	0.00000007			6.95	0.00000009		
0	R3	6.99	0.00000010	0.00000008	±0.000000013	6.97	0.00000009	0.00000009	±0.000000004
	R1	6.93	0.00000008			6.85	0.00000006		
	R2	6.94	0.00000008			6.89	0.00000007		
1	R3	6.96	0.00000009	0.00000008	±0.000000004	6.83	0.00000005	0.00000006	±0.000000010
	R1	7.02	0.00000010			7.02	0.00000010		
	R2	7.05	0.00000011			6.98	0.00000009		
2	R3	7.09	0.00000012	0.00000011	±0.000000007	7.23	0.00000014	0.00000011	±0.000000024
	R1	7.11	0.00000014			7.03	0.00000011		
	R2	7.13	0.00000012			6.74	0.00000002		
3	R3	7.02	0.00000010	0.00000012	±0.000000019	6.88	0.00000007	0.00000006	±0.000000044
	R1	6.89	0.00000007			6.87	0.00000006		
	R2	6.92	0.00000008			6.89	0.00000007		
4	R3	6.97	0.00000009	0.00000008	±0.000000011	6.91	0.00000008	0.00000007	±0.000000006
	R1	7.12	0.00000012			6.93	0.00000008		
	R2	7.11	0.00000012			7.04	0.00000011		
5	R3	7.23	0.00000014	0.00000013	±0.000000010	7.11	0.00000012	0.00000010	±0.000000020
	R1	7.21	0.00000014			7.23	0.00000014		
	R2	7.18	0.00000013			7.04	0.00000011		
6	R3	7.13	0.00000012	0.00000013	±0.000000006	7.12	0.00000012	0.00000012	±0.000000016
	R1	6.88	0.00000007			7.32	0.00000015		
	R2	6.93	0.00000008			7.28	0.00000015		
7	R3	6.98	0.00000009	0.00000008	±0.000000014	7.24	0.00000014	0.00000015	±0.000000005
	R1	7.14	0.00000013			6.88	0.00000007		
	R2	7.18	0.00000013			6.93	0.00000008		
8	R3	7.24	0.00000014	0.00000013	±0.000000007	6.98	0.00000009	0.00000008	±0.000000014
	R1	7.55	0.00000017			7.14	0.00000013		
	R2	7.78	0.00000018			7.18	0.00000013		
9	R3	7.69	0.00000018	0.00000018	±0.000000006	7.24	0.00000014	0.00000013	±0.000000007
	R1	7.48	0.00000017			7.23	0.00000014		
10	R2	7.54	0.00000017	0.00000017	±0.000000004	7.26	0.00000014	0.00000014	±0.000000003

APPENDIX H

Carotenoid Content of Selected Microalgae Strains Grown at Different Levels of Carbon Dioxide

a) *Chlorella vulgaris* UMACC 001

Day	treatment	sample	452nm	Carotenoid (µg/mL)	Mean	STDEV
	Control (no aeration)	R1	0.201	0.078		
		R2	0.201	0.078		
		R3	0.201	0.078	0.078	±0.000
	0.04% CO_2	R1	0.201	0.078		
		R2	0.201	0.078		
		R3	0.201	0.078	0.078	±0.000
	5% CO_2	R1	0.201	0.078		
		R2	0.201	0.078		
		R3	0.201	0.078	0.078	±0.000
	10% CO_2	R1	0.208	0.0800		
		R2	0.201	0.078		
0		R3	0.201	0.078	0.079	±0.001
	Control (no aeration)	R1	0.021	0.08106		
		R2	0.023	0.08878		
		R3	0.019	0.07334	0.081	±0.008
	0.04% CO_2	R1	0.023	0.08878		
		R2	0.032	0.12352		
		R3	0.025	0.0965	0.103	±0.018
	5% CO_2	R1	0.048	0.18528		
		R2	0.036	0.13896		
		R3	0.045	0.1737	0.166	±0.024
	10% CO_2	R1	0.048	0.18528		
		R2	0.042	0.16212		
2		R3	0.045	0.1737	0.174	±0.012
	Control (no aeration)	R1	0.028	0.10808		
		R2	0.041	0.15826		
		R3	0.041	0.15826	0.142	±0.029
	0.04% CO_2	R1	0.068	0.26248		
		R2	0.057	0.22002		
		R3	0.070	0.2702	0.251	±0.027
	5% CO_2	R1	0.083	0.32038		
		R2	0.079	0.30494		
		R3	0.099	0.38214	0.336	±0.041
	10% CO_2	R1	0.071	0.27406		
		R2	0.073	0.28178		
4		R3	0.069	0.26634	0.274	±0.008

Chlorella vulgaris UMACC 001 (continue)

Day	treatment	sample	452nm	Carotenoid (µg/mL)	Mean	STDEV
6	Control (no aeration)	R1	0.050	0.193		
		R2	0.049	0.18914		
		R3	0.048	0.18528	0.189	±0.004
	0.04% CO_2	R1	0.093	0.35898		
		R2	0.078	0.30108		
		R3	0.11	0.4246	0.362	±0.062
	5% CO_2	R1	0.132	0.50952		
		R2	0.172	0.66392		
		R3	0.149	0.57514	0.583	±0.077
	10% CO_2	R1	0.128	0.49408		
		R2	0.133	0.51338		
		R3	0.126	0.48636	0.498	±0.014
8	Control (no aeration)	R1	0.061	0.23546		
		R2	0.065	0.2509		
		R3	0.064	0.24704	0.244	±0.008
	0.04% CO_2	R1	0.131	0.50566		
		R2	0.126	0.48636		
		R3	0.168	0.64848	0.547	±0.089
	5% CO_2	R1	0.185	0.7141		
		R2	0.405	1.5633		
		R3	0.285	1.1001	1.126	±0.425
	10% CO_2	R1	0.232	0.89552		
		R2	0.271	1.04606		
		R3	0.154	0.59444	0.845	±0.230
10	Control (no aeration)	R1	0.105	0.4053		
		R2	0.093	0.35898		
		R3	0.097	0.37442	0.380	±0.024
	0.04% CO_2	R1	0.343	1.32398		
		R2	0.29	1.1194		
		R3	0.357	1.37802	1.274	±0.136
	5% CO_2	R1	0.539	2.08054		
		R2	0.536	2.06896		
		R3	0.485	1.8721	2.007	±0.117
	10% CO_2	R1	0.477	1.84122		
		R2	0.537	2.07282		
		R3	0.438	1.69068	1.868	±0.192

b) *Chlorella* UMACC 275

Day	treatment	sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
0	Control (no aeration)	R1	0.009	0.03474	0.035	±0.000
		R2	0.009	0.03474		
		R3	0.009	0.03474		
	0.04% CO_2	R1	0.009	0.03474	0.035	±0.000
		R2	0.009	0.03474		
		R3	0.009	0.03474		
	5% CO_2	R1	0.009	0.03474	0.035	±0.000
		R2	0.009	0.03474		
		R3	0.009	0.03474		
	10% CO_2	R1	0.009	0.03474	0.035	±0.000
		R2	0.009	0.03474		
		R3	0.009	0.03474		
2	Control (no aeration)	R1	0.010	0.03860	0.036	±0.002
		R2	0.009	0.03474		
		R3	0.009	0.03474		
	0.04% CO_2	R1	0.017	0.06562	0.095	±0.026
		R2	0.029	0.11194		
		R3	0.028	0.10808		
	5% CO_2	R1	0.037	0.14282	0.127	±0.013
		R2	0.031	0.11966		
		R3	0.031	0.11966		
	10% CO_2	R1	0.041	0.15826	0.152	±0.006
		R2	0.039	0.15054		
		R3	0.038	0.14668		
4	Control (no aeration)	R1	0.014	0.05404	0.051	±0.002
		R2	0.013	0.05018		
		R3	0.013	0.05018		
	0.04% CO_2	R1	0.048	0.18528	0.174	±0.010
		R2	0.044	0.16984		
		R3	0.043	0.16598		
	5% CO_2	R1	0.047	0.18142	0.185	±0.004
		R2	0.048	0.18528		
		R3	0.049	0.18914		
	10% CO_2	R1	0.054	0.20844	0.207	±0.002
		R2	0.054	0.20844		
		R3	0.053	0.20458		

Chlorella UMACC 275(continue)

Day	treatment	sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
6	Control (no aeration)	R1	0.014	0.05404		
		R2	0.014	0.05404		
		R3	0.013	0.05018	0.053	±0.002
	0.04% CO_2	R1	0.116	0.44776		
		R2	0.080	0.3088		
		R3	0.077	0.29722	0.351	±0.084
	5% CO_2	R1	0.102	0.39372		
		R2	0.116	0.44776		
		R3	0.121	0.46706	0.436	±0.038
	10% CO_2	R1	0.153	0.59058		
		R2	0.155	0.5983		
		R3	0.143	0.55198	0.580	±0.025
8	Control (no aeration)	R1	0.013	0.05018		
		R2	0.019	0.07334		
		R3	0.021	0.08106	0.068	±0.016
	0.04% CO_2	R1	0.098	0.37828		
		R2	0.120	0.4632		
		R3	0.081	0.31266	0.385	±0.075
	5% CO_2	R1	0.207	0.79902		
		R2	0.220	0.8492		
		R3	0.211	0.81446	0.821	±0.026
	10% CO_2	R1	0.325	1.2545		
		R2	0.184	0.71024		
		R3	0.245	0.9457	0.970	±0.273
10	Control (no aeration)	R1	0.024	0.0926		
		R2	0.035	0.1351		
		R3	0.033	0.1274	0.118	±0.023
	0.04% CO_2	R1	0.147	0.5674		
		R2	0.229	0.8839		
		R3	0.189	0.7295	0.727	±0.158
	5% CO_2	R1	0.334	1.2892		
		R2	0.213	0.8222		
		R3	0.278	1.0731	1.062	±0.234
	10% CO_2	R1	0.315	1.2159		
		R2	0.410	1.5826		
		R3	0.325	1.2545	1.351	±0.201

c) *Scenedesmus quadricauda* UMACC 041

Day	treatment	sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
		R1	0.007	0.0270		
		R2	0.007	0.0270		
	Control (no aeration)	R3	0.007	0.0270	0.0270	±0.000
		R1	0.007	0.0270		
		R2	0.007	0.0270		
	0.04%CO_2	R3	0.007	0.0270	0.0270	±0.000
		R1	0.007	0.0270		
		R2	0.007	0.0270		
	5% CO_2	R3	0.007	0.0270	0.0270	±0.000
		R1	0.007	0.0270		
		R2	0.007	0.0270		
0	10% CO_2	R3	0.007	0.0270	0.0270	±0.000
		R1	0.008	0.0309		
		R2	0.018	0.0695		
	Control (no aeration)	R3	0.011	0.0425	0.0476	±0.020
		R1	0.025	0.0965		
		R2	0.020	0.0772		
	0.04%CO_2	R3	0.037	0.1428	0.1055	±0.034
		R1	0.038	0.1467		
		R2	0.032	0.1235		
	5% CO_2	R3	0.034	0.1312	0.1338	±0.012
		R1	0.033	0.1274		
		R2	0.029	0.1119		
2	10% CO_2	R3	0.026	0.1004	0.1132	±0.014
		R1	0.047	0.1814		
		R2	0.052	0.2007		
	Control (no aeration)	R3	0.045	0.1737	0.1853	±0.014
		R1	0.102	0.3937		
		R2	0.109	0.4207		
	0.04%CO_2	R3	0.092	0.3551	0.3899	±0.033
		R1	0.159	0.6137		
		R2	0.169	0.6523		
	5% CO_2	R3	0.168	0.6485	0.6382	±0.021
		R1	0.093	0.3590		
		R2	0.093	0.3590		
4	10% CO_2	R3	0.120	0.4632	0.3937	±0.060

Scenedesmus quadricauda UMACC 041(continue)

