

**EFFECT OF CO₂ CONCENTRATION AND
TEMPERATURE ON GROWTH RATE AND LIPID
CONTENT OF *Isochrysis galbana***

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**Izmir Institute of Technology
December, 2012**

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TEMPERATURE ON GROWTH RATE AND LIPID
CONTENT OF *Isochrysis galbana***

**A Thesis Submitted to
the Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of**

MASTER OF SCIENCE

in Food Engineering

**by
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**December 2012
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ABSTRACT

EFFECT OF CO₂ CONCENTRATION AND TEMPERATURE ON THE GROWTH RATE AND LIPID CONTENT OF *Isochrysis galbana*

The lipid production and biomass growth of microalgae *Isochrysis galbana* was investigated under the effects of different medium temperature and carbon dioxide levels. Algae production was studied in ten-liter, open-air, aerated bioreactors and sampling was done daily to monitor the cell growth. At the end of each fermentation, the dry mass, lipid amount and fatty acid profiles were determined. The algae were grown according to designed experiments (central composite design (CCD)) at 15, 22.5 and 30 °C medium and aerated with air containing 5, 10 and 15% v/v CO₂. It was observed that in 15 °C experiments, the fatty acid profile was formed mainly by unsaturated fatty acid (both mono and poly unsaturated) and the amount of saturated fatty acids increased by increased CO₂ levels. The saturated fatty acid content increased with increasing growth temperature. Dominantly observed fatty acids are Pentadecanoic Acid (C15:0), Pentadecanoic Acid (C15:0), Palmitic Acid (C16:0), Linolenic Acid (C18:3), Oleic Acid (C18:1). In the algal productions performed at 22.5 °C and 30 °C medium conditions, the fatty acid content was mainly formed by saturated fatty acids. However, the amount of lipid produced increased with increasing temperatures. The highest cell growth was seen at 15 °C experiments. The lowest growth rate was observed at 30° C experiments.

It was observed that the medium temperature had the largest effect for the production of saturated and unsaturated fatty acids. The carbon dioxide was found effective linearly on the fatty acid production.

ÖZET

CO₂ KONSANTRASYONU VE SICAKLIĞIN *Isochrysis galbana*'nın BÜYÜME HIZI VE YAĞ MİKTARI ÜZERİNDE ETKİSİ

Bu çalışmada, farklı CO₂ seviyelerinde ve farklı ortam sıcaklıklarında *Isochrysis galbana* mikroalginin biyokütle büyümesi ve yağ üretimi incelenmiştir. Alg üretimi on litrelik, üzeri açık, havalandırılmalı biyoreaktörlerde gerçekleştirilmiş, hücre büyümesini gözlemek için günlük örnekleme yapılmıştır. Fermantasyonun sonunda, kuru kütle, yağ miktarı ve yağ asitlerinin içerikleri saptanmıştır. Algler 15, 22.5 ve 30 °C sıcaklıklarda ve havalandırma için hacimce 5, 10 ve %15 içeren CO₂ hava ile büyütülmüştür.

Isochrysis galbana nın 15 °C sıcaklıkta serbest yağ asidi üretiminin ağırlıklı olarak doymamış serbest yağ asitleri (tekli ve çoklu doymamış) olduğu . ve doymuş yağ asidi miktarının artan CO₂ seviyesi ile çoğaldığı gözlemlenmiştir. Doymuş yağ asidi miktarı 22.5 ve 30 °C ortam sıcaklıklarında gerçekleştirilen üretimlerde artmıştır. Pentadecanoik Asit (C15:0), Pentadecanoik Acid (C15:0), Palmitik Asit (C16:0), Linolenik Asit (C18:3), Oleik Asit (C18:1) ağırlıklı olarak gözlenen yağ asitleridir. 15 °C ortam sıcaklığı, *Isochrysis galbana* türünün büyümesi için düşük bir sıcaklıktır. Fakat sadece bu sıcaklık değerinde doymamış yağ asidi üretimi en yüksek seviyededir. En düşük büyüme oranı 30 °C deneylerde gözlemlenmiştir.

Tepki yüzey metodunda kullanılan merkezi birleşik tasarımın sonucu olarak, ortam sıcaklığının doymuş ve doymamış yağ asitlerinin üretiminde en büyük etkiye sahip olduğu görülmüştür. Karbondioksitin ise yağ asidi üretiminde doğrusal etkisi olduğu bulunmuştur.

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CHAPTER 1

INTRODUCTION

One-third of the world's plant biomass is algae. Eukaryotic microalgae formerly known as blue-green algae and *cyanobacteria* represent all photosynthetic prokaryotic and eukaryotic microorganisms like all other algal groups. Because of this characteristic, microalgae production systems are similar. Nowadays algal biotechnology continues to evolve rapidly with increasing importance of microalgae and interest in these organisms. This new focus of research emerge the evaluation of many types of substances derived from different species of algae since the earliest studies.

A Microalgal biotechnology study has been initiated in the early 1950s and attracted the attention of sectors (Sason 1997). These species have economic value and also has wide range of applications. The best quality, increase yield and economic value can be obtained by characteristics of microalgae production within environment. Different species studied in world-wide studies has been widely used in various photo-bioreactors (Gökpınar 1990).

Microalgal species or plankton is the main producers of organic matter in aquatic environments by photosynthesis and they are the most important manufacturer of organic matter in the aquatic habitat. Microalgae can use CO₂, nutrient salts, organic matter, trace elements and convert energy from the sun through photosynthesis. These planktonic forms of organisms cannot move on their own, and suspended and moves with the flow of marine and fresh water. Planktonic forms, which constitute the members of the phytoplankton plant species, are found as single cells or colonies.

Microalgae, belonging to various taxonomic groups, are microscopic single-celled, multi-celled, planktonic or benthic algae. Microalgae are the first staple of shellfish and fish larvae of aquatic animals. In addition it has a great importance for feeding other fish staples like rotifers, *Cladocera*, or copepods (Timur 1992).

Microalgae can produce not only biomass but also complex molecules necessary for life activities with the assistance of any light source (sun or artificial light); by combining various sources such as nutrient salts and CO₂.

It is not clear when human first started to take advantage of microalgae farming, but nearly for the last 100 years they have been in use. The first known pure culture (bacteria-free) dates back to in 1890 by Beijerinck (Andersen 2005), although it is not clearly known. Following this study, in 1910, Allen and Nelson isolated the cultures of *Phaeodactylum* type *tricornutum* (Cirik 1993). Yet microscopic algae are commercially available for only about last 40 years. Nowadays algal production are started to be used in new industries in waste water treatment and energy conversion to biomass. In addition algae cultures are widely used in aquaculture and larval production plants widely. These applications become so successful that in other applications as marine fish farming in the production of phytoplankton species, fish and crustacean larvae, and in young stages of bivalve molluscs used as a source of nutrition (Brown et al.1997). Microalgae first used as indirect feeding in production in fishery products, molluscs and crustaceans. Other than being used as feed for the larvae presence of microalgae also has other uses. Algae indirectly limit the growth of bacteria and reducing the burden of improving the quality of water by nitrogen and phosphorus limitation larval fish cultivation tanks and improving immunological response (Moretti *et al.* 1999).

Phytoplankton species play a crucial role in the food chain as a key nutrient in aquatic environments. These species use ammonia, urea, nitrate and phosphate nutrients, vitamins, trace elements and using some form of organic matter formed by chemical breakdown of dead cells (Gökpinar 1990). The marine algae cultures have a great importance in the economy both in the laboratory and in the natural environment. This importance stems from a wide variety of available application in public areas. Algae have been using as a source of nutrients and algae derived products in chemical industry, medicine, pharmacy and dentistry (Atay 1982).

Phytoplankton or zooplankton is the first step in the food chain for the most marine fish farming as feedstock. For this reason, proper feeding of marine fish larvae production of phytoplankton and zooplankton has a great importance. Phytoplankton production in Turkey, especially fish, sea bream and sea bass larvae is an important step in fish growth (Atay 2000).

Problems in marine fish farming, as well as the culture of oysters and shrimp solved with the development of single-celled algae production methods. Techniques in this problem and how they overcome this problem in Japan stated in detailed and analyzed for shrimp larvae feed by Timur et al.

In any of the applications the main objective of phytoplankton production is to reach the highest cell density in minimum time (Gökpınar 1983). Several factors can affect the growth of phytoplankton cells. These include light, heat, physical factors such as ventilation-mixing, mineral salts, the carbon dioxide gas, chemical factors such as pH and salinity. Among these factors, CO₂ metabolism and algal growth are one of the most important ecological factors. Yet other physical and chemical factors such as temperature, quality and amount of nutrition or duration of sunlight are also identified as other critical factors. And all these information forms the basis of system design and operational parameters for establishing an efficient culture.

Photosynthesis in the cells increased due to CO₂ and results in increasing the capacity of growth rate of synthesis (Gökpınar 1983). Microalgae as aquaculture is not only used to feed the cultures but also important in ensuring the balance of carbon dioxide and oxygen.

To date, much of the research carried out on phytoplankton cultures and natural populations was carried out to understand the environmental conditions suitable for growth. Specific physiological and biochemical studies using planktonic algae cultures for algae applications as organic materials; as a source or as a food supplement for uses for many marine rearing larvae have been evaluated (Gökpınar 1990).

Considering the studies in the literature, in this study the effect of different temperature conditions and CO₂ feed on the biomass and lipid production and fatty acid profile was investigated through the controlled experiments. *Isochrysis galbana* was chosen for its high oil content up to 23 – 37 % dry weight, high reproduction rate, reliability and sustainability in standardized commercial growing systems. In the following chapters details on experimental studies, results and discussions on findings were stated.

CHAPTER 2

ALGAE GROWTH AND LIPID PRODUCTION

Microalgae biomass contains products with high commercial importance like proteins, lipids, carbohydrates and pigments (Torzillo 2004).

The success of commercial production of microalgae is based on many factors. One of them is a large-scale algal culture system, the development of less expenditure efficient way to get the product. The optimal development of each species of algae shows its own specific culture media that meets the requirements.

Photosynthetic growth requires light, carbon dioxide, water and inorganic salts. The basic elements such as nitrogen, phosphorus, iron must be provided to ensure the growth of algae cells. The microelements such as nitrate and phosphate are usually supplemented with sea water to grow microalgae (Grima 1998).

Dry weight of microalgal biomass contains about 50% carbon, is obtained by carbon dioxide (Miron 2003). Carbon dioxide is continuously fed during the daytime hours. For the production of microalgal biomass through the use of large-scale pools and daytime light is required. Almost 25% of the biomass produced during the night due to respiration during the day is lost. It is largely due to the difference between growth temperature and environmental temperature during night time (Grima 1998).

Currently algae are used as fish feed. Bivalve molluscs, penaeid, shrimp and marine fish hatcheries use microalgae as larvae feed. The cultured species in clear tanks or ponds and are either harvested through the ponds. On a large scale Alga culture has an importance on typing of aquaculture in some places (McHugh 2003). Yet microalgae are a promising alternative source for many applications in wide range of areas for instance agriculture, pharmaceuticals, energy production, pollution control and various other uses. Micro-algae have been cultured for many years both pharmaceutical and for health food purposes but never in a large scale commercially. The lipids contained in some species of microalgae are very similar to vegetables making it very suitable for to be produced. Algae have many advantages compared to traditional agriculture. Competitive demand on energy crops and food is one of the main global debates of the last decade. Algae have major advantages that can be answer for this problem. First of

all do not require soil for growth, thus algae do not compete with agricultural products. Furthermore they have high production per acre yield and they have less water consumption compared to land crops. And even algae are potentially more cost effective than conventional farming for many applications because of their comparably higher growth rate.

