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Characterization of microalgae species from Qatar coastal waters for animal feed production

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By

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THESIS APROVAL

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ABSTRACT

Food security worldwide is field of major research and investigation to improve and find new resource production systems for a continuously growing world population. The state of Qatar being an arid and with limited arable lands is increasingly reliant on imported food products and has recently engaged extensive efforts to advance its challenging strategy to sustainably enhance its food security. Microalgae found in the local environment and adapted to wide range of environmental conditions are considered promising candidate to play a central role in this food security strategy since no arable land neither freshwater are needed for its cultivation. We aim in this study to identify the species and culture condition for the obtaining of microalgal biomass as source for feedstock production. *Tetraselmis* and *Nannochloris* isolates were selected from the Qatar University Culture Collection of Cyanobacteria and Microalgae (QUCCCM) based on preliminary results and extensive available literature review. Salinity and CO_2 enrichment experiments were conducted with several levels (i.e. 35, 40 and 45 psu salinities and 3%, 5% and 10% CO_2 enrichment), using photobioreactors cultivation system. Results suggest that 35 psu salinity and 5% CO_2 enrichment cultivation conditions are favorable for the protein hyper-producer *Tetraselmis* strain, while 40 psu salinity and 3% CO_2 enrichment are more suitable for the lipid hyper-producer *Nannochloris* strain. Mineral uptake differed between the two species and at some extent between different salinities and CO_2 enrichment culture conditions. *Tetraselmis* contains higher amount of Calcium while *Nannochloris* contains higher amount of Potassium. Mineral profiles of the two species respond differently to salinity and CO_2 enrichment culture conditions. The conducted biochemical characterization of the obtained biomass

suggests that a feed blending using both *Tetraselmis* and *Nannochloris* biomasses would provide high quality products with high protein contents while supplying animals with essential fatty acids (i.e. PUFAs) and mineral ingredients. Recommendations for future research and development efforts are discussed.

Key words: Microalgae, protein, lipids, minerals, Qatar food security, feedstock, salinity, carbon dioxide, productivity, photobioreactor.

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CHAPTER I: Introduction

World population is anticipated to keep significantly expanding during the upcoming century. Will worldwide food production be expanded to accommodate the impending populace development? Since numerous populations are facing shortage in food provision today, it will be important to expand current levels of sustenance production more than to the corresponding population development, to ensure sufficient and sustainable food production and provision.

Whether food supply can keep pace with a stretching human populace is an old inquiry. It is anticipated that populace development might exceed food supply. In the early 1960s, most countries were independent in sustenance, yet alert about a rapid expansion of world population (~2% every year) confirmed to many authors Malthus' expectation. Recently, food productivity per capita has shown to be decreasing (Fuller, 2004). To provide sufficient food resources, we can cultivate more land, or we can increase the yield from every unit of cultivated area. As Fuller, (2004) indicated, the prospect lies in expanding yields. Additionally one can observe that humanity is pushing beyond limits the carrying capacity of most worlds' cultivated systems.

Given the current levels of world population and likely trends it is crucial to anticipate future needs. Agricultural systems are being placed under pressure and the capacity to deliver affordable food and nutritional products for human and animals is dwindling. This makes it imperative for scientists to find alternative solutions to enhance the sustainability of food production systems and processes.

One of interesting solutions is exploiting the potential production of food and animal feed from microalgal biomass (MBD, 2013). Microalgae are characterized by their large number and wide variety of benefits related with how and where they grow; as the earth's most productive and sustainable plants, algae can double their numbers every few hours and can be harvested daily. In contrast, other crops are limited to a single, short lived growth season, needing months to mature. As a result, microalgae have the potential to produce biomass greater than most of cultivated crops. Microalgae characterized by extreme diversity in their chemical composition provide a large spectrum of food and feed co-products with some strains showing high contents in protein and others in oil and/or carbohydrates (algae basics, 2014). Algae cultivation uses land that in many cases is unsuitable for terrestrial farming; thus eliminating competition for agricultural land crops as well as water sources that are not useable for other crops, such as marine. (Alltech, 2014).

Domestic agriculture in Qatar faces development problems mainly related to the scarcity of freshwater for irrigation, the poor quality of the soils, and adverse climatic conditions (Feeding Doha, 2013). On the other hand, population in Qatar is steadily increasing and that put more pressure on the few available agriculture land. The aim of using microalgae for animal feed was to provide animal feed produced locally without competing with human food needs. This project by identifying suitable microalgae strains and biomass production requirements will contribute in reducing Qatar's reliance on imported food and/or animal feed through the realization of the principle of self-sufficiency. Moreover, it will

contribute to the welfare of Qatar, using efficient technology in harmony with the environment following the principles of Qatar National Vision 2030 (QNV 2030).

We hypothesize that local marine microalgae isolates may provide feedstock for the sustainable production of animal feed using non arable land and with no requirement of freshwater resources. Due to harsh weather conditions in the Arabian Gulf region that result in prolonged periods of time for microalgae selection and adaptation, studying these microorganisms and selecting candidate for animal feed would give local biological and technological tool for implementation.

It would be valuable to investigate microalgae products obtained from local isolates. **During this study several algae strains were investigated and an assessment of the biochemical properties of candidate isolates is performed**.

CHAPTER II: Background and Literature Review

Food security

Increasing costs for food production in 2007-2008 and again in 2011 reinvigorated the worldwide dialog on food security and its sustainability. As per the World Bank, by 2008, global food costs had expanded by a disturbing 83 percent throughout the span of the past three years. Worldwide grain stocks have not been sufficiently improved in the interceding years, and food supplies have not kept balanced with the expanding requests of the increasing population. Other more foreseeable problems can be expected such as population growth, changes in consumption patterns and even the impact of climate change disrupting harvests (floods, droughts, etc…) (Lynch, 2013).

Food security in the Gulf Cooperation Council (GCC) region and in Qatar particularly is extremely vulnerable to any external factors affecting food supplies and costs since Qatar imports 90% of its food needs from other countries. Qatar and the Gulf region often do not have the necessary agricultural resources and conditions to produce their own food. The shortage of arable lands, beside, a scarce supply of freshwater, and an extreme harsh climate hinder local food production and make the region one of the most dependent food importers in the world (Feeding Doha, 2013).

Despite all these obstacles, GCC countries react to create long term sustainable sources of food. In the 1970s and 1980s, Saudi Arabia invested heavily in developing a food self-sufficiency program. By the early 1980s, Saudi Arabia was the sixth largest global producer of wheat. The Saudi program had reduced its import dependency but this came

at a great harm to its environment specifically to underground water reservoirs (Mashood, 2013).

Qatar decided to tackle food security through the implementation of a more sustainable approach. The Qatar National Food Security Program (QNFSP) was set up in 2008 as an authorized entity to reach sustainable food security goal. The QNFSP today has two obligations; first, to develop short and long term strategic master plan for addressing food security and the second key part is to aid the overall sustainable growth of Qatar through the implementation of that master plan (qnfsp, 2014).

In addition to the need to secure food for human, animal feed is not less important than its predecessor. Total world feed output is approximately 614 million tons. Animal feed consumption already exceeds direct human food consumption by almost four, and this ratio can only rise as the demand for more animal protein increases. The ability to produce increasing amounts of feeds is one of the greatest challenges facing mankind (IFIF, 2014).

Qatar lacks any substantial animal feed project since the few cultivable lands is used for human food. There is a need to engage animal feed in food security plans to boost food security in Qatar without conflicting with human food needs.

Microalgae are foreseen as possible solution to boost food security in Qatar since demonstrating potential sustainable production of feedstock with no use of arable land, freshwater resources and adapted to the regional harsh climate.

Criteria of microalgae

Regularly, the microalgae selected for fish feeding studies seemed to have been chosen generally for feeding, since they require minimal effort and economically accessible. Indeed, microalgae (e.g. Spirulina, Chlorella and Dunaliella) might be handled by minimal effort in open-raceways systems and are promoted as dry powders, and their nutritional profiles are overall recorded (Andersen, 2005)

Lately there has been extraordinary enthusiasm toward the capability of microalgae as a biochemical microalgae feedstock, and it has frequently been recommended that the protein contents may be a potential feedstock for animal feeds (Becker , 2004).

Microalgae

Microalgae are eukaryotic organism. Cells are bounded by cell wall and the nucleus where DNA is preserved is bounded by a membrane (Figure 1). Other organelles are also bounded by a membrane such as Golgi body, endoplasmic reticulum, vacuoles, mitochondria, centrioles and plastids. Chloroplast is another membranous organelle Microalgae in the cytoplasm that carries out photosynthesis process. (Alanis, 2013).

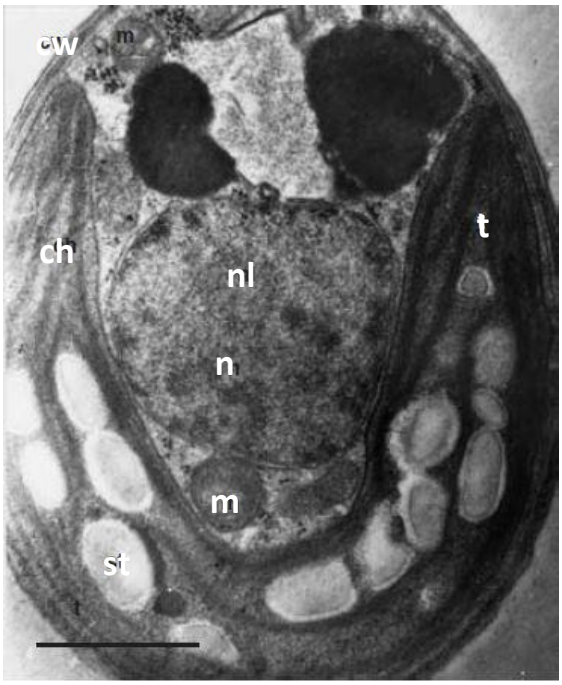

Figure 1: Electron micrograph of a cell of ***Chlorella vulgaris*** in longitudinal section.