Day	treatment	sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
6	Control (no aeration)	R1	0.058	0.2239		
		R2	0.044	0.1698		
		R3	0.045	0.1737	0.1891	±0.030
	0.04%CO_2	R1	0.114	0.4400		
		R2	0.082	0.3165		
		R3	0.112	0.4323	0.3963	±0.069
	5% CO_2	R1	0.202	0.7797		
		R2	0.221	0.8531		
		R3	0.211	0.8145	0.8157	±0.037
	10% CO_2	R1	0.157	0.6060		
		R2	0.155	0.5983		
		R3	0.156	0.6022	0.6022	±0.004
8	Control (no aeration)	R1	0.081	0.3127		
		R2	0.074	0.2856		
		R3	0.079	0.3049	0.3011	±0.014
	0.04%CO_2	R1	0.113	0.4362		
		R2	0.151	0.5829		
		R3	0.125	0.4825	0.5005	±0.075
	5% CO_2	R1	0.296	1.1426		
		R2	0.332	1.2815		
		R3	0.308	1.1889	1.2043	±0.071
	10% CO_2	R1	0.250	0.9650		
		R2	0.244	0.9418		
		R3	0.240	0.9264	0.9444	±0.019
10	Control (no aeration)	R1	0.082	0.3165		
		R2	0.086	0.3320		
		R3	0.091	0.3513	0.3332	±0.017
	0.04%CO_2	R1	0.226	0.8724		
		R2	0.184	0.7102		
		R3	0.164	0.6330	0.7385	±0.122
	5% CO_2	R1	0.322	1.2429		
		R2	0.403	1.5556		
		R3	0.325	1.2545	1.3510	±0.177
	10% CO_2	R1	0.216	0.8338		
		R2	0.352	1.3587		
		R3	0.268	1.0345	1.0757	±0.265

d) *Oocystis* UMACC 074

Day	treatment	sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
		R1	0.007	0.0270		
	Control (no	R2	0.005	0.0193		
	aeration)	R3	0.005	0.0193	0.022	±0.004
		R1	0.007	0.0270		
		R2	0.005	0.0193		
	0.04%CO_2	R3	0.005	0.0193	0.022	±0.004
		R1	0.007	0.0270		
		R2	0.005	0.0193		
	5% CO_2	R3	0.005	0.0193	0.022	±0.004
		R1	0.007	0.0270		
		R2	0.005	0.0193		
0	10% CO_2	R3	0.006	0.0232	0.023	±0.004
		R1	0.019	0.0733		
	Control (no	R2	0.018	0.0695		
	aeration)	R3	0.016	0.0618	0.068	±0.006
		R1	0.024	0.0926		
		R2	0.016	0.0618		
	0.04%CO_2	R3	0.023	0.0888	0.081	±0.017
		R1	0.026	0.1004		
		R2	0.028	0.1081		
	5% CO_2	R3	0.026	0.1004	0.103	±0.004
		R1	0.024	0.0926		
		R2	0.025	0.0965		
2	10% CO_2	R3	0.022	0.0849	0.091	±0.006
		R1	0.021	0.0811		
	Control (no	R2	0.021	0.0811		
	aeration)	R3	0.022	0.0849	0.082	±0.002
		R1	0.053	0.2046		
		R2	0.045	0.1737		
	0.04%CO_2	R3	0.031	0.1197	0.166	±0.043
		R1	0.067	0.2586		
		R2	0.048	0.1853		
	5% CO_2	R3	0.078	0.3011	0.248	±0.059
		R1	0.067	0.2586		
		R2	0.056	0.2162		
4	10% CO_2	R3	0.069	0.2663	0.247	±0.027

Oocystis UMACC 074 (continue)

Day	treatment	sample	452nm	Carotenoid (ug/mL)	Mean	STDEV
	Control (no aeration)	R1	0.028	0.1081		
		R2	0.023	0.0888		
		R3	0.031	0.1197	0.106	±0.016
		R1	0.067	0.2586		
		R2	0.056	0.2162		
	0.04% CO_2	R3	0.071	0.2741	0.250	±0.030
		R1	0.093	0.3590		
		R2	0.089	0.3435		
	5% CO_2	R3	0.099	0.3821	0.362	±0.019
		R1	0.071	0.2741		
		R2	0.083	0.3204		
6	10% CO_2	R3	0.094	0.3628	0.319	±0.044
	Control (no aeration)	R1	0.036	0.1390		
		R2	0.029	0.1119		
		R3	0.039	0.1505	0.134	±0.020
		R1	0.089	0.3435		
		R2	0.095	0.3667		
	0.04% CO_2	R3	0.078	0.3011	0.337	±0.033
		R1	0.132	0.5095		
		R2	0.154	0.5944		
	5% CO_2	R3	0.129	0.4979	0.534	±0.053
		R1	0.119	0.4593		
		R2	0.136	0.5250		
8	10% CO_2	R3	0.159	0.6137	0.533	±0.077
	Control (no aeration)	R1	0.043	0.1660		
		R2	0.041	0.1583		
		R3	0.044	0.1698	0.165	±0.006
		R1	0.119	0.4593		
		R2	0.132	0.5095		
	0.04% CO_2	R3	0.103	0.3976	0.455	±0.056
		R1	0.199	0.7681		
		R2	0.242	0.9341		
	5% CO_2	R3	0.177	0.6832	0.795	±0.128
		R1	0.167	0.6446		
		R2	0.189	0.7295		
10	10% CO_2	R3	0.234	0.9032	0.759	±0.132

APPENDIX I

Biomass Production of Selected Microalgae at Different Levels of Carbon Dioxide on Day 0 and Day 10

a) *Chlorella vulgaris* UMACC 001

Day	treatment	Sample	Pre weight (g) [A]	Post weight (g) [B]	[B] - [A] (g)	dry weight (mg)	biomass(mg/l)	Mean	STDEV
0	Control (no aeration)	R1	0.0890	0.0927	0.0037	3.700	74.000		
		R2	0.0869	0.0907	0.0038	3.800	76.000		
		R3	0.0867	0.0902	0.0035	3.500	70.000	73.333	±3.055
	0.04% CO_2	R1	0.0890	0.0927	0.0037	3.700	74.000		
		R2	0.0869	0.0907	0.0038	3.800	76.000		
		R3	0.0867	0.0902	0.0035	3.500	70.000	73.333	±3.055
	5% CO_2	R1	0.0890	0.0927	0.0037	3.700	74.000		
		R2	0.0869	0.0907	0.0038	3.800	76.000		
		R3	0.0867	0.0902	0.0035	3.500	70.000	73.333	±3.055
	10% CO_2	R1	0.0863	0.0899	0.0036	3.600	72.000		
		R2	0.0868	0.0905	0.0037	3.700	74.000		
		R3	0.0869	0.0904	0.0035	3.500	70.000	72.000	±2.000
10	Control (no aeration)	R1	0.0832	0.0855	0.0023	2.300	46.000		
		R2	0.0861	0.0879	0.0018	1.800	36.000		
		R3	0.0833	0.0854	0.0021	2.100	42.000	41.333	±5.033
	0.04% CO_2	R1	0.0863	0.0964	0.0101	10.100	202.000		
		R2	0.0864	0.0939	0.0075	7.500	150.000		
		R3	0.0878	0.0971	0.0093	9.300	186.000	179.333	±26.633
	5% CO_2	R1	0.0827	0.0956	0.0129	12.900	258.000		
		R2	0.0854	0.1001	0.0147	14.700	294.000		
		R3	0.0871	0.0998	0.0127	12.700	254.000	268.667	±22.030
	10% CO_2	R1	0.087	0.0994	0.0124	12.400	248.000		
		R2	0.0865	0.0985	0.0120	12.000	240.000		
		R3	0.0867	0.0977	0.0110	11.000	220.000	236.000	±14.422

b) *Chlorella* UMACC 275

Day	treatment	Sample	Pre weight (g) [A]	Post weight (g) [B]	[B] - [A] (g)	dry weight (mg)	biomass(mg/l)	Mean	STDEV
	Control (no aeration)	R1	0.0876	0.0908	0.0032	3.200	64.00		
		R2	0.0875	0.0902	0.0027	2.700	54.00		
		R3	0.0863	0.0897	0.0034	3.400	68.00	62.00	±7.211
	0.04% CO_2	R1	0.0876	0.0908	0.0032	3.200	64.00		
		R2	0.0875	0.0902	0.0027	2.700	54.00		
		R3	0.0863	0.0897	0.0034	3.400	68.00	62.00	±7.211
	5% CO_2	R1	0.0876	0.0908	0.0032	3.200	64.00		
		R2	0.0875	0.0902	0.0027	2.700	54.00		
		R3	0.0863	0.0897	0.0034	3.400	68.00	62.00	±7.211
	10% CO_2	R1	0.0874	0.0902	0.0028	2.800	56.00		
		R2	0.0864	0.0894	0.0030	3.000	60.00		
0		R3	0.0870	0.0903	0.0033	3.300	66.00	60.67	±5.033
	Control (no aeration)	R1	0.0867	0.0882	0.0015	1.500	30.00		
		R2	0.0866	0.0882	0.0016	1.600	32.00		
		R3	0.0871	0.0884	0.0013	1.300	26.00	29.33	±3.055
	0.04% CO_2	R1	0.0879	0.0919	0.0040	4.000	80.00		
		R2	0.0862	0.0895	0.0033	3.300	66.00		
		R3	0.0865	0.0903	0.0038	3.800	76.00	74.00	±7.211
	5% CO_2	R1	0.0865	0.0924	0.0059	5.900	118.00		
		R2	0.0874	0.0920	0.0046	4.600	92.00		
		R3	0.0873	0.0934	0.0061	6.100	122.00	110.67	±16.289
	10% CO_2	R1	0.0867	0.0935	0.0068	6.800	136.00		
		R2	0.0865	0.0939	0.0074	7.400	148.00		
10		R3	0.0854	0.0931	0.0077	7.700	154.00	146.00	±9.165

c) *Scenedesmus quadricauda* UMACC 041

Day	treatment	Sample	Pre weight (g) [A]	Post weight (g) [B]	[B] - [A] (g)	dry weight (mg)	biomass(mg/l)	Mean	STDEV
0	control (no aeration)	R1	0.0875	0.0919	0.0044	4.4000	88.0000	86.000	±11.136
		R2	0.0868	0.0916	0.0048	4.8000	96.0000		
		R3	0.0868	0.0905	0.0037	3.7000	74.0000		
	0.04% CO_2	R1	0.0875	0.0919	0.0044	4.4000	88.0000	86.000	±11.136
		R2	0.0868	0.0916	0.0048	4.8000	96.0000		
		R3	0.0868	0.0905	0.0037	3.7000	74.0000		
	5% CO_2	R1	0.0875	0.0919	0.0044	4.4000	88.0000	86.000	±11.136
		R2	0.0868	0.0916	0.0048	4.8000	96.0000		
		R3	0.0868	0.0905	0.0037	3.7000	74.0000		
	10% CO_2	R1	0.0875	0.0919	0.0044	4.4000	88.0000	86.000	±11.136
		R2	0.0868	0.0916	0.0048	4.8000	96.0000		
		R3	0.0868	0.0905	0.0037	3.7000	74.0000		
10	control (no aeration)	R1	0.0867	0.0891	0.0024	2.4000	48.0000	52.000	±5.292
		R2	0.0866	0.0895	0.0029	2.9000	58.0000		
		R3	0.0868	0.0893	0.0025	2.5000	50.0000		
	0.04% CO_2	R1	0.0886	0.0970	0.0084	8.4000	168.0000	155.333	±20.232
		R2	0.0857	0.0940	0.0083	8.3000	166.0000		
		R3	0.0883	0.0949	0.0066	6.6000	132.0000		
	5% CO_2	R1	0.0890	0.0988	0.0098	9.8000	196.0000	257.333	±56.757
		R2	0.0876	0.1030	0.0154	15.4000	308.0000		
		R3	0.0866	0.1000	0.0134	13.4000	268.0000		
	10% CO_2	R1	0.0871	0.0971	0.0100	10.0000	200.0000	196.000	±6.928
		R2	0.0867	0.0961	0.0094	9.4000	188.0000		
		R3	0.0868	0.0968	0.0100	10.0000	200.0000		

d) *Oocystis* UMACC 074

Day	treatment	Sample	Pre weight (g) [A]	Post weight (g) [B]	[B] - [A] (g)	dry weight (mg)	biomass(mg/l)	Mean	STDEV
0	Control (no aeration)	R1	0.0873	0.0937	0.0064	6.4	128.00	126.00	±2.00
		R2	0.0866	0.0929	0.0063	6.3	126.00		
		R3	0.0867	0.0929	0.0062	6.2	124.00		
	0.04% CO_2	R1	0.0873	0.0937	0.0064	6.4	128.00	126.00	±2.00
		R2	0.0866	0.0929	0.0063	6.3	126.00		
		R3	0.0867	0.0929	0.0062	6.2	124.00		
	5% CO_2	R1	0.0873	0.0937	0.0064	6.4	128.00	126.00	±2.00
		R2	0.0866	0.0929	0.0063	6.3	126.00		
		R3	0.0867	0.0929	0.0062	6.2	124.00		
	10% CO_2	R1	0.0873	0.0937	0.0064	6.4	128.00	126.00	±2.00
		R2	0.0866	0.0929	0.0063	6.3	126.00		
		R3	0.0867	0.0929	0.0062	6.2	124.00		
10	control (no aeration)	R1	0.0855	0.0879	0.0024	2.4	48.00	44.00	±10.58
		R2	0.0867	0.0883	0.0016	1.6	32.00		
		R3	0.0859	0.0885	0.0026	2.6	52.00		
	0.04% CO_2	R1	0.0870	0.0928	0.0058	5.8	116.00	111.33	±19.43
		R2	0.0871	0.0916	0.0045	4.5	90.00		
		R3	0.0856	0.0920	0.0064	6.4	128.00		
	5% CO_2	R1	0.0867	0.0947	0.0080	8.0	160.00	170.00	±11.14
		R2	0.0862	0.0953	0.0091	9.1	182.00		
		R3	0.0870	0.0954	0.0084	8.4	168.00		
	10% CO_2	R1	0.0868	0.0938	0.0070	7.0	140.00	151.33	±13.32
		R2	0.0862	0.0936	0.0074	7.4	148.00		
		R3	0.0858	0.0941	0.0083	8.3	166.00		