Envisaged applications of commercially produced algae are very wide. In food industry algae are used as a support from lack of food proteins in children and adults. In food industry algae is widely used as nutrition supplements In order to support healthy food products and their usage as a wealthy food products. Algae are used directly or as a bioactive compound source. Applications of algal based B-carotene, fikobilin or glycerol applications in the treatment of skin cancer and the use of isotopic compounds of medical used in research in medicine, in food coloring, cosmetic, fertilizer, analytical reagent and even biodiesel production are common examples in industry (Becker 1995). A generalized chemical content of microalgae is shown in Figure 2.1.

Microalgae are used by humans in many ways as fertilizers, soil conditioners and livestock feed. Microalga is consumed especially in Asia as food source. They provide many vitamins including: A, B1, B2, B6, niacin and C, and are rich in iodine, potassium, iron, magnesium and calcium (Simoons 1991) besides commonly cultivated microalgae, including both and cyanobacteria, are sold as nutritional supplements, such as Spirulina, (Morton 2008) Chlorella and the Vitamin-C supplement, Dunaliella, which are high with beta-carotene content. Including fat choy, a cyanobacterium considered a vegeTable; Japan (Mondragon 2003). It is also used along the west coast of North America from California to British Columbia, in Hawaii and by the Maori of New Zealand. Another application in food industry is sweetening by vegetarianism by containing the long-chain, essential omega-3 fatty acids, Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA), in addition to vitamin B12. The oils from some microalgae have high levels of unsaturated fatty acids (Bigogno 2002). Algae has arisen in last years as common source of omega-3 fatty acids for vegetarians who cannot get long-chain EPA and DHA from other vegetarian sources such as flaxseed oil, which only contains the short-chain Alpha-Linolenic acid (ALA) (Nesheim 2007). And also agar, a gelatinous material extracted from microalgae is a number of uses especially in chemistry (Lewis 1988). It is a proper medium for bacteria (McHugh 2003).

Algae also help to end the competition between energy crops and food. Algae based fuels has great strategic importance and it is directly related to the potential to

produce more biomass per unit area in a year traditional agricultural plants. The break-even point for algae-based bio fuels is estimated to occur in about ten to fifteen years because of lack of petroleum (Barbosa 2011). Further algae contain no sulfur therefore it does not cause any SO₂ emissions

Algae can also be used for pollution control. Algae can be used gas effluent treatments. High CO₂ tolerance of algae in gas streams allows high – efficiency CO₂ mitigation due to and algae can reduce industrial emissions of CO₂ can be productively through these processes. Another application of algae is waste water treatment. They can be both used to reduce contaminants or particles in the water by adsorption and absorption mechanisms by fixing these contaminants in produced algae. These applications are now mostly used in aquariums and ponds can be filtered using algae, which absorb nutrients from the water in a device called an algae scrubber, also known as an "ATS". According to the Agricultural Research Service researchers, it was founded that 60 – 90% of nitrogen effluent and 70 – 100% of phosphorus effluent can be captured from manure effluents using. Researchers found that algae can be used to reduce the nutrient runoff from agricultural fields and increase the quality of water flowing into water sources as river or lakes. Further nitrogen included household waste can be disabled using microalgae. It can be reduce the effect of pollution ratio.

Algae produced in these processes can be used as feedstock in their other applications currently available. Algae have been used as an alternative to chemical dyes and coloring agents by including natural pigments (Arad 1998). Another example for other applications is stabilizing substances. Carrageenan, from the red alga *Chondrus crispus*, is used as a stabilizer in milk products. Further algae have been implemented in the production of biodegradable plastics by Cereplast, Inc. An agreement has also been reached with the US Military to introduce more biodegradable plastics as it attempts to move away from petroleum based plastics and utilize more environmentally friendly alternatives (Andersen 2005).

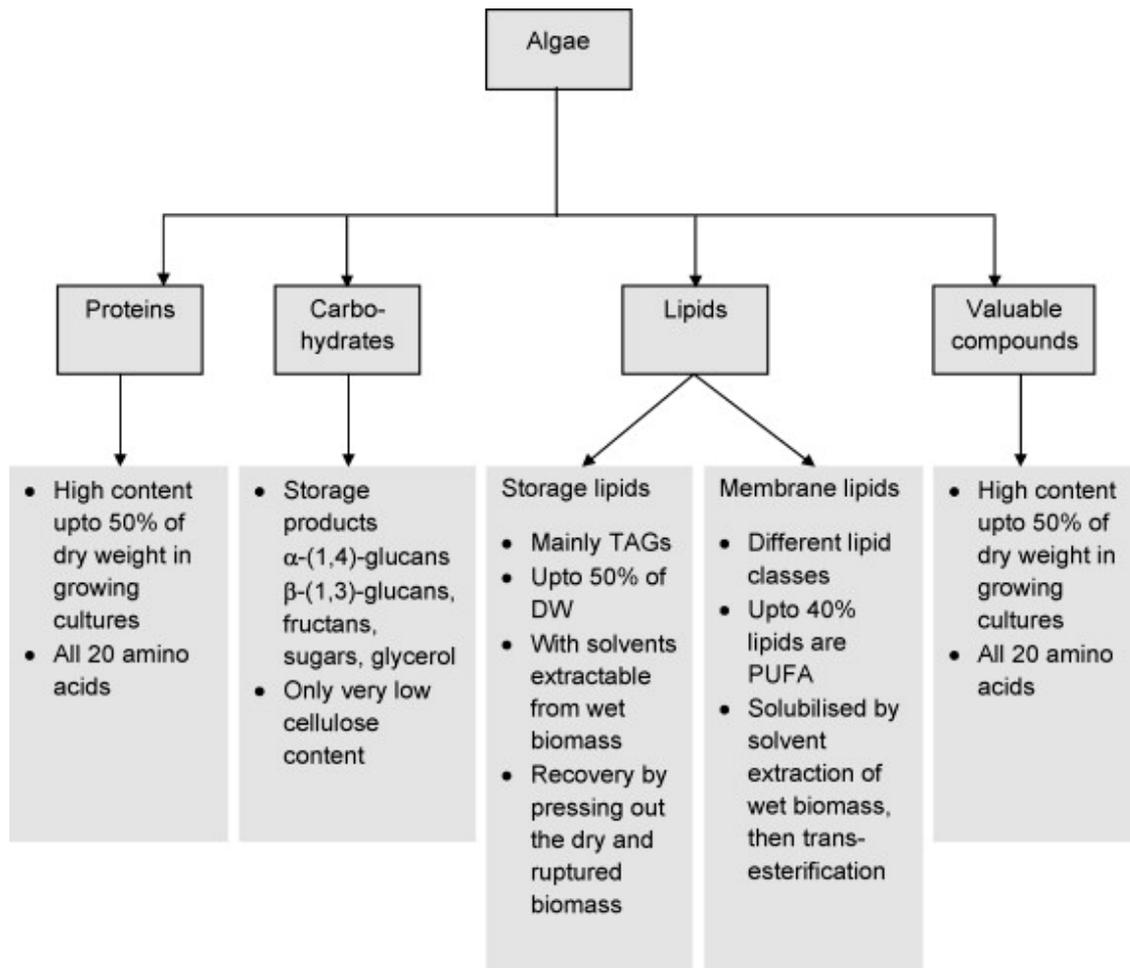


Figure 2.1. Generalized chemical contents of microalgae
(Source: Zhu 1997)

2.1. Algal Growth

The lifetime of algae has 4 main phases. These phases' covers lag phase, exponential phase, stationary phase and death phase. In lag phase little increase in cell density occurs. Following this phase in the exponential phase the cell density starts increasing as a function of time according to a logarithmic function. Then in stationary phase the limiting factors and the growth rate are balanced so no increase or decrease is observed. In death phase cell density decreases rapidly and the culture eventually collapses as seen in Figure 2.2. Parameters affecting algae growth are detailed in this section.

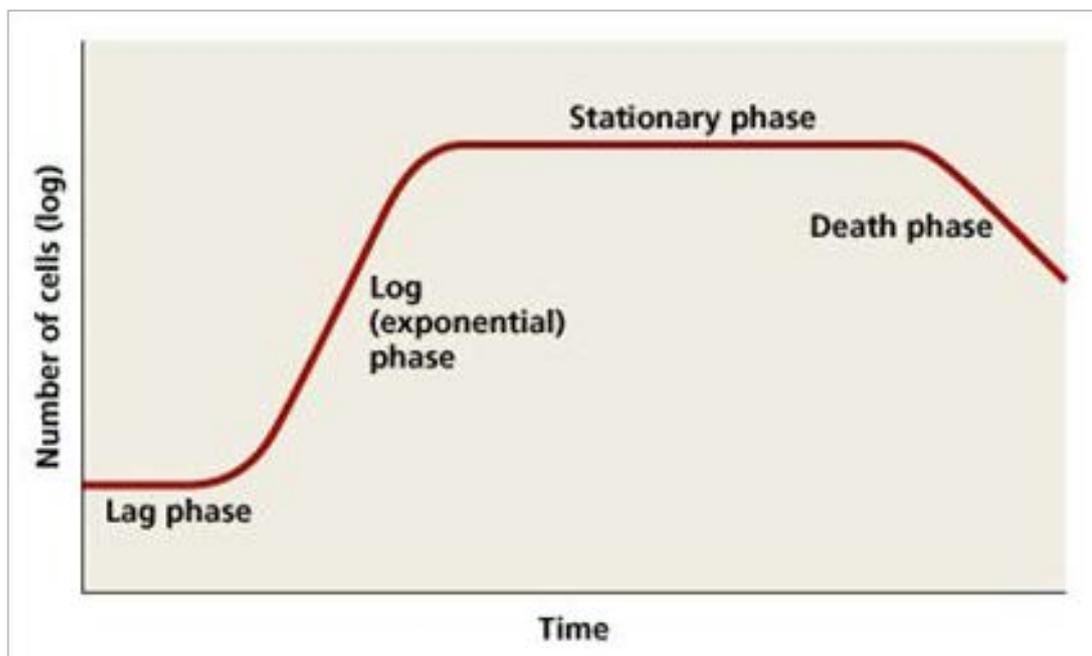


Figure 2.2. Life time of an algal growth.
(Source: FAO 2008)

The most important parameters regulating algal growth are nutrient quality and quantity, light, pH, turbulence, salinity and temperature. The optimum parameters and tolerance ranges changes with species. The ranges of the most important growth factors are provided in Table 2.1. Parameters that are optimal conditions generated media studies may not be optimal for another study (Coutteau 1996).

Table 2.1. Physical and chemical conditions for *Isochrysis galbana* reported in the literature (J. Marchetti 2012, Morais 2007, Yoshioka 2012).

Parameters	Range	Result
Temperature (°C)	16 – 27	18 – 24
Salinity (g.L ⁻¹)	12 – 40	20 – 24
Light intensity (lux) (depends on volume and density)	1,000 – 10,000	2,500 – 5,000
Photoperiod (light: dark, hours)	16:8 (minimum)	24:0 (maximum)
pH	7 – 9	8.2 – 8.7

Among these factors light is a mandatory parameter to sustain the vitality of photosynthetic organisms. Photosynthetic growth of each cell is proportional to the energy of the light used for the productions of the light should be provided at optimum levels. Light intensity plays a very important role in the culture and the culture related to the density and depth of the container, to ensure penetration of the light intensity with high concentrations of deep and cell cultures is increased. The light source may be natural or artificial. Fluorescent lamps typically used for artificial lighting. For cultures artificially illuminated, lighting time should be 18 hours minimum per day (Coutteau 1996). The growth accelerates with the increase of light during growth of photosynthetic organisms. At a certain point, increases the amount of light reaches a level of saturation. From this point on, the cells release energy as heat. By continuing to the high light levels, deterioration of the balance of the organism due to the high amount of energy produced irreversible inhibition occurs, and damages in the phase of photo-inhibition occur. Light inhibition, the effect starts to show in a few minutes, and in some cultures may damage more than 50% in 15 – 20 minutes (Torzillo 2003, Pulz 2001, Graham 2009, Tanaka 1995).