Abbreviations: ch - chloroplast, cw - cell wall, m - mitochondria, n - nucleus, nl -nucleolous, st - starch grains (leucoplasts) and t - thylakoids. Scale = 1 m (Alanis, 2013)**.**

Microalgae Chemical composition

All microalgae have plastids, which are bodies with chlorophyll that achieve photosynthesis. At the same time, the different lines of microalgae have distinctive content of chlorophyll pigments. All microalgae include at different relative extents: proteins, carbohydrates, fats, minerals and nucleic acids (Table 1 and 2). While the rates differ from one microalga to another, several may contain unsaturated fats up to 40% of their general biomass (Alanis, 2013).

A. Proteins

The assessment of protein contents in microalgae revealed most extreme values in the exponential development stage. Fernández-Reiriz *et al*. (1989) discovered the same pattern for the prasinophyte *Tetraselmis suecica*; however, the diatoms *Phaeodactylum tricornutum* and *Chaetoceros calcitrans* did not change their protein content over development. Fernández-Reiriz *et al*. (1989) additionally reported a maximum in protein contents at the transitional development stage. Silva *et al*. (2009) also reported higher protein contents for cells during exponential development period of the prasinophyte *T. gracilisand*. The diatom *Cyclotella cassia*, contain an extensive high protein rate (62 and 55%, dew.). For sugars, distinct patterns were discovered, compared to protein. For all species, aside from *Chaetoceros sp*., expanding convergences of starches were found during all development phases. These findings were highlighted in numerous studies (Fernandez-Reiriz *et al.*, 1989; Laurence *et al*, 1997; Knuckey *et al.*, 2002). As indicated by Enrich *et al*. (1986), when the rate of cell division in microalgae is limited by nutrient depletion, cells adjust their metabolic system. Protein and chlorophyll diminishes in nutrient limitation conditions and the concentration of lipid and/or carbohydrates increases (Gouveia L. , 2011).

B. Lipids

Changes in lipid contents in *Chlorella sp*. and *Rhodomonas sp*, are as those reported by Fernández-Reiriz *et al*. (1989) with the prasinophyte *Tetraselmis suecica* and the cryptomonad Rhodomonas.

As feed source, *Bellerochea* and *Chaetoceros sp*., for example, could be viewed as promising species, since they have the three essential fatty acids (FA); Arachidonic acid (AA), Eicosapentaenoic acid (EPA), and Docosahexaenoic acid (DHA) in all development stages. *Chlorella sp*. should not to be established for utilization in marine aquaculture as a monoalgal feed regime, since showing poor source of Polyunsaturated Fatty Acids (PUFA), revealing low concentrations on the three essential FA in stationary development stage. Consideration toward the consumption of *Rhodomonas sp*. is identically depicted as for Chlorella sp. The cryptomonad displays the three key FA in low concentrations (Knuckey, 2002).

C. Pigments

Pigments are colorful chemical compounds which absorb and reflect certain wavelengths of visible light. Pigments contribute in the photosynthetic system of microalgae as light energy absorber. The main pigments are grouped in chlorophylls and carotenoids and phycobilins.

All higher plants and photosynthetic algae contain chlorophylls, while carotenoids are present in most algae, and phycobilins are only in cyanobacteria and in some red algae. Pigments are used as food and feed additives and as health promoting supplements. Cultivation conditions controls the pigment content in biomass. Especially several secondary pigments accumulate in higher amounts under stress conditions, while chlorophylls in general degrade under stress and therefore their content in biomass decreases significantly (Spolaore *et al*., 2006) (Alanis, 2013).

D. Vitamins and minerals

Microalgae biomass is considered a valuable source of almost all essential vitamins (e.g. A, B1, B2, B6, B12, C, E, nicotinate, biotin folic, acid and pantothenic acid) and a significant mineral content (e.g. Na, K, Ca, Mg, Fe, Zn and trace minerals). Spirulina as an example contains high levels of vitamin B12 and Iron in some microalgae, which makes them particularly suitable as nutritional supplements for vegetarian individuals. The vitamin content of an alga depends on the genotype, the light intensity, the stage in the growth cycle and the nutritional status of the alga. The vitamin content is therefore can be manipulated by varying the factors that we mentioned (Gouveia, 2008).

Table 1: Concentration of chlorophyll a, protein, carbohydrate, and lipid in 16 species of micro-algae normally used in aquaculture (modified from Brown, 1991).

Algal class Species	Dry weight ($pg.cell^{-1}$)	Chi a	Protein	Carbohydrate	Lipid
	Weight of constituent ($pg.cell^{-1}$)				
Bacillariophyceae					
Chaetoceros calcitrans	11.3	0.34	3.8	0.68	1.8
Chaetoceros gracilis	74.8	0.78	9.0	2.0	5.2
Nitzchia closterium	-	-	-	-	-
Phaeodactylum tricornutum	76.7	0.41	23.0	6.4	10.7
Skeletonema costatum	52.2	0.63	13.1	2.4	5.0
Thalassiosira pseudonana	28.4	0.27	9.7	2.5	5.5
Chlorophyceae					
Dunaliella tertiolecta	99.9	1.73	20.0	12.2	15.0
Nannochloris atomus	21.4	0.080	6.4	5.0	4.5
Cryptophyceae					
Chroomonas salina	122.5	0.98	35.5	11.0	14.5
Eustigmatophyceae					
Nannochloropsis oculata	6.1	0.054	2.1	0.48	1.1
Prasinophyceae					
Tetraselmis Chui	269.0	3.83	83.4	32.5	45.7
Tetraselmis suecica	168.2	1.63	52.1	20.2	16.8
Prymnesiophyceae					
Isochrysis galbanum	30.5	0.30	8.8	3.9	7.0
Isochrysis aff. *Galbana* (T-	29.7	0.29	6.8	1.8	5.9

	iso)					
	Pavlova lutheri	102.3	0.86	29.7	9.1	12.3
	Pavlova salina	93.1	0.34	24.2	6.9	11.2
		Percentage of dry weight				
Bacillariophyceae						
	Chaetoceros calcitrans	11.3	3.01	34	6.0	16
	Chaetoceros gracilis	74.8	1.04	12	4.7	7.2
	Nitzchia closterium	-	-	26	9.8	13
	Phaeodactylumtricornutum	76.7	0.53	30	8.4	14
	Skeletonema costatum	52.2	1.21	25	4.6	10
	Thalassiosira pseudonana	28.4	0.95	34	8.8	19
Chlorophyceae						
	Dunaliella tertiolecta	99.9	1.73	20	12.2	15
	Nannochloris atomus	21.4	0.37	30	23.0	21
Cryptophyceae						
	Chroomonas salina	122.5	0.80	29	9.1	12
Eustigmatophyceae						
	Nannochloropsis oculata	6.1	0.89	35	7.8	18
Prasinophyceae						
	Tetraselmis Chui	269.0	1.42	31	12.1	17
	Tetraselmis suecica	168.2	0.97	31	12.0	10
Prymnesiophyceae						
	Isochrysis galbanum	30.5	0.98	29	12.9	23
	Isochrysis aff. *Galbana* (T-iso)	29.7	0.98	23	6.0	20
	Pavlova lutheri	102.3	0.84	29	9.0	12
	Pavlova salina	93.1	0.98	26	7.4	12

Table 2: Cellular density (10^6 cells ml^{-1}) and proximate composition (pg $cell^{-1}$) of four marine micro-algae grown in different culture media (Algal-1 is a commercial nutrient) modified from (Herrero *et al.*, 1991)

	Cellular density	**Protein**	**Carbohydrates**	**Lipids**
T. suecica				
Walne	2.29	13.31	6.20	7.04
ES	2.58	16.98	6.93	7.22
F/2	2.38	21.75	8.37	7.92
Algal-1	4.11	32.22	8.83	8.65
D. tertiolecta				
Walne	4.04	13.37	13.22	22.28
ES	4.24	14.88	15.73	23.94
F/2	4.97	13.26	17.91	23.67
Algal-1	8.45	18.82	11.08	18.18
I. galbanum				
Walne	10.11	5.17	4.28	25.95

	ES	12.09	7.23	5.21	28.38
	F/2	10.81	8.13	5.59	26.82
	Algal-1	16.15	9.57	4.28	20.68
P. tricornutum					
	Walne	19.01	2.65	6.42	6.51
	ES	16.23	5.21	9.20	6.45
	F/2	24.65	3.34	6.90	5.52
	Algal-1	39.04	4.20	5.98	5.79

Animal feed from microalgae

Feed quantity and quality are the most vital exogenous parameters affecting animal health. Food security and safety concerns require the provision of better quality feed ingredients and microalgae biomass is a potential suitable feedstock or as feed supplement. The diversity of microalgae in terms of biochemical composition is an advantage compared with higher plants (Gouveia, 2011). The majority of the raw materials utilized presently for animal feed products are mostly higher plants like corn, soybeans, sorghum, oats, and grain. The utilization of microalgae for animal feed began in the early 70s. Microalgae diverse biochemical profiles are basis for the potential improvement of feed nutritional products, enhancing their impact in creature wellbeing.