APPENDIX J

Biochemical Composition of Selected Microalgae Strains at Different Levels of Carbon Dioxide on Day 0 and Day 10

a) Protein Content

i) *Chlorella vulgaris* UMACC 001

Day	Treatment	Sample	595nm	protein in 100 µg (µg)	protein in 10 ml (µg)	protein in 10 ml (g)	Dry weight (g)	protein (g)/ dry weight(g)	% protein (g)/ dry weight (g)	%Mean protein	STDEV
		R1	0.173	22.032	2203.175	0.00220	0.0037	0.5954526	59.545		
	Control (no	R2	0.176	22.508	2250.794	0.00225	0.0037	0.6083226	60.832		
	aeration)	R3	0.175	22.349	2234.921	0.00223	0.0037	0.6040326	60.403	60.260	±0.655
		R1	0.173	22.032	2203.175	0.00220	0.0037	0.5954526	59.545		
		R2	0.176	22.508	2250.794	0.00225	0.0037	0.6083226	60.832		
	0.04% CO_2	R3	0.175	22.349	2234.921	0.00223	0.0037	0.6040326	60.403	60.260	±0.655
		R1	0.173	22.032	2203.175	0.00220	0.0037	0.5954526	59.545		
		R2	0.176	22.508	2250.794	0.00225	0.0037	0.6083226	60.832		
	5% CO_2	R3	0.175	22.349	2234.921	0.00223	0.0037	0.6040326	60.403	60.260	±0.655
		R1	0.174	22.190	2219.048	0.00222	0.0036	0.6164021	61.640		
		R2	0.175	22.349	2234.921	0.00223	0.0036	0.6208113	62.081		
0	10% CO_2	R3	0.175	22.349	2234.921	0.00223	0.0036	0.6208113	62.081	61.934	±0.255
		R1	0.044	1.556	155.556	0.00016	0.0021	0.0740741	7.407		
	Control (no	R2	0.046	1.873	187.302	0.00019	0.0021	0.0891912	8.919		
	aeration)	R3	0.056	3.460	346.032	0.00035	0.0021	0.164777	16.478	10.935	±4.859
		R1	0.277	38.540	3853.968	0.00385	0.0090	0.4282187	42.822		
		R2	0.343	49.016	4901.587	0.00490	0.0090	0.5446208	54.462		
	0.04% CO_2	R3	0.338	48.222	4822.222	0.00482	0.0090	0.5358025	53.580	50.288	±6.481
		R1	0.553	82.349	8234.921	0.00823	0.0134	0.6145463	61.455		
		R2	0.557	82.984	8298.413	0.00830	0.0134	0.6192845	61.928		
	5% CO_2	R3	0.498	73.619	7361.905	0.00736	0.0134	0.5493959	54.940	59.441	±3.905
		R1	0.320	45.365	4536.508	0.00454	0.0118	0.3844498	38.445		
		R2	0.288	40.286	4028.571	0.00403	0.0118	0.3414044	34.140		
10	10% CO_2	R3	0.354	50.762	5076.190	0.00508	0.0118	0.4301856	43.019	38.535	±4.440

ii) *Chlorella* UMACC 275

Day	Treatment	Sample	595nm	protein in 100 ug (ug)	protein in 10 ml (ug)	protein in 10 ml (g)	Dry weight (g)	protein (g)/ dry weight(g)	% protein (g)/ dry weight (g)	%Mean protein	STDEV
		R1	0.127	14.730	1473.016	0.001473016	0.0031	0.475	47.517		
	Control (no aeration)	R2	0.128	14.889	1488.889	0.001488889	0.0031	0.480	48.029		
		R3	0.129	15.048	1504.762	0.001504762	0.0031	0.485	48.541	48.03	±0.512
		R1	0.127	14.730	1473.016	0.001473016	0.0031	0.475	47.517		
		R2	0.128	14.889	1488.889	0.001488889	0.0031	0.480	48.029		
	0.04% CO_2	R3	0.129	15.048	1504.762	0.001504762	0.0031	0.485	48.541	48.03	±0.512
		R1	0.127	14.730	1473.016	0.001473016	0.0031	0.475	47.517		
		R2	0.128	14.889	1488.889	0.001488889	0.0031	0.480	48.029		
	5% CO_2	R3	0.129	15.048	1504.762	0.001504762	0.0031	0.485	48.541	48.03	±0.512
		R1	0.127	14.730	1473.016	0.001473016	0.0031	0.475	47.517		
		R2	0.128	14.889	1488.889	0.001488889	0.0031	0.480	48.029		
0	10% CO_2	R3	0.129	15.048	1504.762	0.001504762	0.0031	0.485	48.541	48.03	±0.512
		R1	0.078	6.952	695.238	0.000695238	0.0015	0.463	46.35		
	Control (no aeration)	R2	0.069	5.524	552.381	0.000552381	0.0015	0.368	36.83		
		R3	0.085	8.063	806.349	0.000806	0.0015	0.538	53.76	45.64	±8.488
		R1	0.144	17.429	1742.857	0.001742857	0.0037	0.471	47.10		
		R2	0.143	17.270	1726.984	0.001726984	0.0037	0.467	46.68		
	0.04% CO_2	R3	0.179	22.984	2298.413	0.002298413	0.0037	0.621	62.12	51.97	±8.795
		R1	0.272	37.746	3774.603	0.003774603	0.0055	0.686	68.63		
		R2	0.249	34.095	3409.524	0.003409524	0.0055	0.620	61.99		
	5% CO_2	R3	0.247	33.778	3377.778	0.003377778	0.0055	0.614	61.41	64.01	±4.009
		R1	0.286	39.968	3996.825	0.003996825	0.0073	0.548	54.75		
		R2	0.269	37.270	3726.984	0.003726984	0.0073	0.511	51.05		
10	10% CO_2	R3	0.287	40.127	4012.698	0.004012698	0.0073	0.550	54.97	53.59	±2.200

iii) *Scenedesmus quadricauda* UMACC 041

Day	Treatment	Sample	595nm	protein in 100 ug (ug)	protein in 10 ml (ug)	protein in 10 ml (g)	Dry weight (g)	protein (g)/ dry weight(g)	% protein (g)/ dry weight (g)	%Mean protein	STDEV
0	Control (no aeration)	R1	0.171	21.714	2171.429	0.002171	0.0043	0.50498	50.498		
		R2	0.189	24.571	2457.143	0.002457	0.0043	0.57143	57.143		
		R3	0.178	22.825	2282.540	0.002283	0.0043	0.53082	53.082	53.575	±3.349
	0.04%CO_2	R1	0.201	26.476	2647.619	0.002648	0.0043	0.61573	61.573		
		R2	0.189	24.571	2457.143	0.002457	0.0043	0.57143	57.143		
		R3	0.178	22.825	2282.540	0.002283	0.0043	0.53082	53.082	57.266	±4.246
	5% CO_2	R1	0.201	26.476	2647.619	0.002648	0.0043	0.61573	61.573		
		R2	0.189	24.571	2457.143	0.002457	0.0043	0.57143	57.143		
		R3	0.178	22.825	2282.540	0.002283	0.0043	0.53082	53.082	57.266	±4.246
	10% CO_2	R1	0.201	26.476	2647.619	0.002648	0.0043	0.61573	61.573		
		R2	0.189	24.571	2457.143	0.002457	0.0043	0.57143	57.143		
		R3	0.178	22.825	2282.540	0.002283	0.0043	0.53082	53.082	57.266	±4.246
10	Control (no aeration)	R1	0.072	6.000	600.000	0.000600	0.0026	0.23077	23.077		
		R2	0.062	4.413	441.270	0.000441	0.0026	0.16972	16.972		
		R3	0.062	4.413	441.270	0.000441	0.0026	0.16972	16.972	19.007	±3.525
	0.04%CO_2	R1	0.203	26.794	2679.365	0.002679	0.0078	0.34351	34.351		
		R2	0.269	37.270	3726.984	0.003727	0.0078	0.47782	47.782		
		R3	0.247	33.778	3377.778	0.003378	0.0078	0.43305	43.305	41.813	±6.839
	5% CO_2	R1	0.445	65.206	6520.635	0.006521	0.0129	0.50548	50.548		
		R2	0.469	69.016	6901.587	0.006902	0.0129	0.53501	53.501		
		R3	0.476	70.127	7012.698	0.007013	0.0129	0.54362	54.362	52.803	±2.001
	10% CO_2	R1	0.324	46.000	4600.000	0.004600	0.0098	0.46939	46.939		
		R2	0.339	48.381	4838.095	0.004838	0.0098	0.49368	49.368		
		R3	0.343	49.016	4901.587	0.004902	0.0098	0.50016	50.016	48.774	±1.622

iv) *Oocystis* UMACC 074

Day	Treatment	Sample	595nm	protein in 100 ug (ug)	protein in 10 ml (ug)	protein in 10 ml (g)	Dry weight (g)	protein (g)/ dry weight(g)	% protein (g)/ dry weight (g)	%Mean protein	STDEV
	Control (no aeration)	R1	0.246	33.619	3361.905	0.003362	0.0063	0.5336	53.36		
		R2	0.252	34.571	3457.143	0.003457	0.0063	0.5488	54.88		
		R3	0.253	34.730	3473.016	0.003473	0.0063	0.5513	55.13	54.46	±0.954
	0.04%CO_2	R1	0.246	33.619	3361.905	0.003362	0.0063	0.5336	53.36		
		R2	0.252	34.571	3457.143	0.003457	0.0063	0.5488	54.88		
		R3	0.253	34.730	3473.016	0.003473	0.0063	0.5513	55.13	54.46	±0.954
	5% CO_2	R1	0.246	33.619	3361.905	0.003362	0.0063	0.5336	53.36		
		R2	0.252	34.571	3457.143	0.003457	0.0063	0.5488	54.88		
		R3	0.253	34.730	3473.016	0.003473	0.0063	0.5513	55.13	54.46	±0.954
	10% CO_2	R1	0.246	33.619	3361.905	0.003362	0.0063	0.5336	53.36		
		R2	0.252	34.571	3457.143	0.003457	0.0063	0.5488	54.88		
0		R3	0.253	34.730	3473.016	0.003473	0.0063	0.5513	55.13	54.46	±0.954
	Control (no aeration)	R1	0.094	9.492	949.206	0.000949	0.0022	0.4315	43.15		
		R2	0.089	8.698	869.841	0.000870	0.0022	0.3954	39.54		
		R3	0.103	10.921	1092.063	0.001092	0.0022	0.4964	49.64	44.11	±5.119
	0.04%CO_2	R1	0.203	26.794	2679.365	0.002679	0.0056	0.4785	47.85		
		R2	0.198	26.000	2600.000	0.002600	0.0056	0.4643	46.43		
		R3	0.179	22.984	2298.413	0.002298	0.0056	0.4104	41.04	45.11	±3.589
	5% CO_2	R1	0.329	46.794	4679.365	0.004679	0.0085	0.5505	55.05		
		R2	0.318	45.048	4504.762	0.004505	0.0085	0.5300	53.00		
		R3	0.334	47.587	4758.730	0.004759	0.0085	0.5599	55.99	54.68	±1.529
	10% CO_2	R1	0.273	37.905	3790.476	0.003790	0.0076	0.4987	49.87		
		R2	0.289	40.444	4044.444	0.004044	0.0076	0.5322	53.22		
10		R3	0.301	42.349	4234.921	0.004235	0.0076	0.5572	55.72	52.94	±2.934

b) Carbohydrate Content

i) *Chlorella vulgaris* UMACC 001

Day	Treatment	Sample	OD 485nm	carbohydrate in 500 ug (ug)	carbohydrate in 10 ml (ug)	carbohydrate in 10 ml (g)	Dry weight (g)	carbohydrate (g)/dry weight(g)	% carbohydrate (g) /dry weight (g)	Mean carbohydrate (mg/L)	STDEV
	Control (no aeration)	R1	0.397	4.357	87.137	0.000087	0.0037	0.024	2.36		
		R2	0.404	4.433	88.655	0.000089	0.0037	0.024	2.40		
		R3	0.405	4.444	88.872	0.000089	0.0037	0.024	2.40	2.38	±0.026
	$0.04\%CO_2$	R1	0.397	4.357	87.137	0.000087	0.0037	0.024	2.36		
		R2	0.404	4.433	88.655	0.000089	0.0037	0.024	2.40		
		R3	0.405	4.444	88.872	0.000089	0.0037	0.024	2.40	2.38	±0.026
	5% CO_2	R1	0.397	4.357	87.137	0.000087	0.0037	0.024	2.36		
		R2	0.404	4.433	88.655	0.000089	0.0037	0.024	2.40		
		R3	0.405	4.444	88.872	0.000089	0.0037	0.024	2.40	2.38	±0.026
	10% CO_2	R1	0.403	4.422	88.438	0.000088	0.0036	0.025	2.46		
		R2	0.412	4.520	90.390	0.000090	0.0036	0.025	2.51		
0		R3	0.388	4.259	85.184	0.000085	0.0036	0.024	2.37	2.44	±0.073
	Control (no aeration)	R1	0.131	1.472	29.436	0.000029	0.0021	0.014	1.40		
		R2	0.136	1.526	30.521	0.000031	0.0021	0.015	1.45		
		R3	0.121	1.363	27.267	0.000027	0.0021	0.013	1.30	1.38	±0.079
	$0.04\%CO_2$	R1	0.642	7.014	140.282	0.000140	0.0090	0.016	1.56		
		R2	0.662	7.231	144.620	0.000145	0.0090	0.016	1.61		
		R3	0.663	7.242	144.837	0.000145	0.0090	0.016	1.61	1.59	±0.029
	5% CO_2	R1	0.862	9.400	188.004	0.000188	0.0134	0.014	1.40		
		R2	0.889	9.693	193.861	0.000194	0.0134	0.014	1.45		
		R3	0.767	8.370	167.397	0.000167	0.0134	0.012	1.25	1.37	±0.104
	10% CO_2	R1	0.662	7.231	144.620	0.000145	0.0118	0.012	1.23		
		R2	0.686	7.491	149.826	0.000150	0.0118	0.013	1.27		
10		R3	0.598	6.537	130.738	0.000131	0.0118	0.011	1.11	1.20	±0.084