Being living organisms microalgae are affected from different temperature ranges. Temperature has a direct impact on their metabolism and physiological activity. Therefore, considering the optimum temperature of the cells must perform the species to be produced. Especially most of the commercial produced algae live in warm temperature range (Cohen 1989). Temperature increases when the respiratory rate increases therefore, increased respiration leads to loss of biomass. Temperature is effective on the chemical compositions of microalgae (Tortizillo 1991).

Another important environmental factor is pH. All species reproduce specifically to specific pH range. PH of the culture medium for many algal species is between in the range of 7-9. The optimum range varies from 8.2 to 8.7. Lack of appropriate pH for culture causes to break down of cells content by passing through the environment and leads to the death of culture, the increase in pH occurs over time in very dense cultures (Coutteau 1996).

Outside temperature and light, mixing plays one of the most important roles in growing. Mixing keeps medium away from sedimentation. Thanks to mixing, uniform distribution is provided. Thus, all cells may benefit from light and nutrients equally. Therewithal intense mixing may damage flagella so some species may be damaged because of intensive mixing (Becker 1995, Coutteau 1996). CO₂ concentration in the air

is % 0.03 at sea level but it is not optimal for highest productivity (Becker, 1995).because of instant growing phase of algae, absorption of CO₂ or bicarbonate (soluble form of CO₂ in water) from medium let of by the media due to OH-ions so that the pH tends to increase. PH must be stabilized in order to stop depletion between optimum values (Becker, 1995).

Although CO₂ and O₂ in air are crucial for algae other nutrients such as macro nutrients, micro-elements and vitamins are necessary for Phototrophic production of microalgae. It is a known fact that, of each species in the production of various substances needed by different concentrations nutrient. Microalgae requirement depends on the environmental and physiological parameters. According to (Vonshak 1986), factors in the development of media for the production of algal nutrients are summarized below.

- Total salt concentration
- Carbon source
- Selection of suitable and economical source of nitrogen.
- Concentration of the major elements such as; Potassium, magnesium, sodium, sulfate, and phosphate.
- PH value of the environment.
- Trace elements in the environment must be in.
- The addition of organic compounds and growth promoting substances. (Vitamins, hormones, etc.).

In theory, the microalgae are autotrophic, heterotrophic and photoheterotrophic and each species may in the industrial production of these types of food available (Borowitzka 1999, Becker 1995). The focus of this study, lipid production from algae will be detailed in the next section.

2.1.1. Raceway Ponds

Depth of the raceway pond, which is usually light, will include a closed-loop recirculation channel. Mixing and circulation generated by a paddlewheel. However, in open systems efficient production of microalgae is very difficult to live in the selective environments. Recently, in the important applications of the pharmaceutical and

cosmetic industry, especially given the quality and purity of microalgae products need to be open systems include high standards in production that cannot be captured (Borowitzka 1999, Andersen 2005, Moraes 2002).



Figure 2.3. A raceway pond.
(Source: Patringenaru 2009)

In addition, the use of light as a technique to yield the depth of the culture system should not be too much larger areas of open space they occupy as seen in Figure 2.3. There are also some disadvantages additions to the benefits of open systems. Vary during the year of production and production efficiency all year round to meet the operating expenses. For Raceway ponds, no cooling is achieved not only by evaporation. In these systems, seasonal temperature variations occur. Consisting of water loss through evaporation is very important. This reduces the effect of loss of CO₂. Other disadvantages the risks of contamination and other pollution.

2.1.2. Closed Systems

Closed systems are widely used in other production systems. In these systems, microalgae culture cut contact with external environment and the atmosphere.

Closed systems, especially in the emerging needs of industrial markets pigments and oils used in obtaining valuable microalgal products. They provide a controlled environment for a full contamination from the external environment that may photobioreactors of different microorganisms provide a protective shield against the risk of interference environment. Closed systems are designed to ensure that encountered to prevent these problems;

- To ensure that effective use of light and the surface to volume ratio,
- The opportunity to work in high density cultures,
- High spatial and volumetric production,
- Providing Balanced, high-quality and stable production,
- To prevent the risk of contamination,

Be allowed to transfer to a high rate of CO₂ (Olaizola 2003, Pirt 1983, Torzillo 1991).

All of these designs reduce the light path, so that the basic rule is to provide the necessary access to all the cells in the light. In addition, the distribution of light in these systems, providing good mixing and gas transfer can provide optimum conditions (Borowitzka 1999, Pulz 2001, Torzillo 2003).

CHAPTER 3

LITERATURE REVIEW

Algae have been known as a potential source of lipids due to their short growth period. Observations have shown that lipid production from agricultural plants would only meet 50% of only transport fuel demands even if 24% of the total cropland were used for this purpose. On the other hand, lipids gained from microalgae may meet 50% of the fuel demand with only 3% of the total cropland. Since research has shown that the oil content in microalgae can exceed 80% of the biomass it shows how microalgae have the potential for high oil productivities (Christi 2007). The Table 3.1 shows the comparison of the lipid yield for some sources.

Table 3.1. Comparison of some sources of biodiesel
(Source: Christi 2007)

Crop	Oil yield (L/ha)
Corn	172
Soybean	446
Canola	1190
Jatropha	1892
Coconut	2689
Palm	5950
Microalgae	58.700 – 136.900

Microalgae adapt to environmental conditions by reacting with changing their biochemical composition. “Especially, algae resist to conditions to adapt to changing conditions and stresses in their environment. Various types of lipids act as structural components of the plasma membranes and organelles of the cells and signaling components in the intracellular communication pathways. As part of membranes, lipids provide barriers to separate the various compartments within the cells.” (Thompson 1996). During photosynthesis, lipids are observed extensively around membranes of

chloroplast. In addition, chloroplast proteins require lipids cells to be able to combine during light-harvesting mechanism. Environmental stress conditions may cause changes in production of certain lipids for protecting the cell. Lipids have vital importance in renewability of the light harvesting mechanism. Lipids are metabolized within algae and some green algae, in particular as energy hosts in the form of triglycerides (Thompson 1996). Table 3.2. represents the oil content of some microalgae.

Table 3.2. Oil content of some microalgae
(Source: Christi 2007)

Microalga	Oil content (% dry wt)
<i>Botryococcus braunii</i>	25 – 75
<i>Chlorella sp.</i>	28 – 32
<i>Cryptocodinium cohnii</i>	20
<i>Cylindrotheca sp.</i>	16 – 37
<i>Dunaliella primolecta</i>	23
<i>Isochrysis sp.</i>	25 – 33
<i>Monallanthus salina N</i>	20
<i>Nannochloris sp.</i>	20 – 35
<i>Nannochloropsis sp.</i>	31 – 68
<i>Neochloris oleoabundans</i>	35 – 54
<i>Nitzschia sp.</i>	45 – 47
<i>Phaeodactylum tricornutum</i>	20 – 30
<i>Schizochytrium sp.</i>	50 – 77
<i>Tetraselmis sueica</i>	15 – 23

It is important to choose the right micro algal specie and growth parameters for high lipid production. For this study microalga *Isochrysis galbana* was chosen to study the effect of temperature and CO₂ Tolerance.

Microalgal cells at different growth stages and environmental conditions show significantly different fatty acid compositions (Emdadi, 1989). These changes among population can be caused by many culture conditions including nutrient status,

temperature, salinity, pH, photoperiod, light intensity and light quality (Yongmanitchai 1989, Roessler, 1990). *Isochrysis* cell structure has attracted the attention of many researchers. Earlier studies (Green 1977, Hori 1985) on *Isochrysis galbana* mainly focused on flagellar root systems. Cells were motile ellipsoidal, usually solitary, and: 5-6 microns long and 2.5-3 microns thick were found to be 2-4 microns wide. About 7 microns long, the length is approximately equal to the two smooth flagella, and also added to the cells with shortened haptonema (Liu 2001).

Effect of many parameters on growth studied in the literature. The effect of light, salinity, and nitrogen on lipid production by *Nannochloropsis sp.* studied by Pal et al and reported that salinity and light intensity was especially important food lipid production under nitrogen depletion (Pal 2011). Further in another study Chiu et. all shows that other than these parameters high CO₂ low lipid content gained needed to optimize the condition for long-term biomass and lipid yield from *N. Oculata* (Chiu 2009). Similarly optimization studies for *Isochrysis sp.* (J. Marchetti 2012, Yoshioka 2012) are also carried. These studies shows that frequency of intermitted light was affects growth and fatty acid profile The light condition did not affect the ratio of lipid classes or the fatty acid composition of total lipids, neutral lipids, glycolipids, or phospholipids from *I. galbana*. The amounts of neutral lipids, glycolipids, and phospholipids obtained from culture medium were the highest under blue intermittent light. The highest amounts of phospholipids and DHA were recovered from *I. galbana* cultured under blue intermittent light. Another study on that effect of nutrient for *Isochrysis galbana* by Lin et all reports that; the ratio of SFA+MUFA to PUFA was highest in NH₄NO₃ modified Walne culture and minimal in Walne culture. They also reports the effects of temperature and growth phase on glycolipids grown at 15 °C results in higher monounsaturated and saturated fatty acids.

Ultrastructural study and lipid formation of *Isochrysis sp. CCMP1324*, The DHA yield increased with cultivation time until the eighth day. DHA amounts in the cells were reached under aeration with 10% CO₂ (Liu 2001). These studies and their experimental conditions are given in Table 3.3

Table 3.3. Selected studies from the literature with experimental conditions.

Species	Growth medium	Light	T (°C)	Gas feed	Reference
<i>Nannochloropsis sp.</i>	nitrogen (0.8-1.4 g/l)	Continuous	25	2% CO ₂ in air.	Pal 2011
<i>Isochrysis galbana</i>	F/2.	12:12 L: D cycle.	20	Air	Lee 2011
<i>Nannochloropsis oculata</i>	F/2	NA	26	2-15% CO ₂ in air	Chiu 2009
<i>Isocrysis galbana</i>	F/2	NA	25	Air	Liu 2001
<i>Nannochloropsis sp.</i>	F/2	Continuous	27	1.5% CO in air	Wu 2001
<i>Nannochloropsis sp.</i>	NA	NA	NA	Air	Wiyarmo 2011
<i>Nannochloropsis gaditana</i>	F/2	Continuous	23	Air	Simionato 2012
<i>Spirulina sp.</i>	Zarrouk	14:10 L:D cycle.	30	20 mM/(L d) CO ₂	Zeng 2012
<i>Isochrysis galbana</i>	F/2	12:12 L:D cycle.	20 °C	Air	Yoshioka 2012
<i>Isochrysis affinis galbana</i>	F/2	Continuous	30	Air	Marchetti 2012
<i>Synechocystis sp.</i>	F/2	Continuous	22-39	Air	Martinez 2010

Isochrysis galbana has been used as a marine culture feed due to its high content of protein and polyunsaturated fatty acids (PUFAs) (Jeffrey et al. 1994). *Isocrysis galbana* is marine planktonic algae, belonging to these systematic;

- › *Eukaryota*
- › *Haptophyceae*
- › *Isochrysidales*
- › *Isochrysidaceae*
- › *Isochrysis*

The reported characteristics of *I. galbana* are;

- High lipid content up to 15 – 40 % dry weight.
- High CO₂ tolerance up to 2 – 20 % concentration.
- Short growth duration.
- Growth capacity at high cell concentrations.
- Tolerance for different temperature limits.