The dietary plan of microalgae is made up for the most part of proteins, carbohydrates, lipids, pigments and minerals. All species of microalgae have comparative amino acids organization and are rich in essential amino acids. Feeds mixes contain a mixture of different raw materials that are specific to fulfil the prerequisites of the targeted animal. Feeds are mixed from different raw materials and added substances. As per any dietary plan, microalgae supplements must fulfill the Safety and International Standards controlling feed processing. (IFIF, 2014)

In spite of the fact that the utilization of entire microalgae in animal diets has long been considered, since the 1950's, only recent studies has demonstrated attempts to supplement microalgae in animal diets (Group, 2001)

In the last decade, microalgae attracted a major interest for biofuels production. Lately, focus was expanded towards using microalgae in food, feed, chemical and pharmaceutical sector as well (Wijffels & Barbosa, 2010). About 30% of the world algae production is used in animal feed after it have been tested for their biochemical compositions to be used as livestock feed supplement or as primary livestock feed (Becker, 2007). Edible seaweeds is used as food due to lower calorie, high concentration of minerals, vitamins and proteins and a low fat content (Dhargalkar & Verlecar, 2009). *Spirulina* is still used in food supplements due to its excellent nutrient compounds and digestibility (Kumar *et al.*, 2005). *Spirulina* contains high amount of protein (60–70 wt%). In addition, it contains a rich source of vitamins, especially vitamin B12 and pro-vitamin A (b-carotene) and minerals (Thajuddin & Subramanian, 2005). *Spirulina* can be cultivated in high saline water and alkaline conditions compared to other microorganisms, which give an advantage to function as a feedstock for livestock feed. Another suggested microalga as potential feed is *Chlorella* because it is nutrients richness (Spolaore *et al*., 2006). Moreover, microalgae also play a key role in high grade animal nutrition food, from aquaculture to farm animals. Comprehensive nutritional and toxicological evaluations have proved suitability of algae biomass as a valuable feed supplement or substitute for conventional animal feed sources (Dhargalkar & Verlecar, 2009). Aquaculture is the largest application of microalgae as feed source. Their importance in aquaculture is not surprising considering the fact that they are natural food

for these organisms. Microalgae species *Hypnea cervicornis* and *Cryptonemia crenulata* particularly rich in protein were tested in shrimp diets (da Silva & Barbosa , 2008). Amount of algae in fish meal resulted in significant increase in shrimp growth rates and weights. Tilapia fish was observed when supplied with algae as nutritional food source in feed and its shows better growth weights and protein efficiencies ratio (Azaza *et al.*, 2008). Beside its importance in aquaculture, algae were reported to contain up to 5–10% of proteins which can directly be used to replace conventional protein sources in poultry feed (Spolaore, et. al, 2006). Ginzberg *et al*. (2000) studied role of algae, *Porphyridium sp*. as feed supplement on metabolism of chicken. The results indicate that about 10% reductions in cholesterol of egg yolk and color of egg yolk became darker; indicating higher carotenoid was produced. Belay *et al.* (1996) reviewed potential of *Arthrospira* (*Spirulina*) in animal feed. *Arthrospira* is widely used as food additive and can replace 50% of protein diets in existing feeds. Due to economic concerns, it was concluded that protein sources from soya and fish meal were preferable compared to *Arthrospira* (Dhargalkar & Verlecar, 2009). Another study on the addition of *Laminaria digitata* suggested that algae supplemented feed increased pig weight up to 10% on a daily basis (He et al, 2002). Recently, microalgae *Isochrysis galbana* and *Diacronema vlkianum* attracted attention due to their ability to produce long chain PUFA, mainly EPA and also DHA that are accumulated as oil droplets (Gouveia *et al*, 2008).

Microalgae Nutritional Requirements

Several studies were carried out to investigate and identify specific requirements by microalgae. Vonshak, (1986) designed novel medium recipes after the investigation of the basic requirements for microalgae, which are:

- Carbon source (carbon dioxide or bicarbonate).
- Nitrogen source (nitrate, ammonia and urea).
- Trace elements and chelating agents (e.g. EDTA).
- Vitamins.
- Hydrogen potential (pH).
- Salt content, which will be determined by the natural habitat of the microalgae.
- Other ionic components based on cellular composition.

Using Vonshak, (1986) guidelines, several culture media were designed, tested and established. Some of the culture media are general and sustain growth of many microalgae types, while others are specific. A comprehensive list of recipes can be found in (CCAP, 2013). A few of them are of interest to this particular study and are described next:

- BG-11 (Allen, 1968) is a growth medium that has been used extensively for cyanobacteria and freshwater green algae. Marine organisms can be cultured in this media even after the addition of NaCl.

- F/2 medium (RRL, 1962) is a widely used, general enriched seawater medium optimized for growing coastal marine algae. (Alanis, 2013)

Conditions for Growth and Maintenance of Cultures

Beside the nutritional requirements mentioned previously, certain physical conditions must be provided to sustain micro algal growth and viability maintenance. Species differ in their optimal parameters along with the tolerated ranges and must be determined

individually (Lavens *et al.*, 1996).The most important abiotic conditions to be considered are discussed next.

A. Light

Fixation of carbon dioxide is the main source of energy. Light is essential for photosynthesis reaction that are responsible of carbon dioxide fixation in microalgae. Three variables are interested when setting the appropriate level of light to the media which are: light intensity, spectral quality and photoperiod requirements of the strain (Lavens, 1996).

Usually, light intensity is the biggest challenge when culturing microalgae due to their growth and proliferation which raises biomass density, and reduces the penetration of light with the same strength throughout the culture. Therefore, bottom layer are shaded by the surface layer, and thus leads to unequal distribution of light. To avoid growth inhibition, at least 100-200 µmol photon $s^{-1}m^{-2}$ must be provided for proper cultivation of microalgae (Lavens, 1996). Ogbonna *et al*. (1997) studied the growth rate of Chlorella sp. by increasing the light intensity from 163 µmol photon s^{-1} m^{-2} to 310 µmol photon s^{-1} m^{-2}, the growth increased from 2 g $L^{-1}d^{-1}$ to 4 g $L^{-1}d^{-1}$ demonstrating the importance of light requirements in growth and division of algal cells.

In contrast, greater light intensity may cause photo inhibition if light that is provided exceed the needed and photo inhibition may occur (Adir, 2003). Light behaves as a substrate and that saturating levels are in the range of full sunlight intensity that is why careful attention must be taken when working with culturing systems that use sunlight as the light source, since it might not be the best approach for the cultivated organism.

Fluorescent lights could substitute sun light in those cases. It is recommended to use a photoperiod of 16:8 h light/dark as a minimum for biomass production. In closed photo reactors, the light intensity and duration can be controlled in contrast of open culture systems (Lavens, 1996). (Alanis, 2013)

B. Temperature

For marine non extremophilic microalgae, the optimal temperature between 25°C and 32°C. For example, Dauta *et al*. (1990) studied temperature ranges from 10°C to 35°C for four microalgae strains and determined that optimal growing temperature and light intensity for *Chlorella vulgaris*, *Fragilaria crotonensis*, *Staurastrum pingue* and *Synechocystis minima* were 30°C and 140 µmol/s/m2, 25°C and 150 µmol photon $s^{-1}m^{-2}$, 27°C and 270 µmol photon $s^{-1}m^{-2}$ and 32°C and 125 µmol photon $s^{-1}m^{-2}$, respectively.

It is reported from several studies that low temperatures will not usually kill the algae, but will reduce their growth rate. On the other hand, sustained temperatures above 32°C will kill most types of microalgae. (Alanis, 2013)

C. pH

The pH tolerance for most of microalgae lies between 7 and 9, while the optimal is reported to be 8.2 - 8.7. Inappropriate pH levels may result in culture collapse due to inhibition of cellular processes. To avoid pH increase due to CO_2/HCO_3 imbalance, CO_2 bubbling as a buffer and as substrate too is applied alongside with proper mixing. (Lavens *et al.*, 1996). (Alanis, 2013)

D. Salinity

Salinities between 25 and 30 psu (~ 2.5% and 3.0%) have been reported to be optimal for the cultivation of flagellates, and between 20 and 25 psu (~ 2.0% and 2.5%) for diatoms. While this range serves as a general guide, salinity measurement should be done to specify particular strain environment. (Alanis, 2013)

E. Mixing and aeration

Mixing is important to prevent sedimentation of the microalgae, to guarantee that all cells of the population are equally provided with light and nutrients, and to enhance gas trade between the culture medium and the air. Mixing is accomplished by shacking day by day by hand (test tubes, Erlenmeyer's), circulating air through (packs, tanks), or utilizing rotation wheels and jet pumps (ponds and tanks).

Aeration of cultures with CO_2 enriched air in the form of micro-bubbles is a common strategy for mixing since it serves three purposes. First, addition of CO_2 enriched air is used to increase biomass production; second, CO_2 addition buffers the water against pH changes as a result of the CO_2/HCO_3 balance and third, mixing comes along as a further benefit. (Alanis, 2013)

CHAPTER III: METHODOLOGY

The objective of this study is to investigate microalgae strains from Qatar university culture collection (QUCCCM). The selection is based on preliminary experiment and literature. The potentiality of these microalgae as source of food is to be examined at various culture conditions.

Research Strategy

A. Selection of microalgae strains from QUCCCM native to Qatar with high protein content.

B. Examine the yield performance of the QUCCCM selected strains under different culturing settings.

- Growth rates and yield performance.

C. Provide biochemical characterization and adequacy as biomass for animal feed production.

- Total protein and lipid determination
- Minerals.

Culture Media

The culture media used for inoculum cultivation is modified BG11 (Table 3) which is a general media recipe for fresh water microalgae. Modification was made by adding sea water instead of fresh water to suit marine microalgae cultivation. For salinity and CO_2 enrichment experiments, the media that is used is Guillard's F\2 (Table 3) which is general and wide used media for marine microalgae cultivation.