ii) *Chlorella* UMACC 275

Day	Treatment	Sample	OD 485nm	carbohydrate in 500 ug (ug)	carbohydrate in 10 ml (ug)	carbohydrate in 10 ml (g)	Dry weight (g)	carbohydrate (g)/dry weight(g)	% carbohydrate (g) /dry weight (g)	Mean carbohydrate (mg/L)	STDEV
	Control (no aeration)	R1	0.223	2.295363	45.907258	0.000046	0.0031	0.015	1.48		
		R2	0.192	1.982863	39.657258	0.000040	0.0031	0.013	1.28		
		R3	0.293	3.001008	60.020161	0.000060	0.0031	0.019	1.94	1.57	±0.336
	0.04% CO_2	R1	0.223	2.295363	45.907258	0.000046	0.0031	0.015	1.48		
		R2	0.192	1.982863	39.657258	0.000040	0.0031	0.013	1.28		
		R3	0.293	3.001008	60.020161	0.000060	0.0031	0.019	1.94	1.57	±0.336
	5% CO_2	R1	0.223	2.295363	45.907258	0.000046	0.0031	0.015	1.48		
		R2	0.192	1.982863	39.657258	0.000040	0.0031	0.013	1.28		
		R3	0.293	3.001008	60.020161	0.000060	0.0031	0.019	1.94	1.57	±0.336
	10% CO_2	R1	0.223	2.295363	45.907258	0.000046	0.0031	0.015	1.48		
		R2	0.192	1.982863	39.657258	0.000040	0.0031	0.013	1.28		
0		R3	0.293	3.001008	60.020161	0.000060	0.0031	0.019	1.94	1.57	±0.336
	Control (no aeration)	R1	0.098	1.035282	20.705645	0.000021	0.0015	0.014	1.38		
		R2	0.119	1.246976	24.939516	0.000025	0.0015	0.017	1.66		
		R3	0.112	1.176411	23.528226	0.000024	0.0015	0.016	1.57	1.54	±0.144
	0.04% CO_2	R1	0.240	2.466734	49.334677	0.000049	0.0037	0.013	1.33		
		R2	0.227	2.335685	46.713710	0.000047	0.0037	0.013	1.26		
		R3	0.234	2.406250	48.125000	0.000048	0.0037	0.013	1.30	1.30	±0.035
	5% CO_2	R1	0.435	4.432460	88.649194	0.000089	0.0055	0.016	1.61		
		R2	0.478	4.865927	97.318548	0.000097	0.0055	0.018	1.77		
		R3	0.498	5.067540	101.350806	0.000101	0.0055	0.018	1.84	1.74	±0.118
	10% CO_2	R1	0.613	6.226815	124.536290	0.000125	0.0073	0.017	1.71		
		R2	0.668	6.781250	135.625000	0.000136	0.0073	0.019	1.86		
10		R3	0.712	7.224798	144.495968	0.000144	0.0073	0.020	1.98	1.85	±0.137

iii) *Scenedesmus quadricauda* UMACC 041

Day	Species	Sample	OD 485nm	carbohydrate in 500 ug (ug)	carbohydrate in 10 ml (ug)	carbohydrate in 10 ml (g)	Dry weight (g)	carbohydrate (g)/dry weight(g)	% carbohydrate (g)/dry weight (g)	Mean carbohydrate (mg/L)	STDEV
		R1	0.644	7.03579	140.71584	0.000141	0.0043	0.03272	3.27246		
		R2	0.625	6.82972	136.59436	0.000137	0.0043	0.03177	3.17661		
	0.03%CO2 (no aeration)	R3	0.601	6.56941	131.38829	0.000131	0.0043	0.03056	3.05554	3.17	±0.109
		R1	0.644	7.03579	140.71584	0.000141	0.0043	0.03272	3.27246		
		R2	0.625	6.82972	136.59436	0.000137	0.0043	0.03177	3.17661		
	0.04%CO2	R3	0.601	6.56941	131.38829	0.000131	0.0043	0.03056	3.05554	3.17	±0.109
		R1	0.644	7.03579	140.71584	0.000141	0.0043	0.03272	3.27246		
		R2	0.625	6.82972	136.59436	0.000137	0.0043	0.03177	3.17661		
	5% CO2	R3	0.601	6.56941	131.38829	0.000131	0.0043	0.03056	3.05554	3.17	±0.109
		R1	0.644	7.03579	140.71584	0.000141	0.0043	0.03272	3.27246		
		R2	0.625	6.82972	136.59436	0.000137	0.0043	0.03177	3.17661		
0	10% CO2	R3	0.601	6.56941	131.38829	0.000131	0.0043	0.03056	3.05554	3.17	±0.109
		R1	0.098	1.11388	22.277657	0.000022	0.0026	0.00857	0.85683		
		R2	0.118	1.33080	26.616052	0.000027	0.0026	0.01024	1.02369		
	0.03%CO2 (no aeration)	R3	0.132	1.48265	29.652928	0.000030	0.0026	0.01140	1.14050	1.01	±0.143
		R1	0.455	4.98590	99.718004	0.000100	0.0078	0.01278	1.27844		
		R2	0.437	4.79067	95.813449	0.000096	0.0078	0.01228	1.22838		
	0.04%CO2	R3	0.442	4.84490	96.898048	0.000097	0.0078	0.01242	1.24228	1.25	±0.026
		R1	0.689	7.52386	150.47722	0.000150	0.0129	0.01166	1.16649		
		R2	0.698	7.62148	152.4295	0.000152	0.0129	0.01182	1.18162		
	5% CO2	R3	0.723	7.89262	157.85249	0.000158	0.0129	0.01224	1.22366	1.19	±0.030
		R1	0.534	5.84273	116.85466	0.000117	0.0098	0.01192	1.19239		
		R2	0.595	6.50434	130.08677	0.000130	0.0098	0.01327	1.32742		
10	10% CO2	R3	0.563	6.15727	123.14534	0.000123	0.0098	0.01257	1.25659	1.26	±0.068

iv*) Oocystis* UMACC 074

Day	Treatment	Sample	OD 485nm	carbohydrate in 500 ug (ug)	carbohydrate in 10 ml (ug)	carbohydrate in 10 ml (g)	Dry weight (g)	carbohydrate (g) /dry weight(g)	% carbohydrate (g) /dry weight (g)	Mean carbohydrate (mg/L)	STDEV
0	Control (no aeration)	R1	0.655	7.155	143.102	0.000143	0.0063	0.0227	2.27	2.04	±0.198
		R2	0.553	6.049	120.976	0.000121	0.0063	0.0192	1.92		
		R3	0.558	6.103	122.061	0.000122	0.0063	0.0194	1.94		
	0.04% CO_2	R1	0.655	7.155	143.102	0.000143	0.0063	0.0227	2.27	2.04	±0.198
		R2	0.553	6.049	120.976	0.000121	0.0063	0.0192	1.92		
		R3	0.558	6.103	122.061	0.000122	0.0063	0.0194	1.94		
	5% CO_2	R1	0.655	7.155	143.102	0.000143	0.0063	0.0227	2.27	2.04	±0.198
		R2	0.553	6.049	120.976	0.000121	0.0063	0.0192	1.92		
		R3	0.558	6.103	122.061	0.000122	0.0063	0.0194	1.94		
	10% CO_2	R1	0.667	7.285	145.705	0.000146	0.0063	0.0231	2.31	2.13	±0.196
		R2	0.623	6.808	136.161	0.000136	0.0063	0.0216	2.16		
		R3	0.554	6.060	121.193	0.000121	0.0063	0.0192	1.92		
10	Control (no aeration)	R1	0.313	3.446	68.915	0.000069	0.0022	0.0313	3.13	3.34	±0.202
		R2	0.335	3.684	73.688	0.000074	0.0022	0.0335	3.35		
		R3	0.354	3.890	77.809	0.000078	0.0022	0.0354	3.54		
	0.04% CO_2	R1	0.490	5.366	107.310	0.000107	0.0056	0.0192	1.92	1.96	±0.045
		R2	0.513	5.615	112.299	0.000112	0.0056	0.0201	2.01		
		R3	0.501	5.485	109.696	0.000110	0.0056	0.0196	1.96		
	5% CO_2	R1	0.521	5.702	114.035	0.000114	0.0085	0.0134	1.34	1.42	±0.093
		R2	0.543	5.940	118.807	0.000119	0.0085	0.0140	1.40		
		R3	0.592	6.472	129.436	0.000129	0.0085	0.0152	1.52		
	10% CO_2	R1	0.498	5.452	109.046	0.000109	0.0076	0.0143	1.43	1.56	±0.106
		R2	0.556	6.081	121.627	0.000122	0.0076	0.0160	1.60		
		R3	0.567	6.201	124.013	0.000124	0.0076	0.0163	1.63		

c) Lipid Content

i) *Chlorella vulgaris* UMACC 001

Day	treatment	Sample	blank glass vial(g)	blank glass vial + lipid (g)	lipid(g)	dry weight (g)	lipid(g)/dry weight(g)	% lipid/dry weight	mean	STDEV
		R1	3.1474	3.1478	0.0004	0.0037	0.1081	10.81		
	Control (no aeration)	R2	3.1761	3.1767	0.0006	0.0037	0.1622	16.22		
		R3	3.1422	3.1428	0.0006	0.0037	0.1622	16.22	14.41	±3.121
		R1	3.1474	3.1478	0.0004	0.0037	0.1081	10.81		
		R2	3.1761	3.1767	0.0006	0.0037	0.1622	16.22		
	0.04%CO_2	R3	3.1422	3.1428	0.0006	0.0037	0.1622	16.22	14.41	±3.121
		R1	3.1474	3.1478	0.0004	0.0037	0.1081	10.81		
		R2	3.1761	3.1767	0.0006	0.0037	0.1622	16.22		
	5% CO_2	R3	3.1422	3.1428	0.0006	0.0037	0.1622	16.22	14.41	±3.121
		R1	3.1473	3.1478	0.0005	0.0036	0.1389	13.89		
		R2	3.1763	3.1770	0.0007	0.0036	0.1944	19.44		
0	10% CO_2	R3	3.1427	3.1431	0.0004	0.0036	0.1111	11.11	14.81	±4.243
		R1	3.2240	3.2244	0.0004	0.0021	0.1905	19.05		
	Control (no aeration)	R2	3.2056	3.2060	0.0004	0.0021	0.1905	19.05		
		R3	3.2453	3.2456	0.0003	0.0021	0.1429	14.29	17.46	±2.749
		R1	3.1758	3.1782	0.0024	0.0090	0.2667	26.67		
		R2	3.2043	3.2066	0.0023	0.0090	0.2556	25.56		
	0.04%CO_2	R3	3.1218	3.1239	0.0021	0.0090	0.2333	23.33	25.19	±1.697
		R1	3.1998	3.2043	0.0045	0.0134	0.3358	33.58		
		R2	3.1389	3.1435	0.0046	0.0134	0.3433	34.33		
	5% CO_2	R3	3.2203	3.2251	0.0048	0.0134	0.3582	35.82	34.58	±1.140
		R1	3.2204	3.2233	0.0029	0.0118	0.2458	24.58		
		R2	3.1637	3.1670	0.0033	0.0118	0.2797	27.97		
10	10% CO_2	R3	3.1731	3.1766	0.0035	0.0118	0.2966	29.66	27.40	±2.589