3.1. Effect of CO₂ Concentration on the Growth of *Isocrysis galbana*

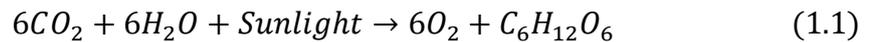
Increased CO₂ in the atmosphere of greenhouse gases is a serious problem for humanity so much. Recently, a lot of research and development approach to reduce CO₂ emissions being studied for use. Various processes in reducing CO₂ emissions have been used; and using the photosynthetic microalgae is considered as a technology that can be applied widely to produce. Made to reduce flue gas CO₂ and photosynthetic algae of various microalgae culture started to become an attractive technology. The microalgal species should be selected for production and growth under high – CO₂ concentration needs to be high. Produced by microalgae carbohydrates, fats and proteins, with the energy, chemicals and foods as well as biomass can be converted. Vitamins and pigments are widely used energy-rich biomass in the production of a fuel source (liquid or gas), health foods and as animal feed is used (Negoro. 1991).

3.2. Effect of Temperature on the Growth of *Isocrysis galbana*

Plants produce sugar and oxygen from carbon dioxide, water and sunlight. This process is called photosynthesis. Photosynthesis is a series of chemical reactions. Heat speeds up chemical reactions by adding kinetic energy to the reactants. Therefore, heat speeds up photosynthesis, unless another factor, such as weak light, limits photosynthesis. Initially, increasing temperature causes net photosynthesis to increase. More kinetic energy leads to more collisions between the reactants in photosynthesis. Cellular respiration increases in the same way, at the same time, for the same reason. Then, net photosynthetic activity reaches a peak. Increasing cellular respiration has limited the accumulation of photosynthetic products. Denatured enzymes do not yet limit photosynthesis. If they did, then cellular respiration also would be limited. Finally, too much heat denatures the enzymes that catalyze both photosynthesis and cellular respiration. Photosynthesis is severely limited, as is cellular respiration.

3.3. Photosynthesis Reaction of *Isocrysis galbana*

Photosynthesis is a metabolic process that converts light energy into chemical energy in the form of carbohydrates. All reactions that occur during photosynthesis can be summarized as follows;



Photosynthesis membranes are the center of the light reactions of Tillakoid. During photosynthesis, light energy, is held by the photosynthetic pigments such as chlorophyll, and carotenoids fikosiyanobilin. All of these pigments are available in many different conjugated bonds. This bond connects the pigments in the visible region of electromagnetic radiation and radiation interactions allow efficiently absorbed. Spectral properties of pigments affect chemical composition as well as the surrounding environment protein structures. Typically, the complex protein pigments allow to more efficient absorption of light energy is broadened bands (Mohanty 1997).

Photosynthesis consists of two separate phases. Light reactions relates to light energy capture, conversion of ATP, and the reduction of energy. This event place with the help of light to transport takes. $NADP^+$ into H_2O electrons. The transportation of electron reactions in the cell membranes, which are scattered tillakoid are placed on. Unlike higher plants, in microalgae, these membranes have not been organized in grana and stroma. These reactions occur in the cytoplasm (Mohanty 1997).

Light absorbed into pigments with antennae, photosystem I and Photosystem II. Activation energy of the reaction carried the load separation starts in center of photosystem. Secondary electron current is reduced to a variety of components of the photosynthetic electron transport chain that is carried out. ATP and NADPH formed in this manner, is used for fixation of CO_2 (Mohanty 1997).

CHAPTER 4

MATERIAL AND METHODS

4.1. Materials

Chemicals used in this study and their details are given in two groups: chemicals used in fatty acid analysis and in growth medium and chemicals are listed in Tables 4.1 and 4.2 respectively.

Table 4.1. Properties of materials used in analysis and extraction.

Chemical	Purity	Producer and Product Code
NaOH	98%	<i>S8045 SIGMA-ALDRICH</i>
Hexane	95%	<i>296090 SIGMA-ALDRICH</i>
MeOH	99.9%	<i>34860 SIGMA-ALDRICH</i>

The *Isochrysis galbana* used in this study (Microalgal strains 100-IGA00) were obtained from Algae Depot Company (Wisconsin, USA). Sea water was obtained from Ege University Fisheries Faculty Urla Facilities (Izmir, Turkey).

Table 4.2. Properties of materials used in growth medium.

Chemical	Purity	Producer and Product Code
NaNO ₃	99.0%	S5022 <i>SIGMA-ALDRICH</i>
NaH ₂ PO ₄ ·H ₂ O	98.0-102.0%	S9638 <i>SIGMA-ALDRICH</i>
Na ₂ SiO ₃ ·9H ₂ O	44-47%	307815 <i>SIGMA-ALDRICH</i>
FeCl ₃ ·6H ₂ O	97%	236489 <i>SIGMA-ALDRICH</i>
Na ₂ EDTA·2H ₂ O	99.0-101.0%	E5134 <i>ALDRICH</i>
CuSO ₄ ·5H ₂ O	99.99%	451657 <i>SIGMA</i>
Na ₂ MoO ₄ ·2H ₂ O	98%	243655 <i>SIGMA-ALDRICH</i>
ZnSO ₄ ·7H ₂ O	Reagent grade	32047 Fluka
CoCl ₂ ·6H ₂ O	98.0%	60818 <i>SIGMA-ALDRICH</i>
MnCl ₂ ·4H ₂ O	99%	244589 <i>ALDRICH</i>
Vitamin B12	98.5%	V2876 <i>SIGMA</i>
Biotin	99%	B4501 <i>SIGMA-ALDRICH</i>
Thiamine HCl	Reagent grade	51483 <i>FLUKA</i>
FeCl ₃ ·6H ₂ O	97%	157740 <i>SIGMA-ALDRICH</i>
Na ₂ EDTA·2H ₂ O	99.0-101.0%	E5134 <i>SIGMA</i>
CuSO ₄ ·5H ₂ O (9.8 g/L dH ₂ O)	99.99%	451657 <i>ALDRICH</i>
Na ₂ MoO ₄ ·2H ₂ O (6.3 g/L dH ₂ O)	98%	243655 <i>SIGMA-ALDRICH</i>
ZnSO ₄ ·7H ₂ O (22.0 g/L dH ₂ O)	Reagent grade	32047 <i>FLUKA</i>
CoCl ₂ ·6H ₂ O (10.0 g/L dH ₂ O)	98.0%	60818 <i>SIGMA-ALDRICH</i>
MnCl ₂ ·4H ₂ O (180.0 g/L dH ₂ O)	99%	244589 <i>ALDRICH</i>
NaNO ₃ (75.0 g/L dH ₂ O)	99.0%	S5506 <i>SIGMA-ALDRICH</i>
NaH ₂ PO ₄ ·H ₂ O (5.0 g/L dH ₂ O)	99.0%	S8282 <i>SIGMA-ALDRICH</i>
Na ₂ SiO ₃ ·9H ₂ O (30.0 g/L dH ₂ O)	Reagent grade	307815 <i>ALDRICH</i>

4.2. Methods

Two factors studied on algae growth (biomass), lipid production and fatty acid profiles were Temperature and CO₂ level.

A face centered Central Composite Design was applied to observe the effects of CO₂ and temperature. Upper and lower limits were determined considering typical

industry effluent of CO₂ and water temperature averages in the region. Temperature was studied between 15 °C and 30 °C; CO₂ was studied between 5% and 10%. Among 12 experiments 4 of them were performed at the mid values of each factor (10 % CO₂ and 22.5 °C). Details of experiments are given in Table 4.3. The response variables are quantified as optical density (OD) and cell count, dry weight, specific growth rate, lipid production, % of fatty acids, lipid characterization.

Table 4.3. Central Composite Design Experiments

Experiment Code	CO₂ (% volume)	Temperature (°C)
1	5	15
2	15	15
3	5	30
4	15	30
5	5	22.5
6	15	22.5
7	10	15
8	10	30
9	10	22.5
10	10	22.5
11	10	22.5
12	10	22.5

4.2.1. Algae Growth Conditions

F2 medium was used as the growth medium (Guillard 1962, Guillard 1975). A 950 mL of natural sea water was filtered and trace elements and vitamin solutions were added. Growth medium components given in Table 4.4 were added to 950 mL of dH₂O and the final volume filled to 1 liter with filtered natural seawater. Then growth medium was autoclaved. If the algae to be grown do not require silica, then it is recommended that the silica be omitted because it enhances precipitation (Smith 1993).

Table 4.4. Growth medium composition in 1 L of sterilized sea water.
(Source: Guillard 1978)

Chemical	Used Amount
NaNO ₃ (75.0 g/L dH ₂ O)	1.0 mL
NaH ₂ PO ₄ ·H ₂ O (5.0 g/L dH ₂ O)	1.0 mL
Na ₂ SiO ₃ ·9H ₂ O (30.0 g/L dH ₂ O)	1.0 mL
F2 Trace Metal Solution	1.0 mL
F2 Vitamin Solution	0.5 mL
Filtered seawater to	1.0 L

Table 4.5. f/2 Trace Metal Solution.
(Source: Guillard 1978)

Chemical	Used Amount
FeCl ₃ ·6H ₂ O	3.15 g
Na ₂ EDTA·2H ₂ O	4.36 g
CuSO ₄ ·5H ₂ O (9.8 g/L dH ₂ O)	1.0 mL
Na ₂ MoO ₄ ·2H ₂ O (6.3 g/L dH ₂ O)	1.0 mL
ZnSO ₄ ·7H ₂ O (22.0 g/L dH ₂ O)	1.0 mL
CoCl ₂ ·6H ₂ O (10.0 g/L dH ₂ O)	1.0 mL
MnCl ₂ ·4H ₂ O (180.0 g/L dH ₂ O)	1.0 mL
Distilled water	1.0 L

F2 Vitamin Solution was prepared, by the procedure given as in Guillard 1978. Final vitamin solution prepared by dissolving thiamine, adding the amounts of the primary stocks as indicated in Table 4.6 and metal trace solutions in Table 4.5 the mixture filled to final volume to 1 liter with dH₂O. Then medium was filtered, sterilized and stored in refrigerator.

Table 4.6. Growth medium
(Source: Guillard 1978)

Chemical	Concentration (mg/L)
NaNO ₃	75.0
NaH ₂ PO ₄ ·H ₂ O	5.0
Na ₂ SiO ₃ ·9H ₂ O	30.0
FeCl ₃ ·6H ₂ O	3.15
Na ₂ EDTA·2H ₂ O	4.36
CuSO ₄ ·5H ₂ O	9.8
Na ₂ MoO ₄ ·2H ₂ O	6.3
ZnSO ₄ ·7H ₂ O	22.0
CoCl ₂ ·6H ₂ O	10.0
MnCl ₂ ·4H ₂ O	180.0
Vitamin B12	0.5
Biotin	0.05
Thiamine HCl	0.1

4.2.2. Batch Growth Studies

The algal production was initialized in 25 ml flasks. It took almost 5 days to grow high concentrations. Then, the algae were taken into 1 L flasks for 8 days. Later, the flask contents were transferred to 10L reactors containing F/2 medium. It took 12-18 days depending on the environmental and growth conditions.

At each temperature three different cultures of algae, *Isocrysis galbana*, fed with different CO₂ level and a control group without excess CO₂ (300 ppm) were used in the batch growth studies.

The batch growth experiments were conducted in glass tubes consisting of a cylindrical aeration pipes. Glass pipes of diameter 5 mm were used as fixed volume photo bioreactors to hold the microalgal cultures. Erlen mayer flasks were used as batch reactors and glass pipes were sterilized in the autoclave for 30 minutes.

A VWR Scientific Products® Model 71 Immersion Circulator with digital Controller was used to control temperature to the desired level in the reactor during experiments. The blended gas was bubbled into the columns using glass diffuser (Capillary) tubes through panels. CO₂ levels were controlled by a series of valves and each flow line was separately controlled by gas flow meter online. Six cool 40 watts fluorescent lamps were placed adjacent to the plexi-glass tank to provide light for the algae.

Batch experiments to test the applicability in using the algal cultures were conducted using 1000 ml flasks. The initial step of algae production was shown in Figure 4.1.



Figure 4.1. Initial algae growth in batch reactors.

Flat-Plate Photobioreactor;

Reactors of 10 L volume were equipped with pipes including holes at the bottom for the purpose of aeration. A schematic diagram of the flat-plate photo bioreactor system is shown in Figure 4.2.