Table 3: Guillard's F\2 (Guillard, 1962)**and BG-11 SW compositions. (Allen, 1968)**

	Guillard's F/2	BG-11 SW
$NaNO_3$	0.075g	1.5 g
$K_2HPO4.8H_2O$	--------	4×10^{-2}g
$MgSO_4{\cdot}7H_2O$	--------	7.5×10^{-2}g
$CaCl_2{\cdot}2H_2O$	--------	3.6×10^{-2}g
Citric acid	--------	6×10^{-3}g
Ferric ammonium citrate	--------	6×10^{-3}g
EDTA (disodium salt)	--------	1×10^{-3}g
$NaCO_3$	--------	2×10^{-2}g
$NaH_2PO_4.\ 2H_2O$	5.65×10^{-3}g	--------
Vitamin Mix		
Thiamine HCl (B1)	1×10^{-4}g	--------
Biotin	5×10^{-7}g	--------
Trace metal mix A5		
H_3BO_3		2.86×10^{-3}g
$MnCl_2{\cdot}4H_2O$		1.81×10^{-3}g
$ZnSO_4{\cdot}7H_2O$		2.2×10^{-4}g
$NaMoO_4{\cdot}2H_2O$		3.9×10^{-5}g
$CuSO_4{\cdot}5H_2O$		7.9×10^{-5}g
$Co(NO_3)2{\cdot}6H_2O$		4.94×10^{-5}g
EDTA (disodium salt)	4.16×10^{-3}g	
$FeCl_3.6H_2O$	3.15×10^{-3}g	
$CoCl_2.6H_2O$	1×10^{-5}g	
Agar (if needed)		**15.0 g**
Water		**1.0 L**

A. Selection of microalgae strains from QUCCCM

In this study, marine algae were selected on the criteria that they have been referred to as good candidates for protein production and used in aquaculture feeding in the literature (Walne, 1970, Bayne, 1976, and Witt, 1981). For convenience, I chose isolates we already had in the university culture collection of microalgae (QUCCCM). Two marine microalgae isolates that satisfied the criteria were: *Tetraselmis* and *Nannochloris*, which both of them are from the *Chlorophyta* division.

The stock cultures of both species were grown in separate BG-11 SW agar plate media and were kept under controlled environment in growth chambers (Versatile environmental test chamber, MLR-351/MLR-351H ,SANYO, Japan), at 25°C temperature and with a 16 hour light/8 hour dark cycle (fluorescent lamp FL40SS W\37) without CO_2 addition.

After seven days, culture that was grown in BG-11 SW agar plates transferred into 250 ml Erlenmeyer flasks contains 50 ml Gillard's F\2 liquid media. Then the pervious cultures transferred into larger Erlenmeyer flasks after a month with addition of 50 ml Gillard's F\2 liquid media. The last step was repeated after each month until it reaches to 500 ml culture that was used as inoculum in growth experiments under different culturing settings.

B. Examine the yield performance of the QUCCCM selected strains under different culturing settings.

The species cultured in Photobioreactors (parallel system for phototrophic cell process development PBR4, RS232/RS485, DASGIP. Germany), this self-contained system is a controllable environment in which to grow algae, and where the supply of light, nutrients, carbon dioxide, air, and temperature can be regulated. It also offers better control over a range of other growing conditions, like the pH (Figure 2: Photo of DASGIP® multi-controlling system photobioreactors for culturing photosynthetic organism.The two settings that were chosen was salinity and carbon dioxide enrichment due to their importance and direct effect in pond mass culturing. Additional settings were fixed such as temperature at

30° C, pH at 7-8, red and blue LED light at 100 µmol photon $s^{-1}m^{-2}$ (12:12 light and dark cycle), agitation speed at 300 rpm and CO_2 concentration at 5% (12:12 air and no air cycle).

Figure 2: Photo of DASGIP® multi-controlling system photobioreactors for culturing photosynthetic organism.

i. Methods

The characterization of growth and yield performance of selected QUCCCM strains were performed by two methods:

(1) Optical Density (OD750) Standardization

Standardization based on the OD750 (Becker, 1994) of *Tetraselmis* and *Nannochloris* stocks was carried out previous to salinity and CO_2 experiments.

First, stock culture of each species was centrifuged and supernatant discarded. Next, 45 ml of autoclaved and filter sterilized Gillard's F\2 was added to all stocks and the pellet was re-suspended. Then, 10 µl of each were taken and placed in a cuvette to measure

their OD750 in an (6850 spectrophotometer, Janway, USA), being Gillard's F\2 the blank in all cases.

Equation (1) was used to calculate the proper dilutions needed so that the initial

OD750 for all isolates in all conditions tested was the same at the beginning of the characterization experiments.

$$m_1 v_1 = m_2 v_2 \qquad (1)$$

Where *m1* equals the OD750 measured in the spectrophotometer, *m2* represents the concentration wanted in each well at the beginning of the experiment, *V2* represents the total volume that will be used for each well during the experiment and *V1* is the unknown, representing the amount of stock volume that needs to be added to accomplish a standard OD750. Finally, the amount of media needed per well is calculated by subtracting *V1* to the total reaction volume.

(2) Growth rate

The dry weights were used to calculate the growth rate of algae. 10 ml sample was taken after 3 days of culture at every day and dry weight were measured (NERL, 2008). The dry weight was found by placing the sample in a dry (60°C for 24 hours), filter papers with a 1.5µm pore size, 47 mm diameter glass microfiber filter (Whatman, UK), rinsed with distilled water, and dried at 60°C for 24 hours (Figure 3). The change in the weight of the filter papers with the addition of the rinsed algae after drying off all of the water was considered the dry weight.

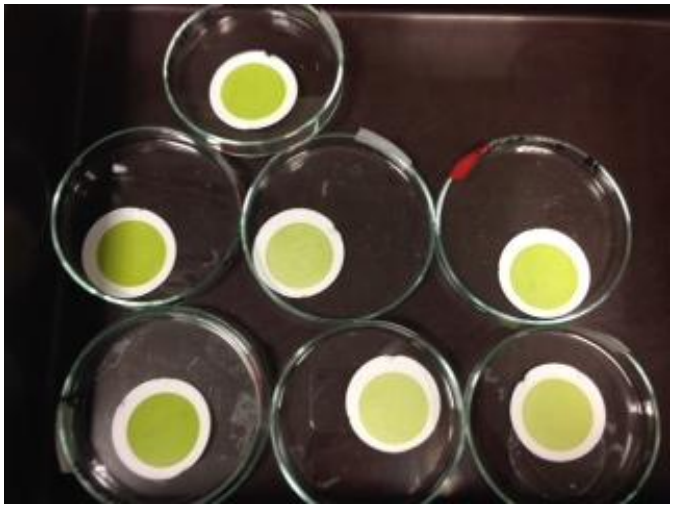

Figure 3: Photo of the filter papers algae samples after drying.

The specific growth rate (μ) is defined as (Shuler, 2002)

$$\mu = \frac{1}{x}\frac{dx}{dt} \qquad (2)$$

Where x is the concentration of algae (g/L) and t is the culture time (day). Hence, from concentration over time data, the specific growth rate can be determined by:

$$\mu = \frac{\ln\frac{x}{x_\circ}}{t} \qquad (3)$$

(3) Productivity

Productivity was calculated from dry weight by using the following equation (Levasseur, 1993)

$$productivity = \frac{x_2 - x_1}{t_2 - t_1} 04)$$

Where x_1 and x_2 = concentration of algae at time1 (t_1) and time2 (t_2) respectively.

ii. Experiments

(1) Salinity experiments

The two species *Tetraselmis* and *Nannochloris* was tested under three different salinity levels; 35 *psu*, 40 *psu* and 45 *psu*. The salinity of Gillard's F\2 liquid media was measured using refractometer (handheld refractometer, REF211ATC, OMEGA, USA). Each salinity level was tested in triplicate.

(2) Carbon dioxide concentration experiments

The two species *Tetraselmis* and *Nannochloris* was tested under three different CO_2 levels; 3 %, 5 % and 10 %. The carbon dioxide concentration was measured using (gassing system MF4, 78543058, DASGIP, Germany) Each CO_2 concentration level was tested in triplicate.

C. Biochemical characterization

Biomass obtained from salinity and CO_2 experiments were harvested and analyzed for total protein, total lipid and minerals content. The biomass was freeze-dried prior to analysis to remove all the moisture and the cells.

Protein

Total protein contents were measured from 2 mg dried biomass by total nitrogen content by elemental analysis (Perkin-Elmer Model 2 400, USA) calibrated using acetanilide as a reference standard). Samples were placed in tin capsules in the oven for combustion at 950 °C using pure oxygen (20 cm^3) as the combustion gas and pure helium as the carrier gas. Nitrogen was measured as N_2 using a thermal conductivity detection system. The

crude protein concentration of the microalgae was estimated according to the following equation: ‘‘protein concentration = nitrogen content × 6.25’’ (Becker, 1994).

Lipid

10 mg of dried biomass is tested for total lipid by Folch method (Folch, 1957) for quantitative extraction of lipids using a chloroform-methanol mixture and a phase partition with water which resulted in quantitative extraction of tissue lipids and removal of water-soluble contaminants.

Minerals

Inductively Coupled Plasma/Optical Emission Spectrometry (ICP-optical) is used to determine concentration of several macro and microelements from a 0.1g sample. (optical emission spectrometer optima 7300DV, Perkin Elmer).

CHAPTER IV: RESULTS and DISCUSSION

Examine the yield performance of the QUCCCM selected strains under different culturing settings.

A. Growth calibration curve

Growth assessment of microalgae cultures is a key procedure to establish culture phases and level of biomass available for potential harvesting. Nevertheless dry weight determination is an impractical and sometime inconsistent methodology, essentially due to filtration process that is time consuming and there is a risk of weight salt particles if filters were not properly washed. In order to make the work easier optical density measured at 750 nm (750nm measures cells density regardless to the effects of sample color) was correlated with dry weight measurements for both considered strains and for all experimental settings (Figures 4 to 7). However correlation levels between optical density and dry weight depends on several aspects, such as the size and shape of the microalgae cells, the wavelength of incident light and particles opacity (Rocha *et al*, 2003).

The result suggests a significant ($p<0.05$) correlation between optical density and dry weight in all treatments. Obtained correlations are significant with a r^2 of 0.883 in *Tetraselmis*, and 0.755 in *Nannochloris* for the salinity experiments.