ii) *Chlorella* UMACC 275

Day	treatment	Sample	blank glass vial(g)	blank glass vial + lipid (g)	lipid(g)	dry weight (g)	lipid(g)/dry weight(g)	% lipid/dry weight	mean	STDEV
0	Control (no aeration)	R1	3.1868	3.1879	0.0011	0.0031	0.355	35.48		
		R2	3.1300	3.1309	0.0009	0.0031	0.290	29.03		
		R3	3.1370	3.1379	0.0009	0.0031	0.290	29.03	31.18	±3.725
	0.04% CO_2	R1	3.1868	3.1879	0.0011	0.0031	0.355	35.48		
		R2	3.1300	3.1309	0.0009	0.0031	0.290	29.03		
		R3	3.1370	3.1379	0.0009	0.0031	0.290	29.03	31.18	±3.725
	5% CO_2	R1	3.1868	3.1879	0.0011	0.0031	0.355	35.48		
		R2	3.1300	3.1309	0.0009	0.0031	0.290	29.03		
		R3	3.1370	3.1379	0.0009	0.0031	0.290	29.03	31.18	±3.725
	10% CO_2	R1	3.1863	3.1871	0.0008	0.0031	0.258	25.81		
		R2	3.1854	3.1863	0.0009	0.0031	0.290	29.03		
		R3	3.1365	3.1374	0.0009	0.0031	0.290	29.03	27.96	±1.862
10	Control (no aeration)	R1	3.1218	3.1220	0.0002	0.0015	0.133	13.33		
		R2	3.1626	3.1628	0.0002	0.0015	0.133	13.33		
		R3	3.1532	3.1533	0.0001	0.0015	0.067	6.67	11.11	±3.849
	0.04% CO_2	R1	3.1868	3.1877	0.0009	0.0037	0.243	24.32		
		R2	3.1281	3.1289	0.0008	0.0037	0.216	21.62		
		R3	3.1863	3.1873	0.0010	0.0037	0.270	27.03	24.32	±2.703
	5% CO_2	R1	3.1282	3.1295	0.0013	0.0055	0.236	23.64		
		R2	3.2099	3.2113	0.0014	0.0055	0.255	25.45		
		R3	3.1276	3.1296	0.0020	0.0055	0.364	36.36	28.48	±6.884
	10% CO_2	R1	3.1989	3.2013	0.0024	0.0073	0.329	32.88		
		R2	3.1757	3.1775	0.0018	0.0073	0.247	24.66		
		R3	3.1886	3.1912	0.0026	0.0073	0.356	35.62	31.05	±5.703

iii) *Scenedesmus quadricauda* UMACC 041

Day	treatment	Sample	blank glass vial(g)	blank glass vial + lipid (g)	lipid(g)	dry weight (g)	lipid(g)/dry weight(g)	% lipid/dry weight	mean	STDEV
0	Control (no aeration)	R1	3.1300	3.1305	0.0005	0.0043	0.1162791	11.627907		
		R2	3.1869	3.1877	0.0008	0.0043	0.1860465	18.604651		
		R3	3.1508	3.1513	0.0005	0.0043	0.1162791	11.627907	13.953	±4.028
	0.04% CO_2	R1	3.1300	3.1305	0.0005	0.0043	0.1162791	11.627907		
		R2	3.1869	3.1877	0.0008	0.0043	0.1860465	18.604651		
		R3	3.1508	3.1513	0.0005	0.0043	0.1162791	11.627907	13.953	±4.028
	5% CO_2	R1	3.1300	3.1305	0.0005	0.0043	0.1162791	11.627907		
		R2	3.1869	3.1877	0.0008	0.0043	0.1860465	18.604651		
		R3	3.1508	3.1513	0.0005	0.0043	0.1162791	11.627907	13.953	±4.028
	10% CO_2	R1	3.1300	3.1305	0.0005	0.0043	0.1162791	11.627907		
		R2	3.1869	3.1877	0.0008	0.0043	0.1860465	18.604651		
		R3	3.1508	3.1513	0.0005	0.0043	0.1162791	11.627907	13.953	±4.028
10	Control (no aeration)	R1	3.1534	3.1537	0.0003	0.0026	0.1153846	11.538462		
		R2	3.2102	3.2107	0.0005	0.0026	0.1923077	19.230769		
		R3	3.1875	3.1879	0.0004	0.0026	0.1538462	15.384615	15.385	±3.846
	0.04% CO_2	R1	3.2036	3.2051	0.0015	0.0078	0.1923077	19.230769		
		R2	3.1885	3.1905	0.0020	0.0078	0.2564103	25.641026		
		R3	3.1643	3.1659	0.0016	0.0078	0.2051282	20.512821	21.795	±3.392
	5% CO_2	R1	3.1702	3.1733	0.0031	0.0129	0.2403101	24.031008		
		R2	3.2038	3.2074	0.0036	0.0129	0.2790698	27.906977		
		R3	3.1285	3.1319	0.0034	0.0129	0.2635659	26.356589	26.098	±1.951
	10% CO_2	R1	3.1299	3.1320	0.0021	0.0098	0.2142857	21.428571		
		R2	3.2207	3.2230	0.0023	0.0098	0.2346939	23.469388		
		R3	3.1661	3.1681	0.0020	0.0098	0.2040816	20.408163	21.769	±1.559

iv) *Oocystis* UMACC 074

Day	treatment	Sample	blank glass vial(g)	blank glass vial + lipid (g)	lipid(g)	dry weight (g)	lipid(g)/dry weight(g)	% lipid/dry weight	mean	STDEV
0	Control (no aeration)	R1	3.1823	3.1833	0.0010	0.0063	0.1587302	15.87		
		R2	3.1787	3.1799	0.0012	0.0063	0.1904762	19.05		
		R3	3.1824	3.1836	0.0012	0.0063	0.1904762	19.05	17.99	±1.83
	0.04% CO_2	R1	3.1823	3.1833	0.0010	0.0063	0.1587302	15.87		
		R2	3.1787	3.1799	0.0012	0.0063	0.1904762	19.05		
		R3	3.1824	3.1836	0.0012	0.0063	0.1904762	19.05	17.99	±1.83
	5% CO_2	R1	3.1823	3.1833	0.0010	0.0063	0.1587302	15.87		
		R2	3.1787	3.1799	0.0012	0.0063	0.1904762	19.05		
		R3	3.1824	3.1836	0.0012	0.0063	0.1904762	19.05	17.99	±1.83
	10% CO_2	R1	3.1823	3.1833	0.0010	0.0063	0.1587302	15.87		
		R2	3.1787	3.1799	0.0012	0.0063	0.1904762	19.05		
		R3	3.1824	3.1836	0.0012	0.0063	0.1904762	19.05	17.99	±1.83
10	Control (no aeration)	R1	3.1390	3.1394	0.0004	0.0022	0.1818182	18.18		
		R2	3.1218	3.1220	0.0002	0.0022	0.0909091	9.09		
		R3	3.1223	3.1226	0.0003	0.0022	0.1363636	13.64	13.64	±4.55
	0.04% CO_2	R1	3.1770	3.1778	0.0008	0.0056	0.1428571	14.29		
		R2	3.1996	3.2003	0.0007	0.0056	0.125	12.50		
		R3	3.1754	3.1763	0.0009	0.0056	0.1607143	16.07	14.29	±1.79
	5% CO_2	R1	3.2209	3.2225	0.0016	0.0085	0.1882353	18.82		
		R2	3.2205	3.2223	0.0018	0.0085	0.2117647	21.18		
		R3	3.2050	3.2064	0.0014	0.0085	0.1647059	16.47	18.82	±2.35
	10% CO_2	R1	3.2113	3.2126	0.0013	0.0076	0.1710526	17.11		
		R2	3.1217	3.1225	0.0008	0.0076	0.1052632	10.53		
		R3	3.1213	3.1228	0.0015	0.0076	0.1973684	19.74	15.79	±4.74

APPENDIX K

<u>Distribution of Fatty Acids of Selected Microalgae Strains at Different Levels of Carbon Dioxide on Day 0 and Day 10</u>

a) *Chlorella vulgaris* UMACC 001

i) *Chlorella vulgaris* UMACC 001 at Control (no aeration)

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	2.25	2.67	2.04	2.32	±0.32	2.67	2.45	1.54	2.22	±0.599
16:0	29.98	28.87	34.31	31.05	±2.87	43.45	41.67	39.89	41.67	±1.780
18:0	18.34	16.75	14.76	16.62	±1.79	21.67	19.87	23.56	21.70	±1.845
Sum	50.57	48.29	51.11	49.99		67.79	63.99	64.99	65.59	
Monounsaturated										
16:1	1.56	1.32	1.75	1.54	±0.22	0.76	1.52	0.65	0.98	±0.474
18:1	0.42	0.51	0.65	0.53	±0.12	0.00	0.00	0.00	0.00	±0.000
Sum	1.98	1.83	2.40	2.07		0.76	1.52	0.65	0.98	
Polyunsaturated										
16:2	11.32	12.34	11.23	11.63	±0.62	0.00	0.00	0.00	0.00	±0.000
16:3	7.75	7.64	6.74	7.38	±0.55	0.00	0.00	3.40	1.13	±1.963
18:2	9.98	8.99	8.34	9.10	±0.83	16.70	17.80	14.56	16.35	±1.648
18:3	18.40	20.91	20.18	19.83	±1.29	14.75	16.69	16.40	15.95	±1.046
Sum	47.45	49.88	46.49	47.94		31.45	34.49	34.36	33.43	1.719
TOTAL	100	100	100	100		100	100	100	100	

ii) *Chlorella vulgaris* UMACC 001 at 0.04% CO_2

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	2.25	2.67	2.04	2.32	±0.321	1.15	2.64	0.98	1.59	±0.913
16:0	29.98	28.87	34.31	31.05	±2.874	31.50	45.24	47.94	41.56	±8.816
18:0	18.34	16.75	14.76	16.62	±1.794	19.23	1.90	23.14	14.76	±11.305
Sum	50.57	48.29	51.11	49.99		51.88	49.78	72.06	57.91	
Monounsaturated										
16:1	1.56	1.32	1.75	1.54	±0.215	1.74	8.99	2.38	4.37	±4.014
18:1	0.42	0.51	0.65	0.53	±0.116	0.00	0.00	0.00	0.00	±0.000
Sum	1.98	1.83	2.4	2.07		1.74	8.99	2.38	4.37	
Polyunsaturated										
16:2	11.32	12.34	11.23	11.63	±0.617	0.00	0.00	0.00	0.00	±0.000
16:3	7.75	7.64	6.74	7.38	±0.554	9.90	19.34	9.70	12.98	±5.509
18:2	9.98	8.99	8.34	9.10	±0.826	11.53	19.56	13.48	14.86	±4.188
18:3	18.4	20.91	20.18	19.83	±1.291	24.95	2.33	2.38	9.89	±13.045
Sum	47.45	49.88	46.49	47.94		46.38	41.23	25.56	37.72	10.844
TOTAL	100	100	100	100		100.00	100.00	100.00	100.00	

iii) *Chlorella vulgaris* UMACC 001 at 5% CO_2

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	2.25	2.67	2.04	2.32	±0.321	7.18	0.90	1.09	3.06	±3.572
16:0	29.98	28.87	34.31	31.05	±2.874	31.42	17.45	23.00	23.96	±7.034
18:0	18.34	16.75	14.76	16.62	±1.794	4.06	2.25	1.47	2.59	±1.329
Sum	50.57	48.29	51.11	49.99		42.66	20.60	25.56	29.61	
Monounsaturated										
16:1	1.56	1.32	1.75	1.54	±0.215	6.60	0.46	5.75	4.27	±3.327
18:1	0.42	0.51	0.65	0.53	±0.116	0.48	0.73	1.20	0.80	±0.366
Sum	1.98	1.83	2.4	2.07		7.08	1.19	6.95	5.07	
Polyunsaturated										
16:2	11.32	12.34	11.23	11.63	±0.617	13.35	4.28	3.21	6.95	±5.571
16:3	7.75	7.64	6.74	7.38	±0.554	18.14	14.20	11.68	14.67	±3.256
18:2	9.98	8.99	8.34	9.10	±0.826	16.06	13.50	11.52	13.69	±2.276
18:3	18.4	20.91	20.18	19.83	±1.291	2.71	46.23	41.08	30.01	±23.779
Sum	47.45	49.88	46.49	47.94		50.26	78.21	67.49	65.32	
TOTAL	100	100	100	100		100	100	100	100	

iv) iii) *Chlorella vulgaris* UMACC 001 at 10% CO_2

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	2.25	2.67	2.04	2.32	±0.321	1.83	1.94	1.61	1.79	±0.168
16:0	29.98	28.87	34.31	31.05	±2.874	21.87	21.68	15.87	19.81	±3.411
18:0	18.34	16.75	14.76	16.62	±1.794	3.25	5.50	2.77	3.84	±1.457
Sum	50.57	48.29	51.11	49.99		26.95	29.12	20.25	25.44	
Monounsaturated										
16:1	1.56	1.32	1.75	1.54	±0.215	3.49	3.75	4.23	3.82	±0.375
18:1	0.42	0.51	0.65	0.53	±0.116	0.00	0.00	0.00	0.00	±0.000
Sum	1.98	1.83	2.4	2.07		3.49	3.75	4.23	3.82	
Polyunsaturated										
16:2	11.32	12.34	11.23	11.63	±0.617	0.46	0.43	0.00	0.30	±0.257
16:3	7.75	7.64	6.74	7.38	±0.554	16.16	15.57	12.48	14.74	±1.976
18:2	9.98	8.99	8.34	9.10	±0.826	7.34	7.19	14.18	9.57	±3.993
18:3	18.4	20.91	20.18	19.83	±1.291	45.60	43.94	48.86	46.13	±2.503
Sum	47.45	49.88	46.49	47.94		69.56	67.13	75.52	70.74	4.317
TOTAL	100	100	100	100		100.00	100.00	100.00	100.00	

b) *Chlorella* UMACC 275

i) *Chlorella* UMACC 275 at Control (no aeration)