The lab-scale flat-plate photobioreactor was made from 0.3 cm thick plexi-glass sheets with a holding capacity of 10 L volume. The tank consisted of rectangular chamber 50 cm long, 10 cm wide and 20 cm in height. Perforated fluoropolymer tubing with 0.48 cm inner diameter and 60 cm length and with 0.05 mm equally spaced holes was used to bubble the gas mixture in the reactor. A Cole-Parmer® Gas Proportioner flow meter (Illinois, USA) was used to mix the air and compressed CO₂ source using a

CONCOA® (Virginia Beach, Virginia USA) regulator. The gas flow rate was maintained 5%, 10% and 15% in order to keep the culture suspended in the medium. Two light panels with three 40 watts GE fluorescent lamps each were placed on the back side of the photobioreactor to provide maximum light intensity.

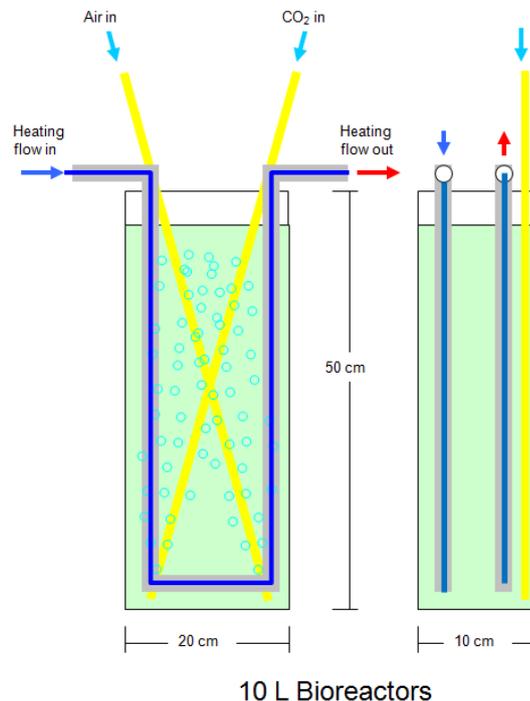


Figure 4.2. Scheme of the flat-plate photobioreactor system

Carbon Dioxide ratio in gas feed was controlled through experiments. The CO₂ was kept at 5, 10, and 15% v/v during the experiments as given in Table 4.3. To provide continuous supply of CO₂ for culture media, 0.98 pure CO₂ was supplied through compressed tanks Praxair® fitted with a CO₂ regulator Reva CO-AR max high purity. The supply of CO₂ was regulated to the desired reading using valves. Compressed room air through a centrally housed system was used to obtain desired gas mixture ratio. The two sources of gas were connected together into the reactors and controlled with a gas flow lines by a flow meter. According to the desired CO₂ concentration, the gas flow of room air and the compressed CO₂ were adjusted. The desired CO₂ concentration as volume percentage obtained by dividing the CO₂ flow by the total flow and multiplied by 100.

The other controlled parameter was temperature. The temperature of the plexi-glass water bath was controlled using the VWR Scientific Products® Model 71

Immersion Circulator with manual controller.

The pH was measured for all the batch and flat-plate photobioreactor experiments (Corning pH meter 340, Illinois, USA). Monitoring of pH was necessary in order to keep the culture in good condition. The pH values were measured 6 times a day for the flue gas measurements. The pH decreased with continuous supply of flue gas. Change due to excess CO₂ caused a difference in growth mediums up to ± 0.6 and pH did not regulated since it's in tolerance limits of *Isochrysis galbana*. Observed pH in the reactors was between 7 to 9. The tolerance pH limits for *Isocrysis galbana* were reported between 7 to 9 as well (Coutteau 1996).

4.2.3. Harvesting and Lipid Extraction

The procedure used for drying the sample and extracting lipids;

1. pH was adjusted to 4.5 by 0.1% HNO₃.
2. 1 M Fe(NO₃)₃ 2.5 mL/L algae was added.
3. pH was adjusted to 8 by 0.1%NaOH solution.
4. Settled algae were separated from the remaining sea water.
5. The algae solution was filtrated by a filter bag
6. The filtered algae was washed with deionized water 10 times
7. Algae was dried under vacuum for 12 hours at 60 °C and 100 milibar
8. Then semi-dried algae were further dried in a well air circulated oven for a night at 40 °C.
9. Totally dried algae was grinded
10. Algae were extracted with hexane with a soxhlet extractor.

4.3. Analysis

4.3.1. Biomass and Lipid Quantification

A UV-Visible Spectrophotometer Shimadzu® UV-1601 (Tokyo, Japan) was used to measure growth rates of *Isochrysis galbana* cultures by measuring the light intensity. As the photosynthetic pigments absorb the light energy from 300 to 800 nm,

all the optical density measurements were done at 688 nm, Standard 10-mm path length cuvettes with a sample holding capacity of 3 ml (Fisher-Scientific® Fisherbrand) were used to hold the samples in the spectrophotometer. For all the batch growth experiments, samples were collected at a 12 hours interval for the optical density measurements. Optical density measurements for the flat-plate photobioreactor were made two to four times a day. Growth rates were compared and analyzed at different environmental conditions for each species of algae and a relationship of optical density and time was plotted. Optical density measurements were taken at equal time intervals to evaluate the growth rates.

An improved Neubauer counting lamella (hemocytometer) was used for counting. It is thick microscope slide engraved with lines that allow the observer to determine the number of cells. Three samples taken from each reactor were counted and then arithmetic average of the values was taken and the sample amount placed into counting chamber was considered in the calculations.

Counting chamber was cleaned and placed in 90% alcohol, cleared of dust and grease definitely. Dimensioned lamella was placed in counting camera and counting chamber was kept for 5 minutes in order to prevent in the collapse of the cells. A binocular microscope (OLYMPUS-CX31) was used for cell count.

Neubauer counting chamber is a depth of 0.1 mm and 9 x 1 mm² square areas. This lamella (hemocytometer) has area of 25 square frames in the center, and each frame is divided into 16 small square areas. Square in the center a total of 400 (16 x 25) is a small square. This is a small volume of one of the frames in 1/4000 mm³, in another words, 2.5 x 10⁻⁷ ml. As a result of counting the number of cells was calculated according to the following formula. (Gökpinar 1990).

$$\text{Cell count (cell per ml)} = \frac{\text{counted cell number} \times 4,000,000}{\text{Counted number of squares}} \quad (4.2)$$

Calculation of specific growth rate;

It was calculated according to the following formula and cell count values and day were used as data.

$$K = \frac{\ln N_t - \ln N_0}{t} \quad (4.3)$$

K is specific growth rate, N_t is the number of cells at the end of the experiment, N_0 is the initial number of cells, t is time.

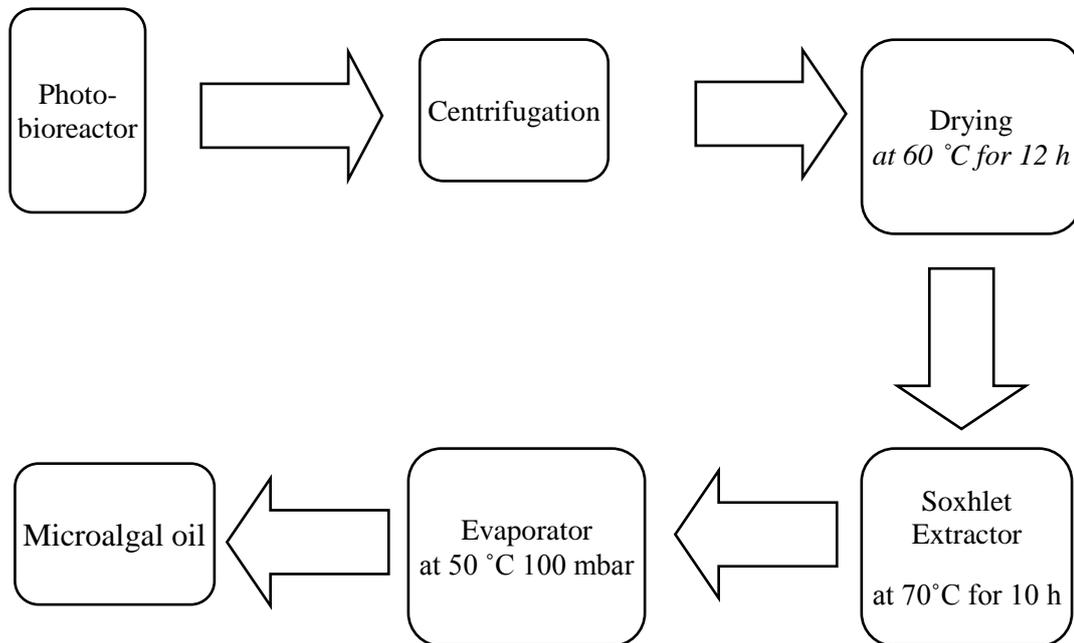


Figure 4.3. Procedure to obtain dry mass and algal oil

The dry mass of all the algal species was determined on triplicate culture samples. Dry mass measurements were conducted on microalgal culture. Equal volumes of the microalgal samples were filtered through a filter paper, which were previously heated in an oven at 110 °C for one hour and weighed for their initial weight. The filters were dried in a Laboratory oven at 110 °C for approximately 24 hours. The filter was taken and cooled to room temperature in desiccators to reach the final weight. The difference in the weights of the filter, after filtration and before filtration, divided by the sample volume filtered gave the dry mass. Procedure can be seen in Figure 4.3.

4.3.2. Fatty Acid Analysis

Lipids were converted to methylesters in order to be injected and analyzed by gas chromatography. First, 1% w/w NaOH to MeOH was added to excess methanol to obtain 30 MeOH/lipid molar ratios and stirred till 1100 rpm for 10 min to form methoxide. After methoxide formation, lipid was added and temperature was increased to 50°C. The reaction medium was stirred at the same speed and the temperature for 4 h

to achieve the complete conversion for the oil. After reaction stopped medium centrifuged at 4000 rpm for 10 min. Then samples were diluted for gas chromatography analysis in MeOH. Methyl esters were analyzed using a GC equipped with FID detector and DB-WAX 122-7032 capillary column with a 60 m column length, 0.25mm column thickness and 0.25 μm film thicknesses. A solution of 37 fatty acid methyl ester (FAME) Supelco 37 compound (Taufkirchen, Germany) were used as detailed in EN-14103 standard. The injection port and detector temperatures are 250 °C. Oven temperature was programmed to start at 120 °C and then heated up to 240 °C with 10 °C/min increment. Column flow was kept 1 ml/min.

4.3.3. CO₂ Solubility: Henry's Law

CO₂ was first needed to dissolve in the growth medium. Solubility of CO₂ for different experimental conditions can be calculated by Henry's Law, which explains the relationship between temperature, solute concentration in gas and solute concentration in liquid.

$$P_A = k_H C_A \quad (4.4)$$

where; P_A is the partial pressure of the solute in the gas above the solution, C_A is the concentration of the solute and k_H is a constant known as the Henry's law constant which depends on the solute, the solvent and the temperature. The value for k_H was taken as 0.123×10^4 (Van Ness 2005). A sample calculation of CO₂ solubility for 10% CO₂ gas flow and 15 °C found is given below, where CO₂ solubility found as 8.13×10^{-5} moles.

$$C_{CO_2} = \frac{P_{CO_2}}{k_H} \quad (4.5)$$

$$C_{CO_2} = \frac{P_{CO_2}}{k_H} = \frac{0.1 \text{ atm}}{0.1230 * 10^4} = 8.13 * 10^{-5}$$

4.3.4 Data Analysis

The data collected as the results of central composite design were evaluated and presented in the analysis of variance (ANOVA) Tables. The significance of parameters in the regression models of each response variable (biomass growth, lipid production, dry weight, fatty acid productions) were decided with respect to p-values. The insignificant terms were removed and the models were re-constructed in case of need. The model quality was evaluated in terms of coefficients of regression such as R^2 , R^2 -adjusted, R^2 -predictions, and also the p-values for lack-of-fit (LOF) parameter. The p-value for LOF should be higher than alpha (α) value, preferably taken as 5% (0.05). Minitab v. 16 (State College, PA, USA) statistical software was used in data analysis.