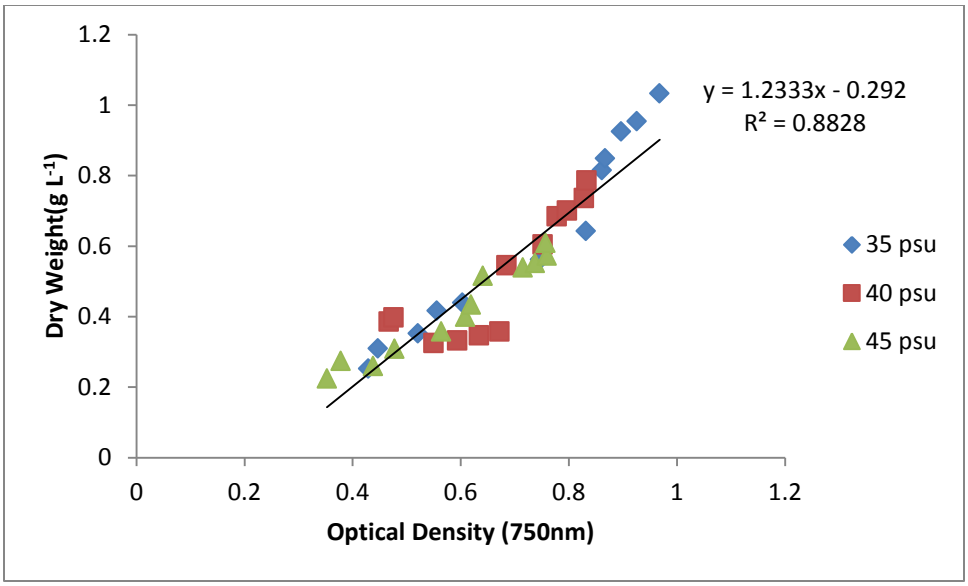

Figure 4: Regression of dry weight (biomass) vs. optical density in *Tetraselmis* cultures under differential salinity treatments.

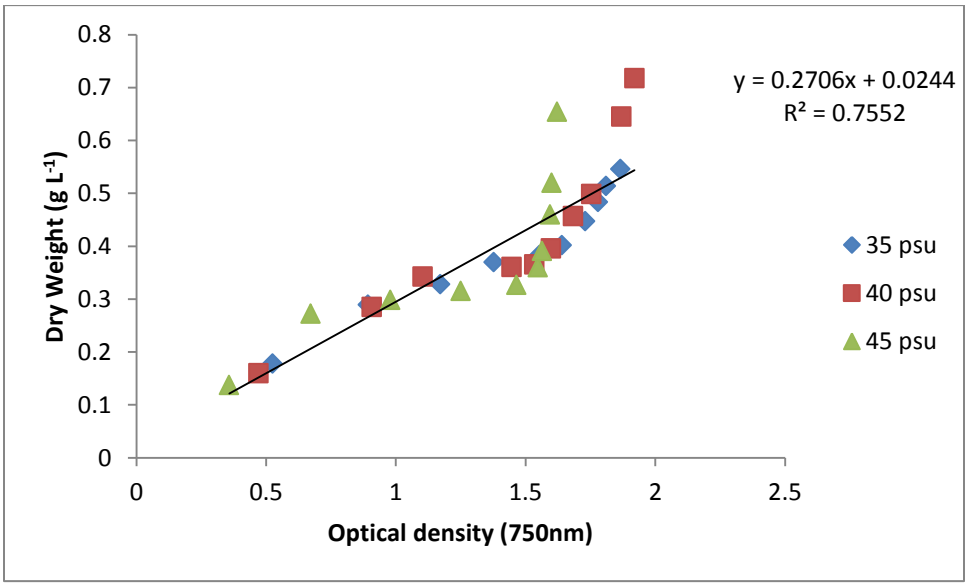

Figure 5: Regression of dry weight (biomass) vs. optical density in *Nannochloris* cultures under differential salinity treatments.

Similarly the obtained correlations for the CO_2 experiments are significant with r^2 = 0.862 for *Tetraselmis*, and 0.872 for *Nannochloris* (Figure 6 and 7). This finding permits the estimation of the dry weight in a culture from optical density measures. The error associated with this estimation may be explained with the probable interference of other factors. According to these results, an increase in optical density and in cellular

concentration represents an increase in biomass, since dry weight is related to biomass quantity.

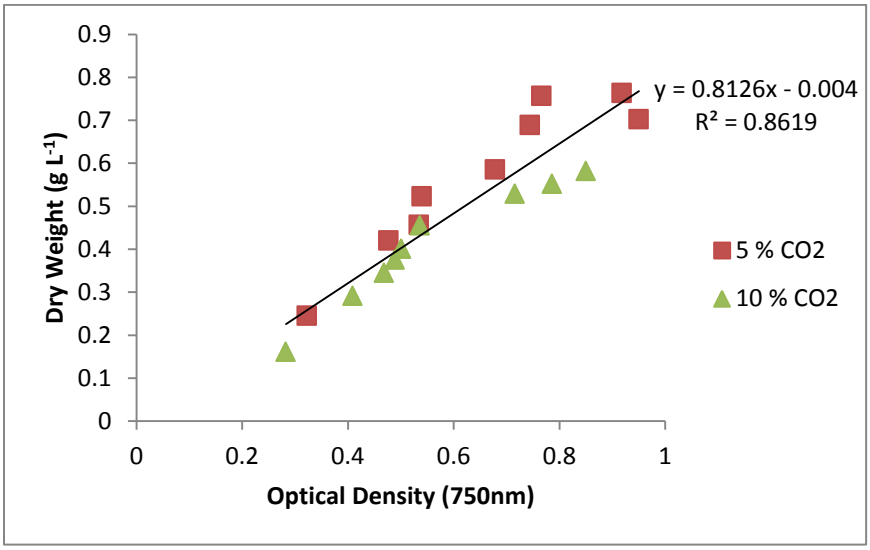

Figure 6: Regression of dry weight (biomass) vs. optical density in *Tetraselmis* cultures under differential CO_2 treatments.

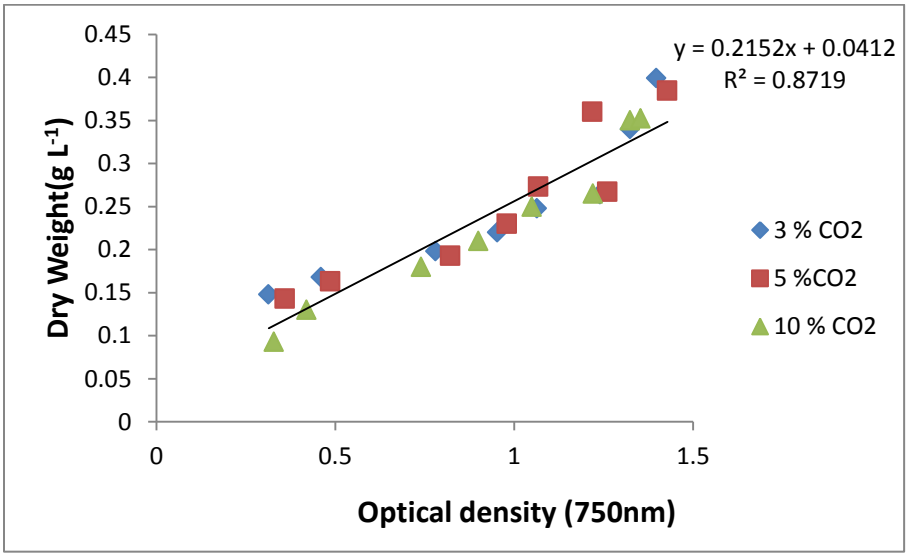

Figure 7: Regression of dry weight (biomass) vs. optical density of *Nannochloris* cultures under differential CO_2 treatments.

These correlations confirmed that optical density values can be used to efficiently assess biomass levels in the cultures without substantial laboratorial work.

B. Salinity experiment

Generally, marine microalgae reveal high tolerance and adaption to wide range of salinities (McLachlan, 1961). Marine microalgae adjust their internal osmotic potential to adapt to wide range of salinities by changing the internal concentrations of glycerol. In response to salinity stress, cells undergo changes in their morphological and developmental features as well as physiological and biochemical processes (Sudhir and Murthy, 2004).

Results on dry weight (g L^{-1}) during the experimental period (days), for each species (*Tetraselmis* and *Nannochloris*) and their productivity (g $L^{-1}day^{-1}$) in cultivations under different salinity levels are reported here below.

1. Tetraselmis

The best salinity condition in terms of dry weight was found to be 35 psu 1.03 g L^{-1} (Figure 8 : Growth rate of salinity experiment using *Tetraselmis*. Three levels of salinity were tested 35psu, 40psu, and 45psu.. A similar favorable 35 psu salinity was obtained by Fabregas *et al*. (1984) and Renaud & Parry (1994) for the same genus. Productivity and biomass was decreased significatively ($p<0.05$) with the increase of salinity to 40 psu and further significant ($p<0.05$) decrease in 45 psu due to high salinity stress that limit the growth.(Figure 9: Productivity (g L-1day-1) of *Tetraselmis* in salinity experiment using three levels (35psu, 40psu and 45psu). .