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	2.55	2.67	2.34	2.52	±0.167	2.43	2.45	1.87	2.25	±0.329
16:0	31.04	29.05	32.56	30.88	±1.760	39.87	41.67	39.89	40.48	±1.034
18:0	16.08	16.75	14.76	15.86	±1.013	5.55	6.78	7.66	6.66	±1.060
Sum	49.67	48.47	49.66	49.27		47.85	50.9	49.42	49.39	
Monounsaturated										
16:1	1.55	1.11	1.75	1.47	±0.327	0.76	1.5	0.78	1.02	±0.433
18:1	1.09	1.23	1.27	1.20	±0.095	0.00	0.00	0.00	0.00	±0.000
Sum	2.64	2.34	3.02	2.67		0.76	1.52	0.78	1.02	
Polyunsaturated										
16:2	0.00	0.00	0.00	0.00	±0.000	0.00	0.00	0.00	0.00	±0.000
16:3	17.04	17.64	16.78	17.15	±0.441	0.00	0.00	0.00	0.00	±0.000
18:2	19.98	18.99	18.35	19.11	±0.821	26.36	27.86	29.88	28.03	±1.766
18:3	10.67	12.56	12.19	11.81	±1.002	25.03	19.72	19.92	21.56	±3.010
Sum	47.69	49.19	47.32	48.07		51.39	47.58	49.8	49.59	1.914
TOTAL	100	100	100	100.00		100	100	100	100	

ii) *Chlorella* sp. UMACC 275 at 0.04%CO_2

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	2.55	2.67	2.34	2.52	±0.167	1.84	2.33	1.98	2.05	±0.252
16:0	31.04	29.05	32.56	30.88	±1.760	29.84	35.63	33.65	33.04	±2.943
18:0	16.08	16.75	14.76	15.86	±1.013	2.23	2.77	3.12	2.71	±0.448
Sum	49.67	48.47	49.66	49.27		33.91	40.73	38.75	37.80	
Monounsaturated										
16:1	1.55	1.11	1.75	1.47	±0.327	8.97	8.76	8.66	8.80	±0.158
18:1	1.09	1.23	1.27	1.20	±0.095	0.00	0.00	0.00	0.00	±0.000
Sum	2.64	2.34	3.02	2.67		8.97	8.76	8.66	8.80	
Polyunsaturated										
16:2	0.00	0.00	0.00	0.00	±0.000	0.00	0.00	0.00	0.00	±0.000
16:3	17.04	17.64	16.78	17.15	±0.441	13.67	9.85	11.87	11.80	±1.911
18:2	19.98	18.99	18.35	19.11	±0.821	20.59	23.14	23.48	22.40	±1.580
18:3	10.67	12.56	12.19	11.81	±1.002	22.86	17.52	17.24	19.21	±3.167
Sum	47.69	49.19	47.32	48.07		57.12	50.51	52.59	53.41	3.380
TOTAL	100	100	100	100		100	100	100	100	

iii) *Chlorella* UMACC 275 at 5%CO_2

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	2.55	2.67	2.34	2.52	±0.167	1.88	1.95	2.66	2.16	±0.432
16:0	31.04	29.05	32.56	30.88	±1.760	28.94	27.40	29.06	28.47	±0.926
18:0	16.08	16.75	14.76	15.86	±1.013	4.08	2.25	3.47	3.27	±0.932
Sum	49.67	48.47	49.66	49.27		34.90	31.60	35.19	33.90	
Monounsaturated										
16:1	1.55	1.11	1.75	1.47	±0.327	7.32	6.46	5.88	6.55	±0.725
18:1	1.09	1.23	1.27	1.20	±0.095	0.00	0.00	0.00	0.00	±0.000
Sum	2.64	2.34	3.02	2.67		7.32	6.46	5.88	6.55	
Polyunsaturated										
16:2	0.00	0.00	0.00	0.00	±0.000	0.00	0.00	0.00	0.00	±0.000
16:3	17.04	17.64	16.78	17.15	±0.441	28.11	25.55	32.02	28.56	±3.258
18:2	19.98	18.99	18.35	19.11	±0.821	19.20	23.43	22.98	21.87	±2.323
18:3	10.67	12.56	12.19	11.81	±1.002	10.47	12.96	3.93	9.12	±4.664
Sum	47.69	49.19	47.32	48.07		57.78	61.94	58.93	59.55	2.148
TOTAL	100	100	100	100		100	100	100	100	

iv) *Chlorella* UMACC 275 at 10%CO_2

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	2.55	2.67	2.34	2.52	±0.17	1.76	4.45	1.38	2.53	±1.67
16:0	31.04	29.05	32.56	30.88	±1.76	26.94	7.57	24.35	19.62	±10.52
18:0	16.08	16.75	14.76	15.86	±1.01	3.04	3.32	1.60	2.65	±0.92
Sum	49.67	48.47	49.66	49.27		31.74	15.34	27.33	24.80	
Monounsaturated										
16:1	1.55	1.11	1.75	1.47	±0.33	7.15	18.95	8.03	11.38	±6.57
18:1	1.09	1.23	1.27	1.20	±0.09	0.00	0.00	0.00	0.00	±0.00
Sum	2.64	2.34	3.02	2.67		7.15	18.95	8.03	11.38	
Polyunsaturated										
16:2	0.00	0.00	0.00	0.00	±0.00	0.00	0.00	17.70	5.90	±10.22
16:3	17.04	17.64	16.78	17.15	±0.44	14.21	37.90	0.00	17.37	±19.15
18:2	19.98	18.99	18.35	19.11	±0.82	18.01	26.48	18.44	20.98	±4.77
18:3	10.67	12.56	12.19	11.81	±1.00	28.89	1.33	28.50	19.57	±15.80
Sum	47.69	49.19	47.32	48.07		61.11	65.71	64.64	63.82	2.41
TOTAL	100	100	100	100		100	100	100	100	

c) *Scenedesmus quadricauda* UMACC 041

i) *Scenedesmus quadricauda* UMACC 041 at Control (no aeration)

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	1.25	1.67	1.34	1.42	±0.221	3.21	3.11	2.54	2.95	±0.361
16:0	28.25	28.87	27.27	28.13	±0.807	18.92	16.78	19.89	18.53	±1.591
18:0	19.33	15.67	16.34	17.11	±1.949	4.45	5.65	5.56	5.22	±0.668
Sum	48.83	46.21	44.95	46.66		26.58	25.54	27.99	26.70	
Monounsaturated										
16:1	0.43	0.78	0.56	0.59	±0.177	0.69	0.89	0.93	0.84	±0.129
18:1	0.39	0.56	0.62	0.52	±0.119	0.00	0.00	0.00	0.00	±0.000
Sum	0.82	1.34	1.18	1.11		0.69	0.89	0.93	0.84	
Polyunsaturated										
16:2	12.89	13.43	11.45	12.59	±1.024	0.44	0.56	0.45	0.48	±0.067
16:3	9.92	6.76	6.56	7.75	±1.885	16.67	17.34	16.78	16.93	±0.359
18:2	10.92	6.88	10.14	9.31	±2.143	18.84	17.65	17.07	17.85	±0.902
18:3	16.62	25.38	25.72	22.57	±5.159	36.78	38.02	36.78	37.19	±0.716
Sum	50.35	52.45	53.87	52.22		72.73	73.57	71.08	72.46	1.267
TOTAL	100	100	100	100		100	100	100	100	

ii) *Scenedesmus quadricauda* UMACC 041 at 0.04%CO_2

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	1.25	1.67	1.34	1.42	±0.221	1.11	0.94	1.25	1.10	±0.155
16:0	28.25	28.87	27.27	28.13	±0.807	21.74	15.76	20.46	19.32	±3.149
18:0	19.33	15.67	16.34	17.11	±1.949	6.08	5.96	3.69	5.24	±1.347
Sum	48.83	46.21	44.95	46.66		28.93	22.66	25.40	25.66	
Monounsaturated										
16:1	0.43	0.78	0.56	0.59	±0.177	3.81	3.98	3.33	3.71	±0.337
18:1	0.39	0.56	0.62	0.52	±0.119	0.52	0.31	0.59	0.47	±0.146
Sum	0.82	1.34	1.18	1.11		4.33	4.29	3.92	4.18	
Polyunsaturated										
16:2	12.89	13.43	11.45	12.59	±1.024	0.35	2.09	0.29	0.91	±1.022
16:3	9.92	6.76	6.56	7.75	±1.885	18.00	18.47	16.85	17.77	±0.833
18:2	10.92	6.88	10.14	9.31	±2.143	15.67	17.31	16.73	16.57	±0.832
18:3	16.62	25.38	25.72	22.57	±5.159	32.72	35.18	36.81	34.90	±2.059
Sum	50.35	52.45	53.87	52.22		66.74	73.05	70.68	70.16	3.187
TOTAL	100	100	100	100		100	100	100	100	

iii) *Scenedesmus quadricauda* UMACC 041 at 5%CO_2

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	1.25	1.67	1.34	1.42	±0.221	1.38	0.93	1.30	1.20	±0.240
16:0	28.25	28.87	27.27	28.13	±0.807	29.24	7.99	28.62	21.95	±12.094
18:0	19.33	15.67	16.34	17.11	±1.949	3.16	11.49	1.75	5.47	±5.264
Sum	48.83	46.21	44.95	46.66		33.78	20.41	31.67	28.62	
Monounsaturated										
16:1	0.43	0.78	0.56	0.59	±0.177	2.41	3.8	2.76	2.98	±0.712
18:1	0.39	0.56	0.62	0.52	±0.119	0.99	0.4	9.00	3.47	±4.801
Sum	0.82	1.34	1.18	1.11		3.4	4.19	11.76	6.45	
Polyunsaturated										
16:2	12.89	13.43	11.45	12.59	±1.024	3.96	0.94	0.60	1.83	±1.850
16:3	9.92	6.76	6.56	7.75	±1.885	28.25	46.95	26.32	33.84	±11.395
18:2	10.92	6.88	10.14	9.31	±2.143	25.39	20.23	24.85	23.49	±2.836
18:3	16.62	25.38	25.72	22.57	±5.159	5.22	7.28	4.80	5.77	±1.327
Sum	50.35	52.45	53.87	52.22		62.82	75.4	56.57	64.93	9.591
TOTAL	100	100	100	100		100	100	100	100	

iv) *Scenedesmus quadricauda* UMACC 041 at 10%CO_2

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	1.25	1.67	1.34	1.42	±0.221	1.28	0.86	0.89	1.01	±0.234
16:0	28.25	28.87	27.27	28.13	±0.807	19.28	18.30	17.98	18.52	±0.677
18:0	19.33	15.67	16.34	17.11	±1.949	5.33	1.61	1.60	2.85	±2.151
Sum	48.83	46.21	44.95	46.66		25.89	20.77	20.47	22.38	
Monounsaturated										
16:1	0.43	0.78	0.56	0.59	±0.177	1.36	3.91	0.86	2.04	±1.636
18:1	0.39	0.56	0.62	0.52	±0.119	0.00	1.92	4.56	2.16	±2.289
Sum	0.82	1.34	1.18	1.11		1.36	5.83	5.42	4.20	
Polyunsaturated										
16:2	12.89	13.43	11.45	12.59	±1.024	0.30	0.30	0.27	0.29	±0.017
16:3	9.92	6.76	6.56	7.75	±1.885	20.00	20.33	20.24	20.19	±0.171
18:2	10.92	6.88	10.14	9.31	±2.143	13.50	10.97	11.12	11.86	±1.419
18:3	16.62	25.38	25.72	22.57	±5.159	38.95	41.80	42.48	41.08	±1.873
Sum	50.35	52.45	53.87	52.22		72.75	73.40	74.11	73.42	0.680
TOTAL	100	100	100	100		100	100	100	100	

d) *Oocystis* UMACC 074

i) *Oocystis* UMACC 074 UMACC 041 at Control (no aeration)