CHAPTER 5

RESULTS AND DISCUSSION

Through this study the effect of different growth medium temperature and CO₂ levels were investigated on the biomass growth, total quantity of oil, and the fatty acid profiles. The lipid content obtained from the algae growth at the end of each batch was extracted and fatty acid profiles were examined. Experimental temperature levels of temperature were chosen as 15, 22.5 and 30 °C. Make up CO₂ gas mixture levels were chosen as 5%, 10% and 15% v/v proportion in air. Other than these experiments also a control group was grown at each temperature level supplied with CO₂ in the air (300ppm) i.e. with no addition of CO₂. The content of this chapter was given as the results of central composite design experiments and the validation experiments.

5.1. CO₂ Solubility in the Growth Medium

Solubility of CO₂ changes with temperature and CO₂ concentration in gas feed. CO₂ solubility decreased with increased temperature, and increases with increased CO₂ concentration in gas feed with Henry Law (Cohen 1989).

As stated in materials and method section CO₂ used as nutrient source has a limitation to dissolve in growth medium by the Henry's law. In Figure 5.1 the solubility level of CO₂ at each experimental condition is shown. The solubility decreased with increased temperature as expected. And also it is clearly seen that concentration of CO₂ in the medium also increased with its increased concentrations in the gas feed.

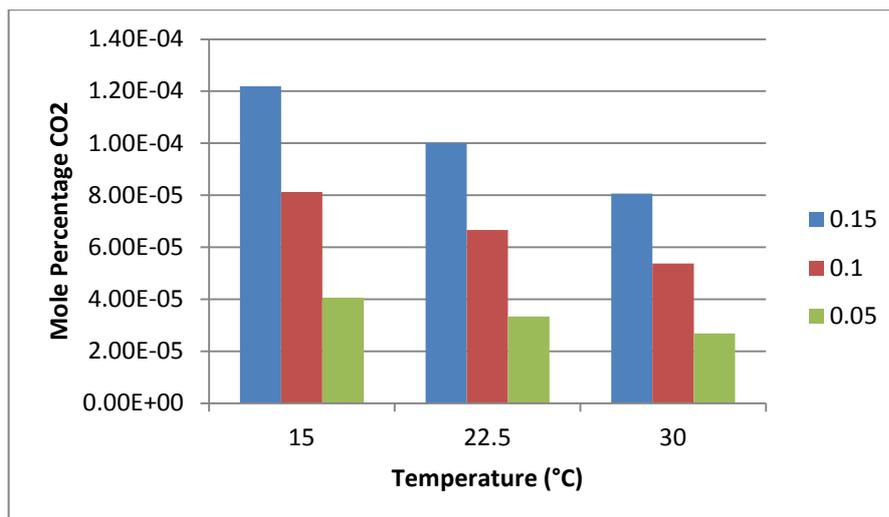


Figure 5.1. CO₂ solubility in water at each experimental case.

In these experiments for different amounts of CO₂, it was observed that required time for cell growth was increased according to other temperatures.

In Figure 5.1 results of experiments at 15 °C with different rates of CO₂ was given. Observations show that, the lowest dry mass was obtained in 15 °C experiments. Among these experiments 15% CO₂ addition has the lowest outcome. In a sense, effect of carbon dioxide was not significantly important in the production at 15 °C.

5.2. Results of Biomass Growth and Lipid Production at Different Temperature and CO₂ Levels

5.2.1. Algae Growth at 15°C

In these experiments for different amounts of CO₂, it was observed that required time for the cell growth was increased according to other temperatures. The highest specific growth was observed at 15 °C.

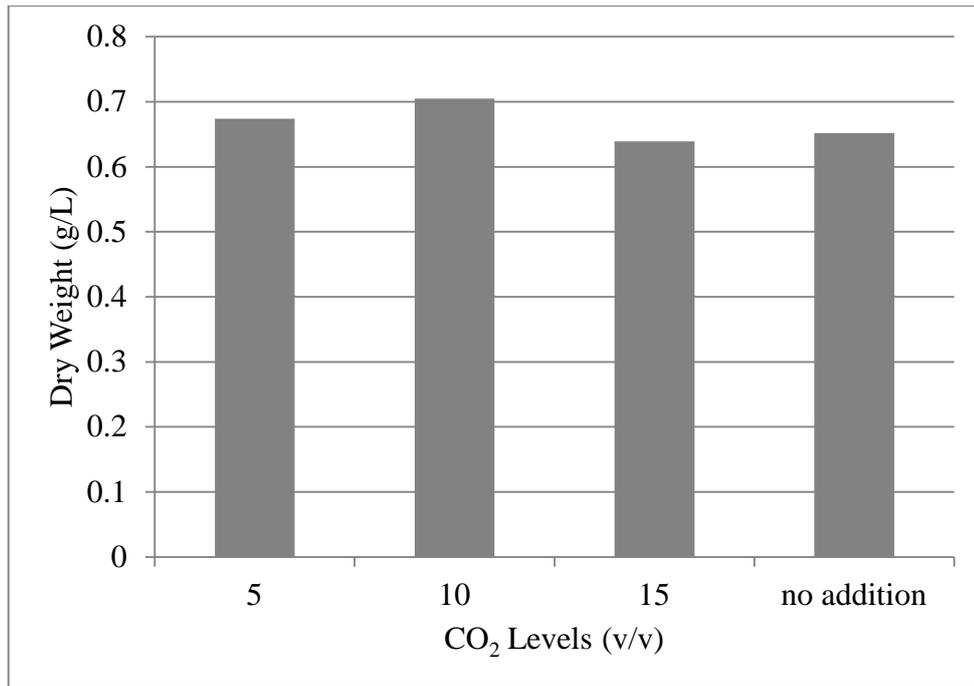


Figure 5.2. Dry weight of *Isochrysis galbana* at 15 °C for different CO₂ levels.

Dry algae weight at 15 changed with between 0.6 and 0.7 g/L for different CO₂ (Figure 5.2). The highest proportion with the amount of 14.2 % was reached. This result shows that, the algae production considering the total amount of oil at 15 °C can be beneficial. Figure 5.3 shows the lipid amount per liter and Figure 5.4 shows the lipid content of *Isocrysis galbana* at 15 °C.

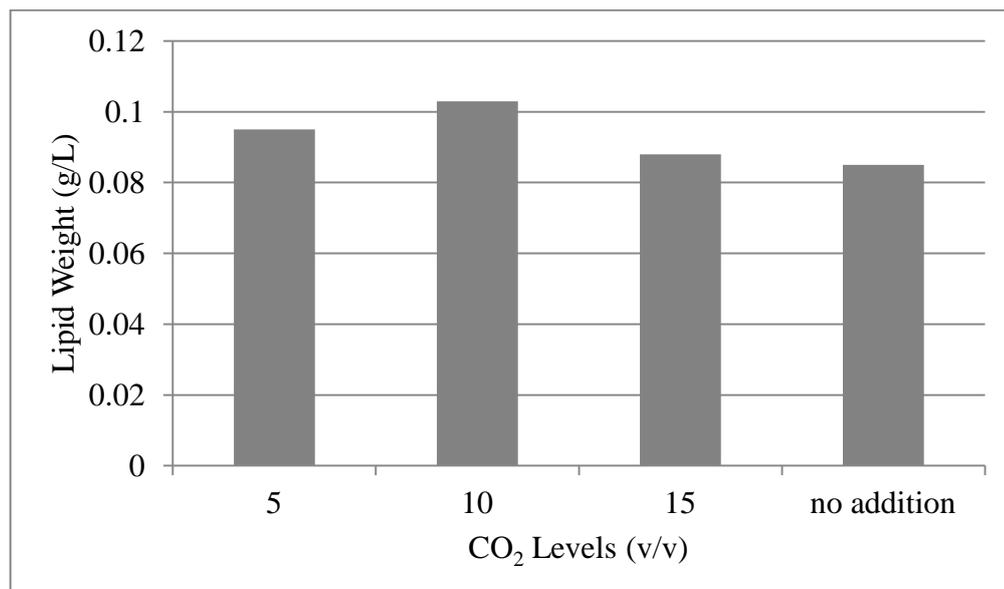


Figure 5.3. Lipid weight of *Isochrysis galbana* at 15 °C for different CO₂ levels.

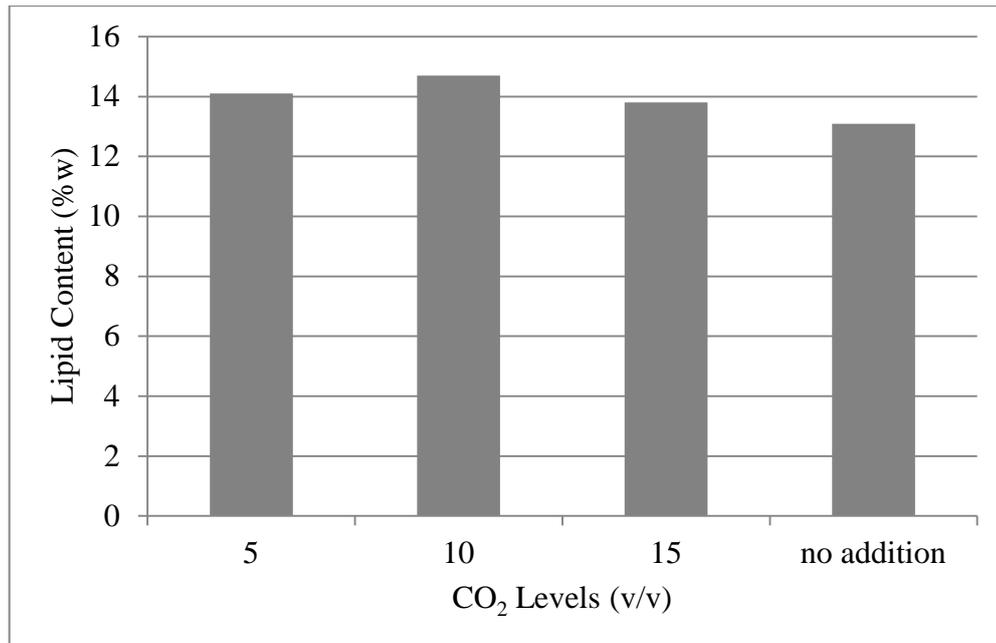


Figure 5.4. Lipid content (%w) of *Isochrysis galbana* at 15 °C for different CO₂ levels.

When lipid content analyzed, 15 °C experiments showed significant difference compared to other conditions in saturated and unsaturated fatty acids. Algae resist to conditions to adapt to changing conditions and stresses in their environment. Various types of lipids act as structural components of the plasma membranes and organelles of the cells and signaling components in the intracellular communication pathways. As part of membranes, lipids provide barriers to separate the various compartments within the cells. (Thompson 1996).