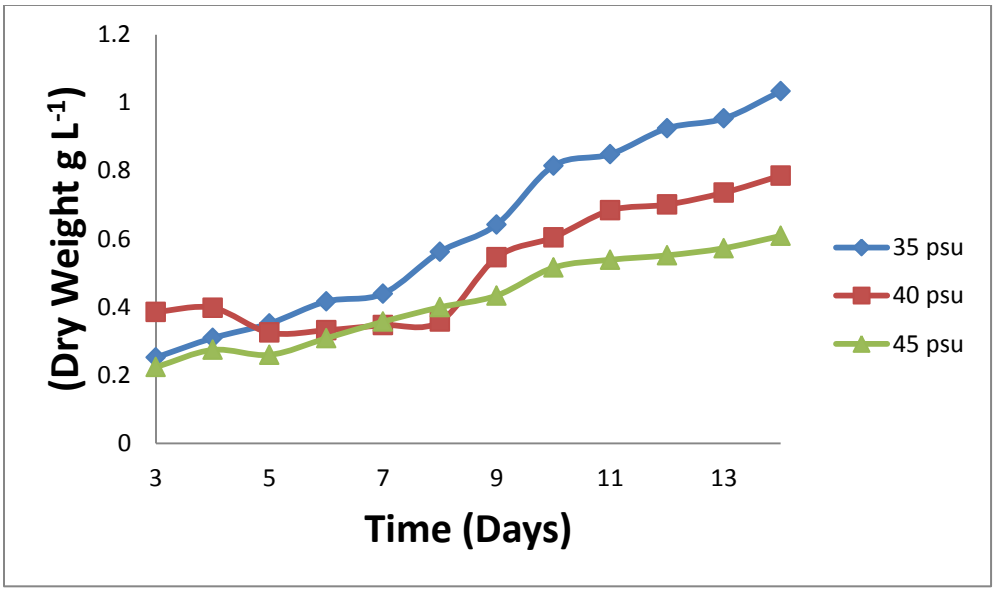

Figure 8 : Growth rate of salinity experiment using *Tetraselmis*. Three levels of salinity were tested 35psu, 40psu, and 45psu.

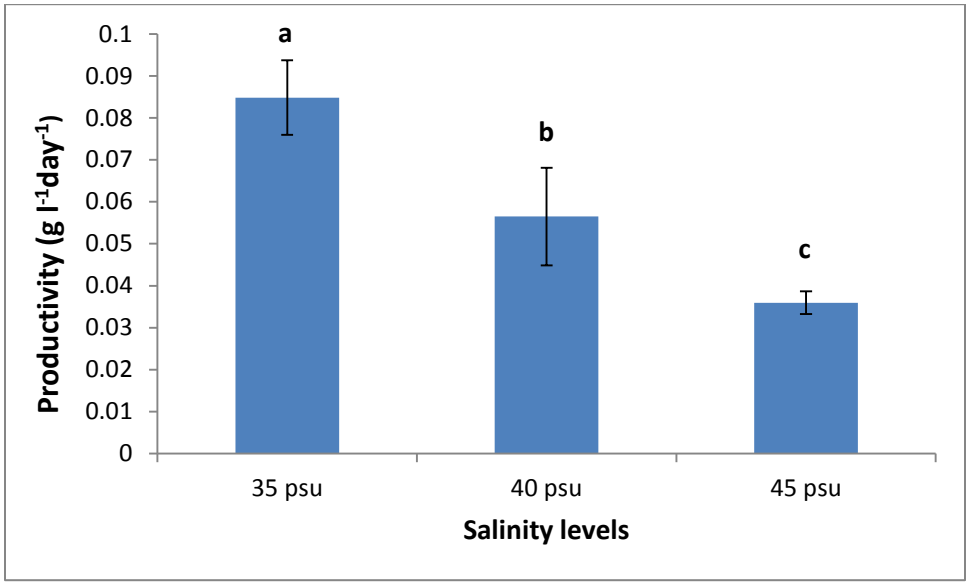

Figure 9: Productivity (g $L^{-1}day^{-1}$) of *Tetraselmis* in salinity experiment using three levels (35psu, 40psu and 45psu).

2. *Nannochloris*

The best salinity conditions were found to be 40 psu 0.718 g L^{-1} dry weight (Figure 10: Growth rate of salinity experiment using *Nannochloris*. Three levels of salinity were tested 35psu, 40psu, and 45psu. , suggesting higher tolerance than *Tetraselmis* strain to relatively higher salinities. Productivity and biomass was decreased significatively ($p<0.05$) at 35 psu and 45 psu. (Figure 11: Productivity (g L-1day-1) of *Nannochloris* in salinity experiment using three levels (35psu, 40psu and 45psu). .

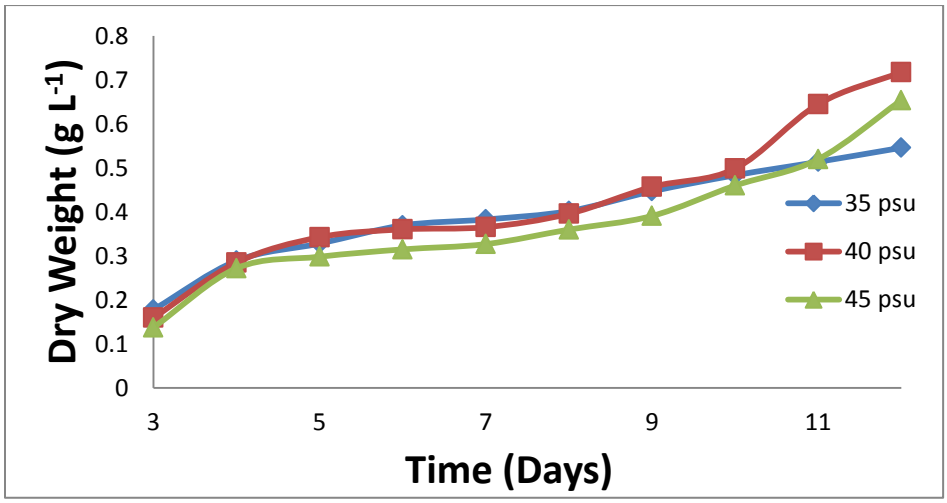

Figure 10: Growth rate of salinity experiment using *Nannochloris*. Three levels of salinity were tested 35psu, 40psu, and 45psu.

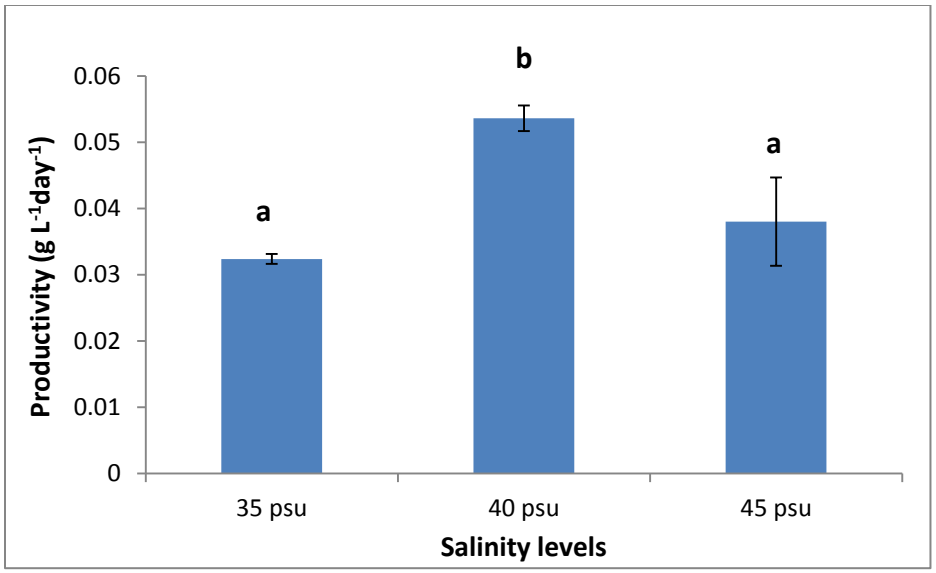

Figure 11: Productivity (g $L^{-1}day^{-1}$) of *Nannochloris* in salinity experiment using three levels (35psu, 40psu and 45psu).

C. CO_2 enrichment experiments

Aeration with adequate mixture of air and carbon dioxide is crucial to maintain microalgae cells in suspension, provide carbon dioxide as carbon source, and remove photosynthetically produced oxygen (Geider *et al.*, 1986). Several studies including Matsumoto *et al*. (1995) found that *Tetraselmis* can tolerate maximally 14% CO_2.

CO_2 is used as buffer to control pH of the culture beside acting as a carbon source. Lower pH effect photosynthysis by decreasing the activity of ribulose 1,5-bisphosphate carboxylase-oxygenase, a key enzyme of photosynthesis (Jensen and Bahr, 1977); causing inhibition of microalgal growth.

1. Tetraselmis

For *Tetraselmis*, the CO_2 condition was found to be 5 % CO_2 with 0.764 g L^{-1}dry weight. The results in our present study showed that CO_2 enrichment below 5 % was limiting for the growth of *Tetraselmis*. While, CO_2 enrichment above 5% may be likewise harmful to

microalgal cells and inhibit their growth. This may be explained by a lower tolerance to lower pH values since high enrichment of CO_2 can result in low pH in the culture medium (Tang *et al*., 2011).

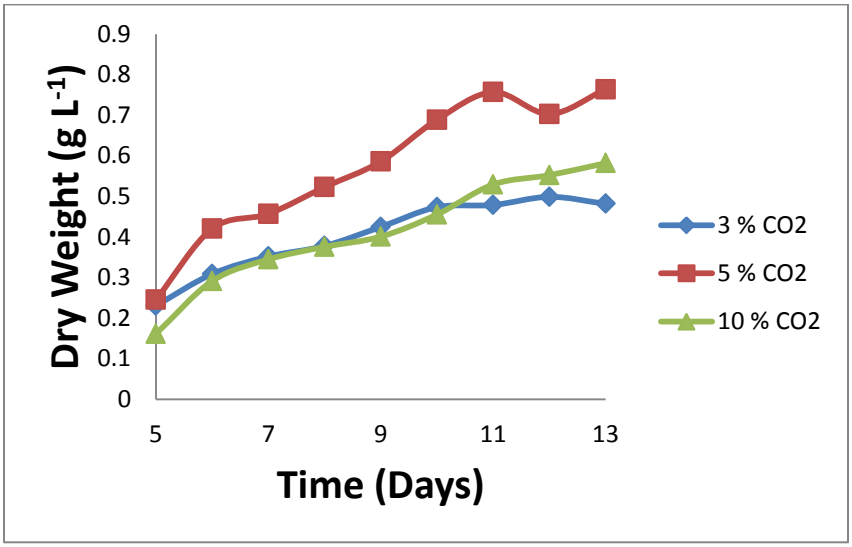

Figure 12: Growth rate CO_2 enrichment experiment using Teraselmis. Three levels of CO_2 were tested 3 %, 5 %, 10 %.