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	2.12	2.31	2.21	2.21	±0.095	2.67	2.45	2.54	2.55	±0.111
16:0	28.29	28.87	29.31	28.82	±0.512	31.25	31.43	32.56	31.75	±0.710
18:0	19.94	15.76	14.76	16.82	±2.748	3.13	3.21	2.98	3.11	±0.117
Sum	50.35	46.94	46.28	47.86		37.05	37.09	38.08	37.41	
Monounsaturated										
16:1	0.32	0.45	0.48	0.42	±0.085	0.76	1.5	0.65	0.98	±0.474
18:1	0.45	0.37	0.42	0.41	±0.040	0	0.0	0	0.00	±0.000
Sum	0.77	0.82	0.90	0.83		0.76	1.52	0.65	0.98	
Polyunsaturated										
16:2	12.21	13.34	12.11	12.55	±0.683	0.00	0.00	0.00	0.00	±0.000
16:3	8.78	6.64	8.86	8.09	±1.259	14.25	14.78	13.78	14.27	±0.500
18:2	10.92	9.06	10.67	10.22	±1.009	16.70	17.80	18.98	17.83	±1.140
18:3	16.97	23.20	21.18	20.45	±3.179	31.24	28.81	28.51	29.52	±1.497
Sum	48.88	52.24	52.82	51.31		62.19	61.39	61.27	61.62	0.500
TOTAL	100.00	100.00	100.00	100.00		100	100	100	100	

ii) *Oocystis* UMACC 074 at 0.04%CO_2

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	2.12	2.31	2.21	2.21	±0.095	2.79	1.96	2.16	2.30	±0.433
16:0	28.29	28.87	29.31	28.82	±0.512	29.95	26.36	20.50	25.60	±4.770
18:0	19.94	15.76	14.76	16.82	±2.748	4.18	9.14	8.97	7.43	±2.816
Sum	50.35	46.94	46.28	47.86		36.92	37.46	31.63	35.34	
Monounsaturated										
16:1	0.32	0.45	0.48	0.42	±0.085	3.62	3.04	6.01	4.22	±1.574
18:1	0.45	0.37	0.42	0.41	±0.040	0.00	0.00	0.00	0.00	±0.000
Sum	0.77	0.82	0.90	0.83		3.62	3.04	6.01	4.22	
Polyunsaturated										
16:2	12.21	13.34	12.11	12.55	±0.683	0.00	0.00	0.00	0.00	±0.000
16:3	8.78	6.64	8.86	8.09	±1.259	13.15	12.69	12.76	12.87	±0.248
18:2	10.92	9.06	10.67	10.22	±1.009	16.23	18.20	18.45	17.63	±1.216
18:3	16.97	23.20	21.18	20.45	±3.179	30.08	28.61	31.15	29.95	±1.275
Sum	48.88	52.24	52.82	51.31		59.46	59.50	62.36	60.44	1.663
TOTAL	100	100	100	100		100	100	100	100	

iii) *Oocystis* UMACC 074 at 5%CO_2

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	2.12	2.31	2.21	2.21	±0.095	1.91	1.82	2.31	2.01	±0.261
16:0	28.29	28.87	29.31	28.82	±0.512	29.45	26.94	22.16	26.18	±3.703
18:0	19.94	15.76	14.76	16.82	±2.748	10.99	13.07	7.23	10.43	±2.960
Sum	50.35	46.94	46.28	47.86		42.35	41.83	31.70	38.63	
Monounsaturated										
16:1	0.32	0.45	0.48	0.42	±0.085	3.12	0.00	5.95	3.02	±2.976
18:1	0.45	0.37	0.42	0.41	±0.040	0.00	0.00	0.00	0.00	±0.000
Sum	0.77	0.82	0.90	0.83		3.12	0.00	5.95	3.02	
Polyunsaturated										
16:2	12.21	13.34	12.11	12.55	±0.683	0.00	4.94	0.00	1.65	±2.852
16:3	8.78	6.64	8.86	8.09	±1.259	11.30	11.84	11.93	11.69	±0.341
18:2	10.92	9.06	10.67	10.22	±1.009	16.58	10.51	19.30	15.46	±4.500
18:3	16.97	23.20	21.18	20.45	±3.179	26.65	30.88	31.12	29.55	±2.514
Sum	48.88	52.24	52.82	51.31		54.53	58.17	62.35	58.35	3.913
TOTAL	100	100	100	100		100	100	100	100	

iv) *Oocystis* UMACC 074 at 10%CO_2

Fatty acid	Day 0					Day 10				
	R1	R2	R3	Mean	STDEV	R1	R2	R3	Mean	STDEV
Saturated										
14:0	2.12	2.31	2.21	2.21	±0.095	2.15	2.11	1.61	1.96	±0.301
16:0	28.29	28.87	29.31	28.82	±0.512	32.34	32.68	31.87	32.30	±0.407
18:0	19.94	15.76	14.76	16.82	±2.748	11.33	12.54	12.77	12.21	±0.774
Sum	50.35	46.94	46.28	47.86		45.82	47.33	46.25	46.47	
Monounsaturated										
16:1	0.32	0.45	0.48	0.42	±0.085	2.45	2.75	3.23	2.81	±0.393
18:1	0.45	0.37	0.42	0.41	±0.040	0.00	0.00	0.00	0.00	±0.000
Sum	0.77	0.82	0.90	0.83		2.45	2.75	3.23	2.81	
Polyunsaturated										
16:2	12.21	13.34	12.11	12.55	±0.683	0.00	0.00	0.00	0.00	±0.000
16:3	8.78	6.64	8.86	8.09	±1.259	11.19	15.57	12.48	13.08	±2.251
18:2	10.92	9.06	10.67	10.22	±1.009	17.34	17.19	14.18	16.24	±1.783
18:3	16.97	23.20	21.18	20.45	±3.179	23.20	17.16	23.86	21.41	±3.692
Sum	48.88	52.24	52.82	51.31		51.73	49.92	50.52	50.72	0.922
TOTAL	100	100	100	100		100	100	100	100	

APPENDIX L

Data for One Way ANOVA and a Tukey's HSD Post hoc Comparison to Determine Significant Differences between Treatment Means

1) One Way ANOVA of *Chlorella vulgaris* UMACC 001

a) Optical Density of *Chlorella vulgaris* UMACC 001

Effect	Univariate Tests of Significance for var 2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	2.883160	1	2.883160	4378.930	0.000000
Treatment	0.552046	3	0.184015	279.482	0.000000
Error	0.005267	8	0.000658		

b) Cell count of *Chlorella vulgaris* UMACC 001

Effect	Univariate Tests of Significance for var 2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	3121200	1	3121200	6054.704	0.000000
Treatment	510938	3	170313	330.383	0.000000
Error	4124	8	515		

c) Chlorophyll a content of *Chlorella vulgaris* UMACC 001

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	122.1374	1	122.1374	365.8810	0.000000
"Var1"	22.7150	3	7.5717	22.6821	0.000288
Error	2.6705	8	0.3338		

d) Carotenoid content of *Chlorella vulgaris* UMACC 001

Effect	Univariate Tests of Significance for Var2 (Spreadsheet3) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	22.92578	1	22.92578	1311.099	0.000000
"Var1"	4.93165	3	1.64388	94.012	0.000001
Error	0.13989	8	0.01749		

e) Biomass Production of *Chlorella vulgaris* UMACC 001 on day 0 at

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	63948.00	1	63948.00	7993.500	0.000000
"Var1"	4.00	3	1.33	0.167	0.915877
Error	64.00	8	8.00		

f) Biomass Production of *Chlorella vulgaris* UMACC 001 on day 10

Effect	Univariate Tests of Significance for Day 0 (Spreadsheet5) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	63948.00	1	63948.00	7993.500	0.000000
"Var1"	4.00	3	1.33	0.167	0.915877
Error	64.00	8	8.00		

g) Protein Content of *Chlorella vulgaris* UMACC 001 on day 10

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	17558.43	1	17558.43	1575.697	0.000000
"Var1"	1697.18	3	565.73	50.768	0.000015
Error	89.15	8	11.14		

h) Carbohydrate content of *Chlorella vulgaris* UMACC 001 on day 0

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	952.7977	1	952.7977	150259.8	0.000000
"Var1"	0.0281	3	0.0094	1.5	0.292142
Error	0.0507	8	0.0063		

i) Carbohydrate content of *Chlorella vulgaris* UMACC 001 on day 10

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	546.8229	1	546.8229	13848.77	0.000000
"Var1"	1.3843	3	0.4614	11.69	0.002706
Error	0.3159	8	0.0395		

j) Lipid content of *Chlorella vulgaris* UMACC 001 on day 0

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	5966.799	1	5966.799	741.1050	0.000000
"Var1"	0.171	3	0.057	0.0071	0.999107
Error	64.410	8	8.051		

k) Lipid content of *Chlorella vulgaris* UMACC 001 on day 10

Effect	Univariate Tests of Significance for Var2 (Spreadsheet6) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	11223.59	1	11223.59	4977.654	0.000000
"Var1"	197.45	3	65.82	29.189	0.000117
Error	18.04	8	2.25		

2) One Way Anova of *Chlorella* UMACC 275

a) Optical Density of *Chlorella* UMACC 275

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	0.473224	1	0.473224	2049.328	0.000000
"Var1"	0.171382	3	0.057127	247.393	0.000000
Error	0.001847	8	0.000231		

b) Cell count of *Chlorella* UMACC 275

Effect	Univariate Tests of Significance for Var2 (Spreadsheet4) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	457861.3	1	457861.3	3591.069	0.000000
"Var1"	130336.7	3	43445.6	340.749	0.000000
Error	1020.0	8	127.5		

c) Chlorophyll a content of *Chlorella* UMACC 275

Effect	Univariate Tests of Significance for Var2 (Spreadsheet6) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	57.52911	1	57.52911	227.8764	0.000000
"Var1"	19.44931	3	6.48310	25.6800	0.000185
Error	2.01966	8	0.25246		

d) Carotenoid content of *Chlorella* UMACC 275

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	7.960141	1	7.960141	263.5787	0.000000
"Var1"	2.523287	3	0.841096	27.8506	0.000138
Error	0.241602	8	0.030200		

e) Biomass Production of *Chlorella* UMACC 275 on day 0

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	45633.33	1	45633.33	1006.618	0.000000
"Var1"	4.00	3	1.33	0.029	0.992668
Error	362.67	8	45.33		

f) Biomass Production of *Chlorella* UMACC 275 on day 10

Effect	Univariate Tests of Significance for Var2 (Spreadsheet6) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	97200.00	1	97200.00	946.7532	0.000000
"Var1"	22498.67	3	7499.56	73.0476	0.000004
Error	821.33	8	102.67		

g) Protein Content of *Chlorella* UMACC 275 on day 0

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	23095.11	1	23095.11	267909.7	0.000000
"Var1"	0.00	3	0.00	0.0	1.000000
Error	0.69	8	0.09		

h) Protein Content of *Chlorella* UMACC 275 on day1 0

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	26745.88	1	26745.88	1865.613	0.000000
"Var1"	176.86	3	58.95	4.112	0.048732
Error	114.69	8	14.34		

i) Carbohydrate Content of *Chlorella* UMACC 275 on day 0

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	615.3258	1	615.3258	1047.363	0.000000
"Var1"	0.0000	3	0.0000	0.000	1.000000
Error	4.7000	8	0.5875		

j) Carbohydrate Content of *Chlorella* UMACC 275 on day 10

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	632.8998	1	632.8998	9207.374	0.000000
"Var1"	2.8119	3	0.9373	13.636	0.001642
Error	0.5499	8	0.0687		

k) Lipid Content of *Chlorella* UMACC 275 on day 0

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	13402.72	1	13402.72	3135.206	0.000000
"Var1"	9.09	3	3.03	0.709	0.573278
Error	34.20	8	4.27		

l) Lipid Content of *Chlorella* UMACC 275 on day 10

Effect	Univariate Tests of Significance for Var2 (Spreadsheet6) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	9876.069	1	9876.069	813.2329	0.000000
"Var1"	382.999	3	127.666	10.5125	0.003776
Error	97.154	8	12.144		

3) One Way Anova of *Scenedesmus quadricauda* UMACC 041

a) Optical density of *Scenedesmus quadricauda* UMACC 041

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	1.663585	1	1.663585	499.5627	0.000000
"Var1"	0.422666	3	0.140889	42.3079	0.000030
Error	0.026641	8	0.003330		

b) Cell count of *Scenedesmus quadricauda* UMACC 041

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	123424.1	1	123424.1	1206.099	0.000000
"Var1"	13128.3	3	4376.1	42.763	0.000029
Error	818.7	8	102.3		

c) Chlorophyll a content of *Scenedesmus quadricauda* UMACC 041

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	44.82716	1	44.82716	247.3174	0.000000
"Var1"	8.00323	3	2.66774	14.7183	0.001276
Error	1.45003	8	0.18125		

d) Carotenoid content of *Scenedesmus quadricauda* UMACC 041

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	9.179347	1	9.179347	314.3266	0.000000
"Var1"	1.736860	3	0.578953	19.8250	0.000463
Error	0.233626	8	0.029203		

e) Biomass Production of *Scenedesmus quadricauda* UMACC 041 on day 0

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	88752.00	1	88752.00	715.7419	0.000000
"Var1"	0.00	3	0.00	0.0000	1.000000
Error	992.00	8	124.00		

f) Biomass Production of *Scenedesmus quadricauda* UMACC 041 on day 10

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	327360.3	1	327360.3	353.2665	0.000000
"Var1"	67046.3	3	22348.8	24.1174	0.000232
Error	7413.3	8	926.7		

g) Protein content of *Scenedesmus quadricauda* UMACC 041 on day 0

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	28407.16	1	28407.16	5185.800	0.000000
"Var1"	10.26	3	3.42	0.624	0.619116
Error	43.82	8	5.48		

h) Protein content of *Scenedesmus quadricauda* UMACC 041 on day 10

Effect	Univariate Tests of Significance for Var2 (Spreadsheet5) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	18474.60	1	18474.60	3005.579	0.000000
"Var1"	785.44	3	261.81	42.594	0.000029
Error	49.17	8	6.15		

i) Carbohydrate content of *Scenedesmus quadricauda* UMACC 041 on day 0

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	1261.238	1	1261.238	39809.44	0.000000
"Var1"	0.000	3	0.000	0.00	1.000000
Error	0.253	8	0.032		

j) Carbohydrate content of *Scenedesmus quadricauda* UMACC 041 on day 10

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	463.9698	1	463.9698	8799.064	0.000000
"Var1"	0.9333	3	0.3111	5.900	0.020013
Error	0.4218	8	0.0527		

k) Lipid content of *Scenedesmus quadricauda* UMACC 041 on day 0

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	5707.649	1	5707.649	543.2101	0.000000
"Var1"	0.000	3	0.000	0.0000	1.000000
Error	84.058	8	10.507		

l) Lipid content of *Scenedesmus quadricauda* UMACC 041 on day 10

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	8957.955	1	8957.955	2026.488	0.000000
"Var1"	92.175	3	30.725	6.951	0.012827
Error	35.363	8	4.420		