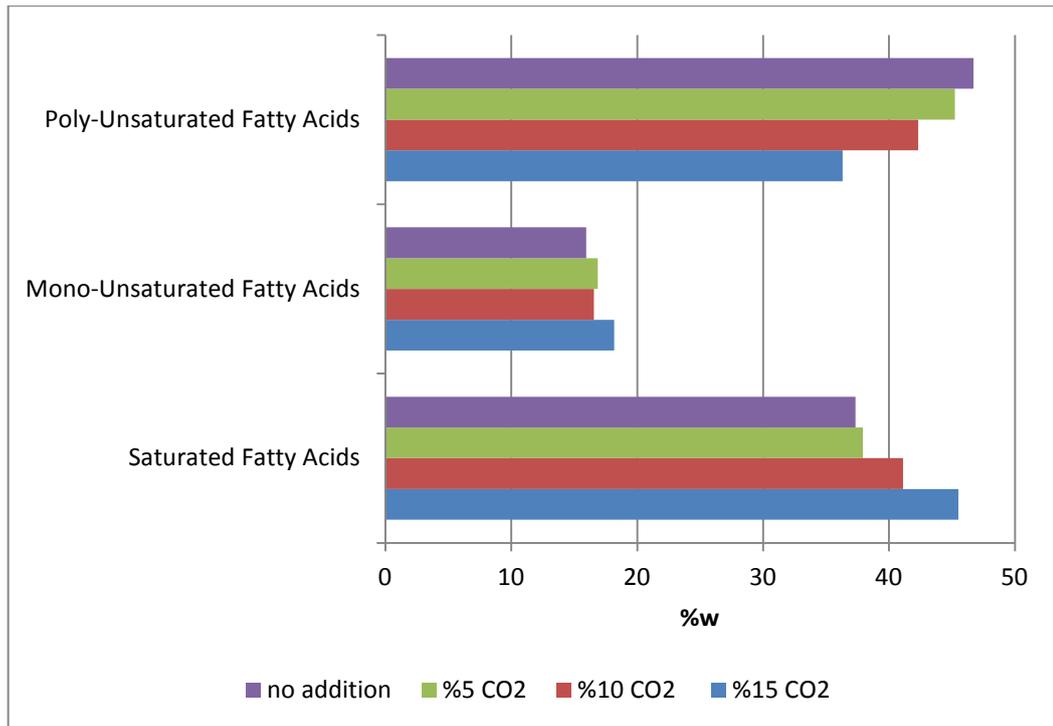


Figure 5.5. Saturated and unsaturated fatty acids distribution at 15 °C

Growth trends were analyzed, the result was the long duration of stationary phase and the production appears to be prolonged. I took 18 days for the cells reach the death phase. considering the production time, does not seem advantageous for the production of dry mass should be considered due to the fact that different fatty acids.

A different fatty acid profile was observed at 15°C compared to those obtained at other temperatures. The amounts of unsaturated fatty acids were higher than the 22.5 °C and 30 °C experiments. Also at 15 °C growths, the polyunsaturated fatty acids were produced more in case of lower CO₂ levels (no-addition and 5% CO₂ experiments) as seen in the Figure 5.5.

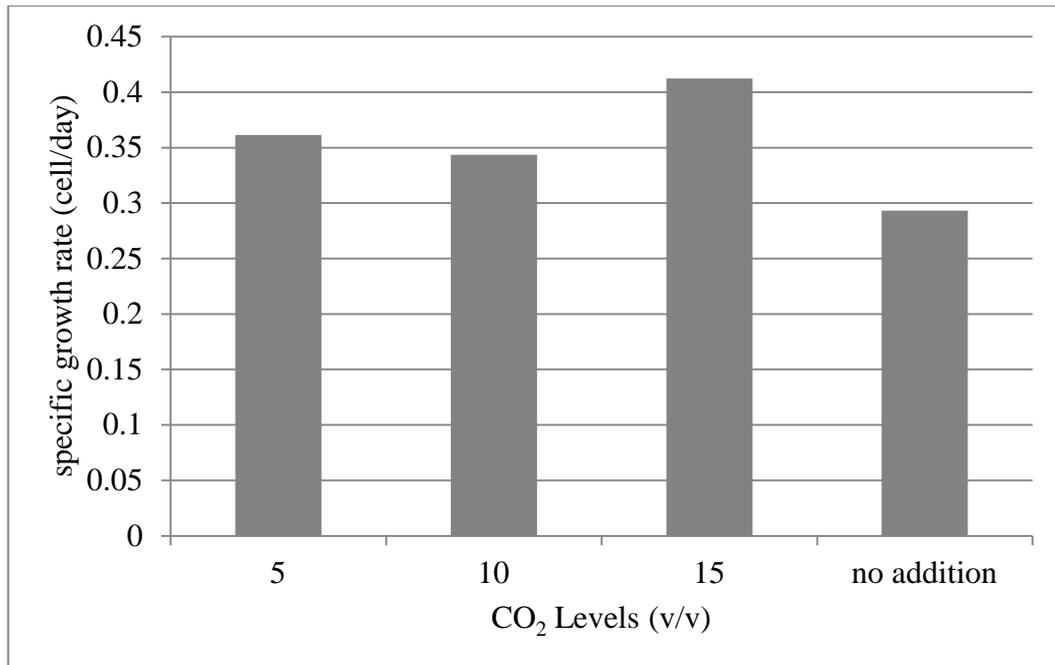


Figure 5.6. Specific growth rate of *Isochrysis galbana* at 15 °C for different CO₂ levels.

Specific growth rates were changing with respect to carbon dioxide levels according to the Figure 5.6. Given the growth curves resulting that, the effect of carbon dioxide has an efficient impact on growth behavior. Without CO₂ addition, life times of algae decreases. Growth behavior at each case can be seen in Figure 5.7.

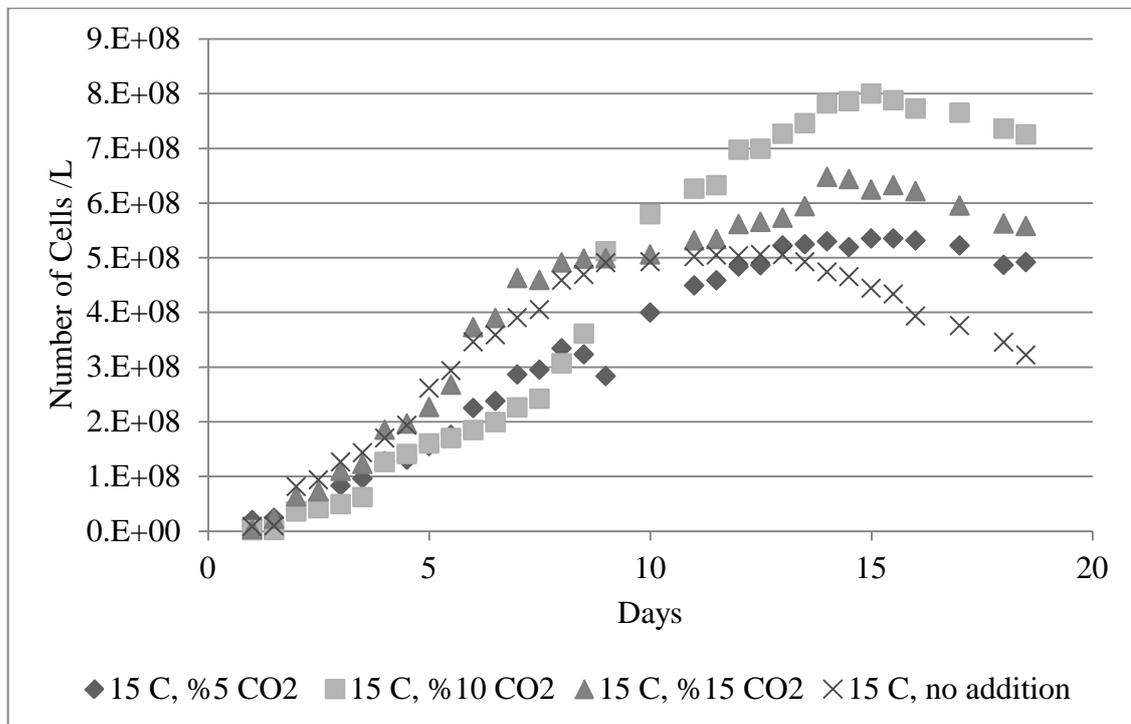


Figure 5.7. Growth of *Isochrysis galbana* at 15 °C.

Table 5.1. Algae growth rates, lipid contents and dry weight at 15 °C.

CO₂ Levels (v/v)	dry weight (g/L)	lipid weight (g/L)	lipid content (w/w %)	Specific growth rate (μ) (day⁻¹)
5%	0.674	0.0095	14.1	0.361
10%	0.705	0.103	14.7	0.343
15%	0.639	0.088	13.8	0.412

Groups without excess CO₂ are compared for it was observed that at 15 °C the highest lipid weight had been reached. At this temperature it was observed that increasing in the amount of excess CO₂ has reducing effect on the amount of lipid. Or in other words 15 °C the addition of CO₂, plays an inhibiting role in lipids with medium chain length lipid production. These effects can be seen in Table 5.1.

5.2.2. Algal Growth at 22.5 °C

Experiments at 22.5 °C shows that difference in growth, high cell density and the high lipid content of different growth mediums with different CO₂ levels clearly. For *Isocrysis galbana* 22.5 °C is approximately the optimal amount in Figure 5.8. The most important result of these experiments was that the highest cell density among all experiments achieved in a short growth time can be at 22.5 °C. The maximum cell density was reached after the twelfth day. Further in these experiments, the effect of CO₂ on growth was clearly observed. Highest dry mass achieved at 10% CO₂ level, yet highest quantities of lipid achieved at 15% CO₂ level. This temperature is close to the mean values of sea water in Turkey (Turkish state meteorological service); this temperature range may be evidence of a positive impact in terms of production efficiency was found. Thus a high rate of biomass to do this result in larger-scale production systems can be achieved.

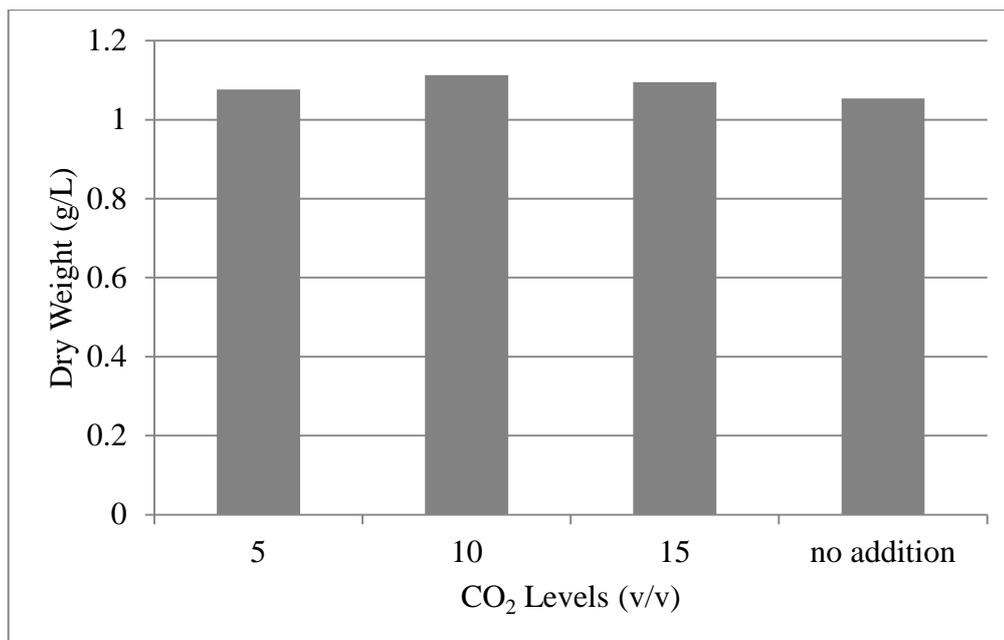


Figure 5.8. Dry weight of *Isochrysis galbana* at 22.5 °C for different CO₂ levels.