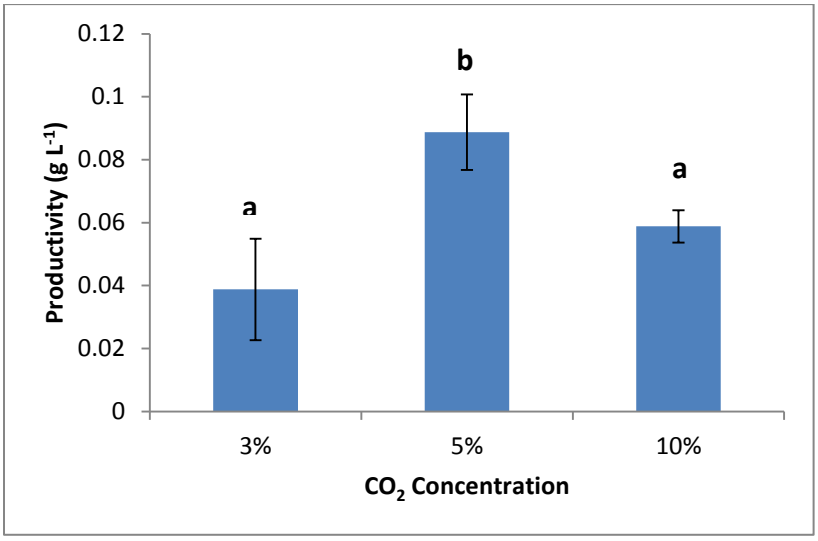

Figure 13; Productivity (g $L^{-1}day^{-1}$) of *Tetraselmis* in CO_2 enrichment experiment under three levels of CO_2 (3 %, 5 % and 10 %).

2. *Nannochloris*

For *Nannochloris*, the best CO_2 condition was found to be 3 % CO_2 with 0.40 g L^{-1}dry weight. Productivity and biomass was decreased with the increase of salinity to 10 % and 5 %; indicating that higher enrichment of CO_2 reduce the pH of the culture which effect the enzymatic activity of the cells. The results showed that *Nannochloris* was less tolerant to higher CO_2 enrichment than *Tetraselmis*.

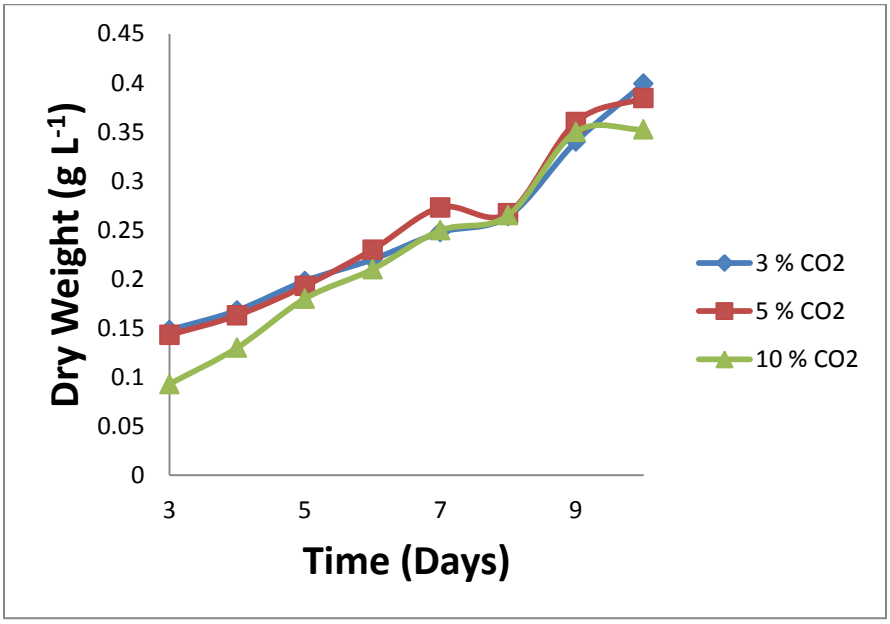

Figure 14: Growth rate for CO_2 enrichment experiment of *Nannochloris* using three levels of CO_2 were tested 3 %, 5 %, 10 %.

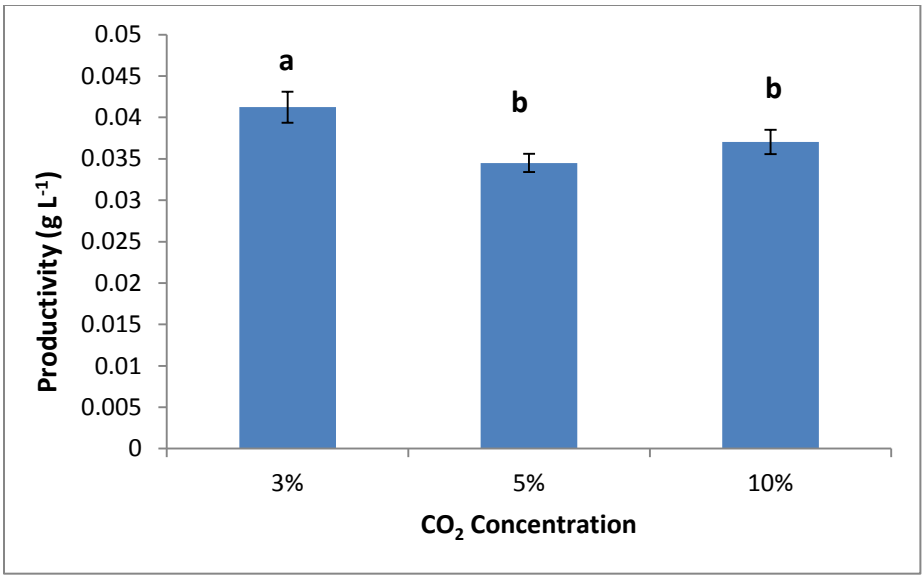

Figure 15: Productivity (g $l^{-1}day^{-1}$) of *Nannochloris* in CO_2 enrichment experiment using three levels of CO_2 (3 %, 5 % and 10 %).

Table 4: Table demonstrates specific growth rate and productivity of the two species in salinity and CO_2 experiments.

	specific growth rate (day^{-1})						Productivity (g L^{-1} day^{-1})					
Species/ treatment	Salinity			CO_2 %			Salinity			CO_2 %		
Level	35 psu	40 psu	45 psu	3%	5%	10%	35 psu	40 psu	45 psu	3%	5%	10%
Tetraselmis	0.122	0.111	0.081	0.094	0.147	0.151	0.085	0.056	0.036	0.039	0.089	0.059
Nannochloris	0.075	0.099	0.111	0.187	0.145	0.06	0.032	0.054	0.038	0.041	0.035	0.037

A comparative analysis of specific growth rate and productivity among both strains suggest that the best salinity condition were found at 35 psu for *Tetraselmis* with specific growth rate of 0.122 day^{-1} and 0.085 g $L^{-1}day^{-1}$, and the best salinity condition were found to be 40 psu for *Nannochloris* with specific growth rate of 0.099 day^{-1} and 0.054 g L^{-1} day^{-1}. The best CO_2 concentration condition for *Tetraselmis* was found at 5% CO_2 with specific growth rate of 0.111 day^{-1} and 0.089 g L^{-1} day^{-1} and the best CO_2 condition

for *Nannochloris* was found at 3% with specific growth rate of 0.075 day^{-1} and 0.041g L^{-1} day^{-1}.

Biochemical characterization

Protein and lipid

Protein content in microalgae is controlled by several factors. Protein content is higher in most microalgae in the exponential development stage. Also environmental factors regulate the uptake of nutrient and direct the cells into specific metabolic pathways to achieve balanced growth (Stross and Pemrick, 1974).

During stress conditions, microalgae preferable metabolic pathway change from protein synthesis mostly to various energy-rich compounds like carbohydrates and lipids and there is a competition between syntheses of such compounds (Siaut *et al*., 2011). Lipids and carbohydrates are the preferred storage products in various stress conditions because they are hydrophobic in nature, have highly reduced states, are efficiently packed in small compartment of cells and can also be used during adverse conditions for cell survival and proliferation (Courchesne *et al*., 2009).

In Table 5, protein content was higher in *Tetraselmis* biomass at a salinity of 35 psu and 3% CO_2 enrichment. While, *Nannochloris* protein content is maximum at 40 psu and 3 % CO_2.

Table 5: Comparative table of protein and lipid ratio of *Tetraselmis* and *Nannochloris* in response to salinity and CO_2 enrichment experiments.

Species	Treatment	Level	Lipid/Protein
Tetraselmis	**Salinity**	**35 psu**	**1:1.38**
		40 psu	**1:1.03**
		45 psu	**1:0.95**

	CO_2 %	3%	1:1.12
		5%	1:1.72
		10%	1:1.21
Nannochloris	Salinity	35 psu	1:0.20
		40 psu	1:0.16
		45 psu	1:0.16
	CO_2 %	3%	1:0.39
		5%	1:0.22
		10%	1:0.28

D. Salinity experiments

In *Tetraselmis*, the protein content was slightly higher than lipids in 35 psu and 40 psu. While, the lipids content was higher in 45 psu; indicating that salinity above 40 psu stress the cells and direct them to produce more lipids in expense of protein.