4) One Way ANOVA of *Oocystis* UMACC 074

a) Optical density of *Oocystis* UMACC 074

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	0.495727	1	0.495727	932.4014	0.000000
"Var1"	0.073043	3	0.024348	45.7949	0.000022
Error	0.004253	8	0.000532		

b) Cell count of *Oocystis* UMACC 074

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	68403.00	1	68403.00	1557.564	0.000000
"Var1"	9133.67	3	3044.56	69.326	0.000005
Error	351.33	8	43.92		

c) Chlorophyll a content of *Oocystis* UMACC 074

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	19.36832	1	19.36832	876.8409	0.000000
"Var1"	4.51535	3	1.50512	68.1396	0.000005
Error	0.17671	8	0.02209		

d) Carotenoid content of *Oocystis* UMACC 074

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	3.546229	1	3.546229	385.0229	0.000000
"Var1"	0.783217	3	0.261072	28.3453	0.000130
Error	0.073683	8	0.009210		

e) Biomass production of *Oocystis* UMACC 074 on day 0

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	190512.0	1	190512.0	47628.00	0.000000
"Var1"	0.0	3	0.0	0.00	1.000000
Error	32.0	8	4.0		

f) Biomass production of *Oocystis* UMACC 074 on day 10

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	170408.3	1	170408.3	862.0995	0.000000
"Var1"	27990.3	3	9330.1	47.2012	0.000020
Error	1581.3	8	197.7		

g) Protein Content of *Oocystis* UMACC 074 on day 0

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	27139.36	1	27139.36	90208.88	0.000000
"Var1"	0.00	3	0.00	0.00	1.000000
Error	2.41	8	0.30		

h) Protein Content of *Oocystis* UMACC 074 on day 10

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	23806.25	1	23806.25	5726.669	0.000000
"Var1"	85.96	3	28.65	6.893	0.013130
Error	33.26	8	4.16		

i) Carbohydrate Content of *Oocystis* UMACC 074 on day 0

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	818.0365	1	818.0365	5249.511	0.000000
"Var1"	0.0726	3	0.0242	0.155	0.923313
Error	1.2466	8	0.1558		

j) Carbohydrate Content of *Oocystis* UMACC 074 on day 10

Effect	Univariate Tests of Significance for Var2 (Spreadsheet5) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	796.0733	1	796.0733	14222.70	0.000000
"Var1"	25.0263	3	8.3421	149.04	0.000000
Error	0.4478	8	0.0560		

k) Lipid content of *Oocystis* UMACC 074 on day 0

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	7546.506	1	7546.506	3937.354	0.000000
"Var1"	0.000	3	0.000	0.000	1.000000
Error	15.333	8	1.917		

l) Lipid content of *Oocystis* UMACC 074 on day 10

Effect	Univariate Tests of Significance for Var2 (Spreadsheet2) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	6431.002	1	6431.002	738.1430	0.000000
"Var1"	30.422	3	10.141	1.1639	0.381805
Error	69.699	8	8.712		

<u>Tukey's HSD Post hoc Comparison between Treatment Means</u>

1) *Chlorella vulgaris* UMACC 001

a) Biomass Production of *Chlorella vulgaris* UMACC 001 on day 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet2) Homogenous Groups, alpha = .05000 Error: Between MS = 8.0000, df = 8.0000		
	Var1	Var2 Mean	1
4	d	72.00000	****
3	c	73.33333	****
2	b	73.33333	****
1	a	73.33333	****

b) Biomass Production of *Chlorella vulgaris* UMACC 001 on day 10

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = .33382, df = 8.0000			
	Var1	Var2 Mean	1	2
1	a	0.861607		****
2	b	3.535313	****	
4	d	4.005810	****	
3	c	4.358533	****	

c) Protein Content of *Chlorella vulgaris* UMACC 001 on day 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = .11592, df = 8.0000			
	Var1	Var2 Mean	1	2
2	b	50.92111	****	
3	c	50.92111	****	
1	a	50.92111	****	
4	d	51.90448		****

d) Protein content of *Chlorella vulgaris* UMACC 001 on day 10

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet2) Homogenous Groups, alpha = .05000 Error: Between MS = 11.143, df = 8.0000				
	Var1	Var2 Mean	1	2	3
1	a	19.03963			****
4	d	38.35310	****		
2	b	45.16202	****	****	
3	c	50.45256		****	

e) Carbohydrate content of *Chlorella vulgaris* UMACC 001 on day 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = .00634, df = 8.0000		
	Var1	Var2 Mean	1
2	b	8.882709	****
3	c	8.882709	****
1	a	8.882709	****
4	d	8.994500	****

e) Carbohydrate content of *Chlorella vulgaris* UMACC 001 on day1 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet2) Homogenous Groups, alpha = .05000 Error: Between MS = .03949, df = 8.0000			
	Var1	Var2 Mean	1	2
4	d	6.289355	****	
3	c	6.709386	****	
1	a	6.755517	****	****
2	b	7.247542		****

f) Lipid content of *Chlorella vulgaris* UMACC 001 on day 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = .11592, df = 8.0000			
	Var1	Var2 Mean	1	2
2	b	50.92111	****	
3	c	50.92111	****	
1	a	50.92111	****	
4	d	51.90448		****

g) Lipid content of *Chlorella vulgaris* UMACC 001 on day 10

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet2) Homogenous Groups, alpha = .05000 Error: Between MS = 2.2548, df = 8.0000				
	Var1	Var2 Mean	1	2	3
1	a	24.65368		****	
2	b	30.11389	****		
4	d	31.54798	****		
3	c	36.01508			****

2) *Chlorella* UMACC 275

a) Biomass Production of *Chlorella* UMACC 275 on day 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = 45.333, df = 8.0000		
	Var1	Var2 Mean	1
4	d	60.66667	****
3	c	62.00000	****
2	b	62.00000	****
1	a	62.00000	****

b) Biomass Production of *Chlorella* UMACC 275 on day 10

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet2) Homogenous Groups, alpha = .05000 Error: Between MS = 0.3020, df = 8.0000				
	Var1	Var2 Mean	1	2	3
1	a	0.118373			****
2	b	0.726967	****		
4	c	1.061500	****	****	
3	d	1.351000		****	

c) Protein Content of *Chlorella* UMACC 275 on day 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = 4.2749, df = 8.0000		
	Var1	Var2 Mean	1
4	d	31.91229	****
3	c	33.92250	****
2	b	33.92250	****
1	a	33.92250	****

d) Protein Content of *Chlorella* UMACC 275 on day10

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet2) Homogenous Groups, alpha = .05000 Error: Between MS = 12.144, df = 8.0000			
	Var1	Var2 Mean	1	2
1	a	19.26555		****
2	b	29.52814	****	
4	d	32.15861	****	
3	c	33.80001	****	

e) Carbohydrate Content of *Chlorella* UMACC 275 on day0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = .58750, df = 8.0000		
	Var1	Var2 Mean	1
2	b	7.160807	****
3	c	7.160807	****
4	d	7.160807	****
1	a	7.160807	****

f) Carbohydrate Content of *Chlorella* UMACC 275 on day 10

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet2) Homogenous Groups, alpha = .05000 Error: Between MS = 14.336, df = 8.0000			
	Var1	Var2 Mean	1	2
1	a	42.47404	****	
2	b	46.14909	****	****
4	d	47.06065	****	****
3	c	53.15789		****

g) Lipid Content of *Chlorella* UMACC 275 on day 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = 4.2749, df = 8.0000		
	Var1	Var2 Mean	1
4	d	31.91229	****
3	c	33.92250	****
2	b	33.92250	****
1	a	33.92250	****

g) Lipid Content of *Chlorella* UMACC 275 on day 10

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet2) Homogenous Groups, alpha = .05000 Error: Between MS = 0.3020, df = 8.0000				
	Var1	Var2 Mean	1	2	3
1	a	0.118373			****
2	b	0.726967	****		
3	c	1.061500	****	****	
4	d	1.351000		****	

3) *Scenedesmus quadricauda* UMACC 041

a) Biomass Production of *Scenedesmus quadricauda* UMACC 041 on day 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = 124.00, df = 8.0000		
	Var1	Var2 Mean	1
2	b	86.00000	****
3	c	86.00000	****
4	d	86.00000	****
1	a	86.00000	****

b) Biomass Production of *Scenedesmus quadricauda* UMACC 041 on day 10

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet2) Homogenous Groups, alpha = .05000 Error: Between MS = 197.67, df = 8.0000				
	Var1	Var2 Mean	1	2	3
1	a	0.333247	****		
2	b	0.738547	****	****	
4	d	1.075653		****	****
3	c	1.351000			****

c) Protein content of *Scenedesmus quadricauda* UMACC 041 on day 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = 5.4779, df = 8.0000		
	Var1	Var2 Mean	1
1	a	47.05310	****
3	c	49.18834	****
4	d	49.18834	****
2	b	49.18834	****

d) Protein content of *Scenedesmus quadricauda* UMACC 041 on day1 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet5) Homogenous Groups, alpha = .05000 Error: Between MS = 6.1468, df = 8.0000			
	Var1	Var2 Mean	1	2
1	a	25.78926		****
2	b	40.25384	****	
4	d	44.29743	****	
3	c	46.60785	****	

e) Carbohydrate content of *Scenedesmus quadricauda* UMACC 041 on day 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = .03168, df = 8.0000		
	Var1	Var2 Mean	1
2	b	10.25198	****
3	c	10.25198	****
4	d	10.25198	****
1	a	10.25198	****

f) Carbohydrate content of *Scenedesmus quadricauda* UMACC 041 on day 10

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet5) Homogenous Groups, alpha = .05000 Error: Between MS = 6.1468, df = 8.0000			
	Var1	Var2 Mean	1	2
1	a	25.78926		****
2	b	40.25384	****	
4	d	44.29743	****	
3	c	46.60785	****	

g) Lipid content of *Scenedesmus quadricauda* UMACC 041 on day 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = 10.507, df = 8.0000		
	Var1	Var2 Mean	1
2	b	21.80911	****
3	c	21.80911	****
4	d	21.80911	****
1	a	21.80911	****

h) Lipid content of *Scenedesmus quadricauda* UMACC 041 on day 10

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet2) Homogenous Groups, alpha = .05000 Error: Between MS = 4.4204, df = 8.0000			
	Var1	Var2 Mean	1	2
1	a	22.98703	****	
2	b	27.78766	****	****
4	d	27.80263	****	****
3	c	30.71101		****

4) *Oocystis* UMACC 074

a) Biomass production of *Oocystis* UMACC 074 on day 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = 4.0000, df = 8.0000		
	Var1	Var2 Mean	1
2	b	126.0000	****
3	c	126.0000	****
4	d	126.0000	****
1	a	126.0000	****

b) Biomass production of *Oocystis* UMACC 074 on day 10

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet2) Homogenous Groups, alpha = .05000 Error: Between MS = 197.67, df = 8.0000				
	Var1	Var2 Mean	1	2	3
1	a	44.0000		****	
2	b	111.3333			****
4	d	151.3333	****		
3	c	170.0000	****		

c) Protein content of *Oocystis* UMACC 074 on day 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = .30085, df = 8.0000		
	Var1	Var2 Mean	1
2	b	47.55643	****
3	c	47.55643	****
4	d	47.55643	****
1	a	47.55643	****

d) Protein content of *Oocystis* UMACC 074 on day1 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet2) Homogenous Groups, alpha = .05000 Error: Between MS = 4.1571, df = 8.0000			
	Var1	Var2 Mean	1	2
1	a	41.60498	****	
2	b	42.18585	****	
4	d	46.68612	****	****
3	c	47.68496		****

e) Carbohydrate content of *Oocystis* UMACC 074 on day 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = .15583, df = 8.0000		
	Var1	Var2 Mean	1
2	b	8.211580	****
3	c	8.211580	****
1	a	8.211580	****
4	d	8.391240	****

f) Carbohydrate content of *Oocystis* UMACC 074 on day 10

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet5) Homogenous Groups, alpha = .05000 Error: Between MS = .05597, df = 8.0000				
	Var1	Var2 Mean	1	2	3
3	c	6.84315	****		
4	d	7.16210	****		
2	b	8.04782		****	
1	a	10.52654			****

g) Lipid content of *Oocystis* UMACC 074 on day 0

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet1) Homogenous Groups, alpha = .05000 Error: Between MS = 1.9166, df = 8.0000		
	Var1	Var2 Mean	1
2	b	25.07739	****
3	c	25.07739	****
4	d	25.07739	****
1	a	25.07739	****

h) Lipid content of *Oocystis* UMACC 074 on day 10

Cell No.	Tukey HSD test; variable Var2 (Spreadsheet2) Homogenous Groups, alpha = .05000 Error: Between MS = 8.7124, df = 8.0000		
	Var1	Var2 Mean	1
1	a	21.48623	****
2	b	22.18214	****
4	d	23.24606	****
3	c	25.68508	****