Comparing *Nannochloris* with Tetraselmis showed that in all salinity levels *Nannochloris* produce more lipid than protein in contrast of *Tetraselmis*. The highest protein content with the lowest lipid content in *Nannochloris* was in 35 psu salinity; indicating that 40 psu and 45 psu are considered high stress conditions for *Nannochloris* as it can be seen from productivity results too.

E. CO_2 concentration experiments

In *Tetraselmis*, the lipid content in 5 % CO_2 concentration was lowest and the protein was the highest indicating that it is the preferable CO_2 concentration among the 3 % and 10%. Insufficient carbon dioxide was implementing stress on the cells; leading them to produce higher lipids and lower protein than preferable 5 % CO_2. On the other hand, 10% CO_2 was stressing the culture by reducing the pH from 7 to 6. As a result less protein was produced in comparison to 3 and 5 % CO_2 concentrations.

Nannochloris produce more lipids than protein in all the experimental conditions. The highest lipid and lowest protein was found in 5 % CO_2. Lower 3 % CO_2 was more favorable and synthesize more protein from 5 % and 10 %. In 10%, the protein was higher the 5 % and the lipid was lower due to change in biochemical composition without change in productivity.

F. Nutritional properties

Not only proteins produced from microalgae are highly valued product but also lipids are valuable as they contain the three essential fatty acids (FA); Arachidonic acid (AA), Eicosapentaenoic acid (EPA), and Docosahexaenoic acid (DHA). The three essential fatty acids are part of polyunsaturated Fatty Acids (PUFA) are reported to produce the best growth for juvenile oysters (*Ostrea edulis*: Enright *et al*., 1986) and larval scallops (*Patinopecten yessoensis*: Whyte, 1989). High dietary protein provides best growth for juvenile mussels (*Myths trossulus*: Kreeger and langdon, 1993).

In the current study, *Tetraselmis* was exhibiting high protein and low lipid synthesis in contrast from *Nannochloris*. *Nannochloris* exhibit higher amount of polyunsaturated fatty acids (PUFA) than *Tetraselmis*. (Unreported data)

We suggest using a mixture of *Tetraselmis* and *Nannochloris* to provide better nutritional diet for animals as it's reported that a mixture of carefully selected microalgae offer better nutritional value than mono-species diet (Brown, 2002).

G. Minerals

Not only microalgae are considered a valuable source of protein or lipid, but also it contains a significant mineral content (e.g. Na, K, Ca, Mg, Fe, Zn and trace minerals). (Gouveia, 2008).

In the current study, we tested several minerals (macroelements) and trace minerals (microelements) in *Tetraselmis* and *Nannochloris* in response to salinity and CO_2 concentration changes (Table 6 & 7).

- **Macroelements**

Generally macroelements concentration showed similar values within experiments (Table 6). The concentration of minerals increases with the increase of productivity.

Results showed that *Tetraselmis* significantly under the same conditions contain more Calcium (2787-15733 ppm) than *Nannochloris* (1630-1881 ppm). Potassium under the same conditions was found more in *Nannochloris* significantly (7455-10145 ppm) than *Tetraselmis* (1330-8078 ppm).

At high salinity, cells accumulate the minerals to compensate the increase of salinity on the cells. This is suggested by the higher concentration of minerals (Na, K, Mg, and Fe) in both *Tetraselmis* and *Nannochloris*.

In *Nannochloris*, salinity has higher impact on mineral profile than CO_2 where salinity influences the osmoregulatory process of cells under salinity stress.

We observe differences in the mineral concentration among the salinities under different treatment. In the CO_2 concentration treatment, we don't observe an effect of CO_2 in the media on the composition of minerals uptake by microalgal cells. This is due to absence of osmosis stress caused by salinity increase.

- **Microelements**

For microelements (heavy metals) concentration showed similar values within experiments under the same conditions for both *Tetraselmis* and *Nannochloris* except zinc profile in response to the increase of CO_2 (Table 7). In the treatments with both *Tetraselmis* and *Nannochloris*, zinc concentration increased with the increase of CO_2 concentration. Zinc uptake impacts the carbon-concentrating mechanism as this effect is highlighted by (Malasarn, 2013).

Overall, the concentration of heavy metals was high and that may be due to the sea water that was used for cultivation. Indeed the used filtered seawater to run the cultivation in laboratory was collected from a coastal region (Al-Khor) that may be polluted from adjacent industrial activities. Further investigation is needed to test the sea water before using it in culturing microalgae and avoid high heavy metals in the final microalgal biomass.

We suggested earlier that using *Tetraselmis* and *Nannochloris* as feed mix is promising option to obtain protein and lipid (specifically PUFA) content. In addition mineral profile of the two species showed different pattern which by combining them in a feed mix provide a balanced mineral content shows great potentiality to prevent mineral deficiency to the animal.

Table 6: Composition of Macroelements in *Tetraselmis* and *Nannochloris* under different culture conditions. Values from the same set of experiment preceded by different letters are considered significatively different (t-test, p<0.05)

			Al	Ca	K	Mg	Na	Fe
			Avg	Avg	Avg	Avg	Avg	Avg
Tetraselmis	Salinity experiment	35 psu	88.05 a	15490.09 a	1807.8 a	1526.55 a	400.56 a	482.17 a
		40 psu	245.22 a	6478.67 a	6340.7 a	2991.04 b	2655.55 b	6934.01 ab
		45 psu	589.65 b	15733.03 a	1913.8 a	2556.57 b	991.40 b	1571.03 b
	CO_2 experiment	3 %	162.77 a	2787.33 a	8078.4 a	2939.37 a	2270.24 a	1073.10
		5 %	87.07 a	14375.97 b	1411.8 b	1941.95 b	1557.31 a	409.83
		10 %	134.66 a	12199.45 b	1330.4 b	2343.84 ab	1337.59 a	647.61
Nannochloris	Salinity experiment	35 psu	84.80 a	1630.62 a	7455.4 a	3170.52 a	2759.95 a	623.47 a
		40 psu	164.66 ab	1757.03 a	7857.2 a	3955.49 a	6369.96 a	1122.41 a
		45 psu	251.95 b	1675.71 a	8108.9 a	3659.13 b	3174.57 a	1450.51 a
	CO_2 experiment	3 %	189.05 a	1985.72 a	8470.5 a	3416.24 a	2682.07 a	1536.60 a
		5 %	180.43 a	1802.45 a	8229.9 a	3406.43 a	2220.26 a	1429.89 a
		10 %	215.94 b	1881.67 a	10145.1 a	3757.19 a	1878.24 a	1113.83 a

Table 7: Composition of Microelements in *Tetraselmis* and *Nannochloris* under different culture conditions.

			Zn	Cd	Cr	Cu	Mn	Pb	As
			Avg	Avg	Avg	Avg	Avg	Avg	Avg
Tetraselmis	Salinity experiment	35 psu	**39.99**	**0.16**	**49.02**	**15.77**	**18.10**	**2.37**	**0.43**
		40 psu	**24.78**	**12.40**	**1232.82**	**43.69**	**75.33**	**6.72**	**0.61**
		45 psu	**42.56**	**0.67**	**83.37**	**28.72**	**23.37**	**5.93**	**0.80**
	CO_2 experiment	3 %	**10.21**	**0.67**	**60.71**	**20.56**	**13.21**	**4.90**	**1.21**
		5 %	**31.59**	**3.47**	**28.66**	**17.10**	**15.43**	**3.21**	**1.25**
		10 %	**45.78**	**1.12**	**68.50**	**20.14**	**13.86**	**3.75**	**0.70**
Nannochloris	Salinity experiment	35 psu	**11.18**	**0.20**	**26.05**	**24.16**	**9.05**	**1.72**	**1.15**
		40 psu	**13.79**	**0.87**	**67.56**	**25.08**	**14.06**	**3.29**	**0.83**
		45 psu	**17.12**	**1.52**	**87.84**	**32.81**	**13.31**	**4.38**	**2.35**
	CO_2 experiment	3 %	**14.48**	**1.62**	**674.74**	**27.98**	**48.49**	**3.35**	**1.55**
		5 %	**10.93**	**0.68**	**68.65**	**18.40**	**14.96**	**3.71**	**0.81**
		10 %	**22.10**	**0.21**	**50.69**	**27.22**	**16.67**	**5.09**	**1.70**

* Concentration of heavy metals is considered high. Further investigation needed for sea water used in culture media.

CHAPTER V: CONCLUSION

- High correlation was found between the different records of optical density and dry weight. Optical density is suggested to be an easy and reliable method to assess the available biomass in the culture.
- Cultivation of *Tetraselmis* under 35 psu and 5% CO_2 allowed better performance when compared with other salinities (i.e. 40 psu and 45 psu) and CO_2 enrichment conditions (i.e. 3% and 10%). *Nannochloris* showed higher growth and productivity at 40 psu and 3% CO_2 cultivation conditions.
- Similarly, protein contents were found to be higher in *Tetraselmis* biomass at a salinity of 35 psu and 3% CO_2 enrichment. While *Nannochloris* protein content were found maximum at 40 psu and 3 % CO_2 in compassion to other salinities and CO_2 enrichments.
- *Tetraselmis* species is a promising source of protein for feed production. Additional blending of *Tetraselmis* by *Nannochloris* may enhance the nutritional quality of the produced feed.
- Generally, macro and microelements are not affected by the changes in salinity and CO_2 enrichment. *Tetraselmis* biomass showed high concentrations of Calcium [2787 to 15733 ppm], while *Nannochloris* biomass showed high concentration of Potassium [7455 to 10145 ppm].
- Zinc uptake was increased with the increase of CO_2 enrichment.
- Future work to be developed includes investigation of amino acids and fatty acids profile of both strains biomass under salinity and CO_2 enrichment conditions. In addition, up-scaling of the experimental unit from 1L photobioreactor into larger

unit would help to assess the economic viability of large scale production of microalgae biomass for feedstock.

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