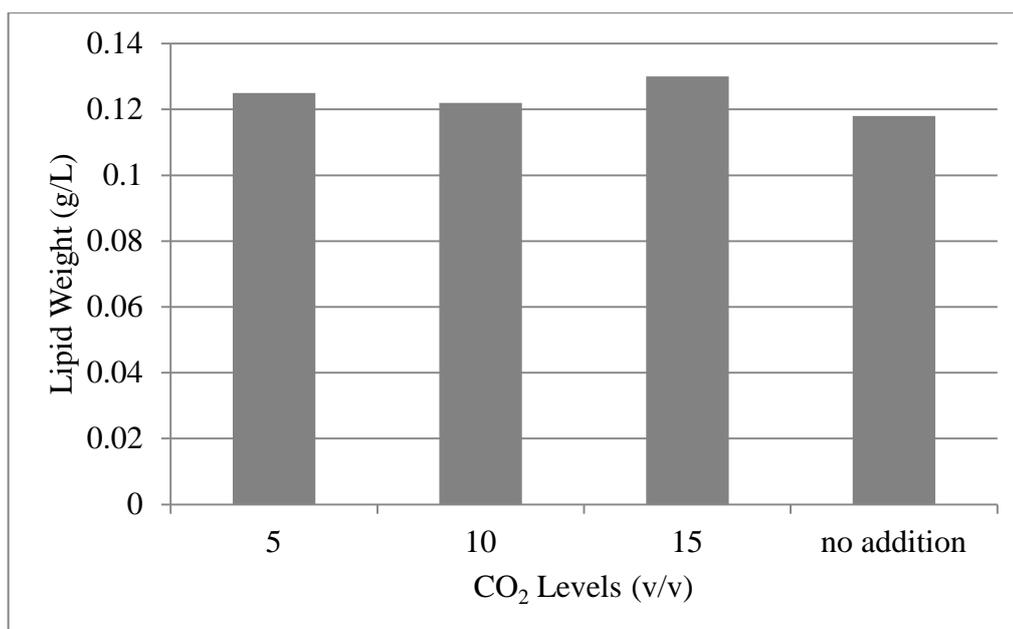


Figure 5.9. Lipid weight of *Isochrysis galbana* at 22.5 °C for different CO₂ levels.

At this temperature, the center point value of the experiments, there was no difference in the diversity of fatty acids is observed. In Figure 5.9 we see the lipid content of 15 °C for different CO₂ levels experiments which have the highest rate of % content among all experiments. Neither temperature nor carbon dioxide diversified the fatty acid profiles. However, at this temperature the lipid content was lower and the increase of oil can be obtained using high amounts of biomass.

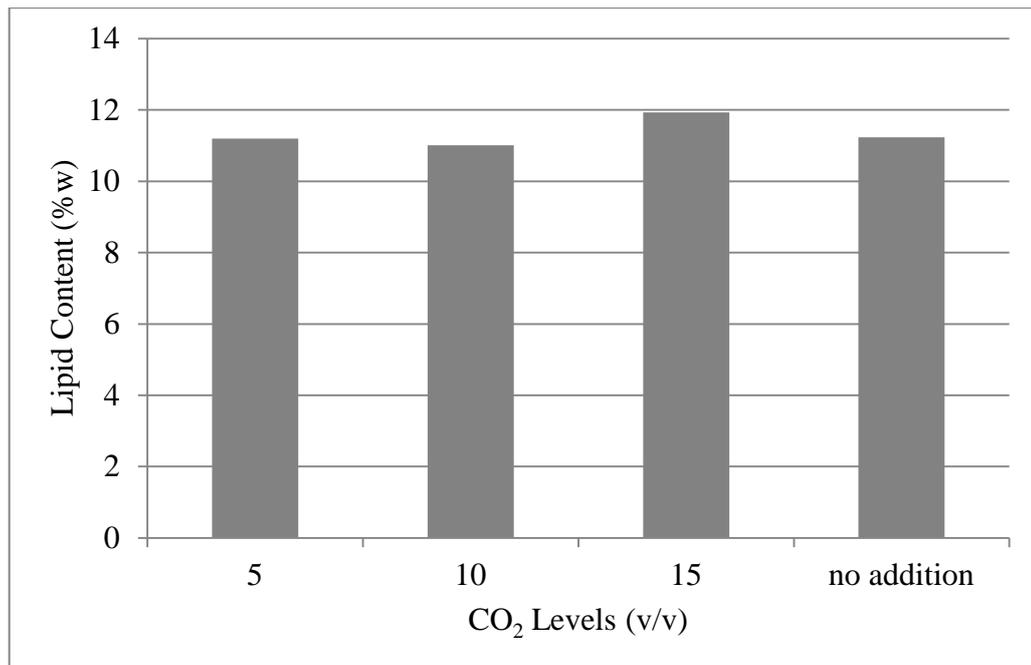


Figure 5.10. Lipid content of *Isochrysis galbana* at 22,5 °C for different CO₂ levels.

At 22.5 °C, it was observed that higher amounts of CO₂ was needed for higher lipid production (Figure 5.10). The reason for this behavior was explained in previous studies as; under stressed conditions results in increase in lipid quantity and this effects lipid profile significantly (Barsanti 2006), by forcing the growth to linear growth phase behavior. Thus plasma membrane difference occurs due to difference in distribution of phospholipids, glycolipids etc. This affects transport properties and light uptake and increasing energy input and CO₂ uptake (Barsanti 2006). In this study, we provide a source of light horizontally to minimize such changes in the resulting biomass.

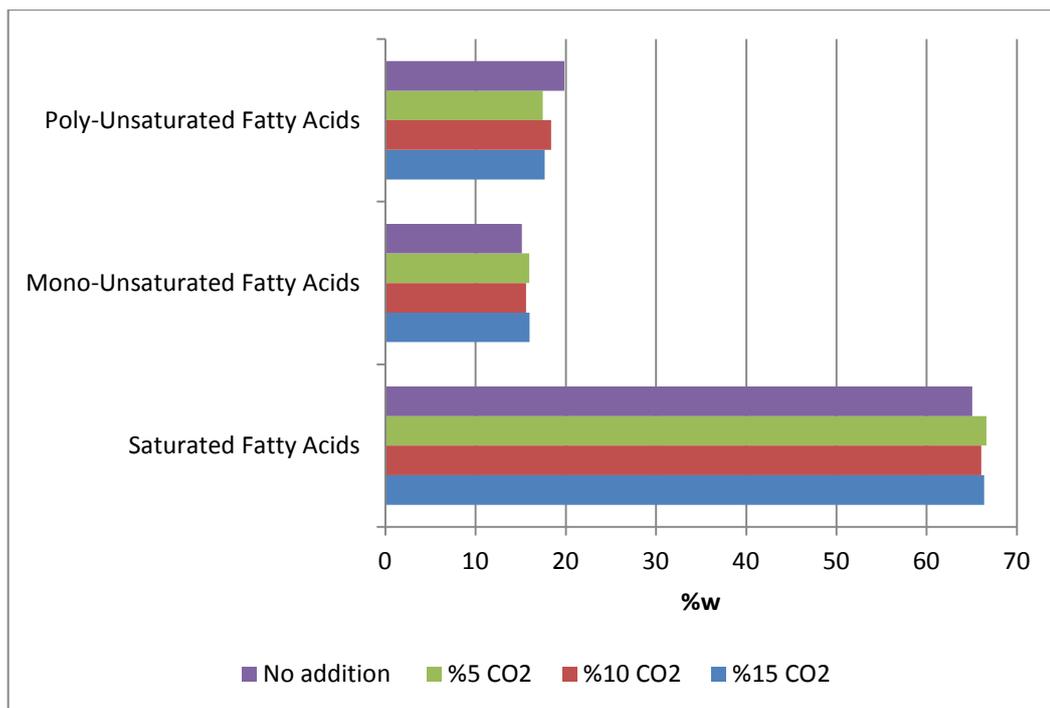


Figure 5.11. Saturated and unsaturated fatty acid distribution at 22.5 °C.

An optimal condition for cell growth as the reason for this is due to the fact that the values primary metabolites create. There is no stress in the cell due to a rapid metabolism of the divided and any changes did not occur. It can be seen in the Figure 5.11, saturated fatty acids are dominant compared with unsaturated fatty acids; the addition of CO₂ did not result in a significant distribution. In the Table 5.2. Summary of the 22.5 °C represented the other result is obtained.

Table 5.2. Lipid production and growth rate data for 22.5 °C.

CO ₂ Levels (v/v)	dry weight (g/L)	lipid weight (g/L)	lipid content %w/w	Specific growth rate (μ) (day ⁻¹)
5%	1.076	0.125	11.2	0.150
10%	1.113	0.122	11.01	0.191
15%	1.095	0.13	11.93	0.196

Particularly 24 carbon fatty acid ratios were decreased from 25% w/w to about 5%. A similar trend was observed in the amounts of dominant fatty acids such as palmitic and linolenic acids. At 22.5 °C and %15 CO₂ v/v of growth conditions 15-carbon pentadecanoic acid and 15 carbon palmitic acid were the significant fatty acids.

The fatty acid profiles at 22.5 °C %10 CO₂ additions showed similar results to other CO₂ levels. Only observed effect was the increase in the biomass content per unit volume. The 22.5°C and %5 CO₂ level experiments have shown that high amounts of biomass production and growth behavior (Figure 5.12). When all the experiments considered at 22.5°C it is observed that some of the fatty acids present in all trials dominantly emergence and fatty acids containing 24 and more carbon were less than experiments at 15 °C. The high carbon fatty acids such as those with 24 C or higher were observed at lower amounts in 22.5 C growth medium. Fatty acid profile at 22.5 °C is given in Figures 5.11 to 5.17.

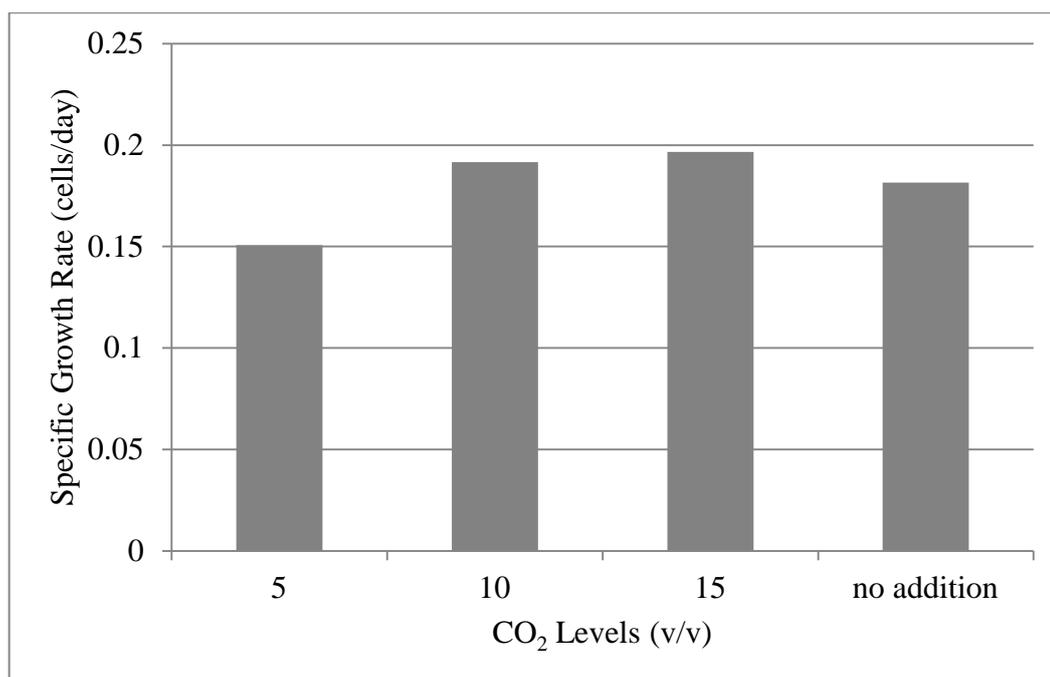


Figure 5.12. Specific growth rate of *Isochrysis galbana* at 22.5 °C for different CO₂ levels.

Growths of 22.5 °C behavior experiments were in close proximity to each other (Figure 5.13) and had specific growth rate were similar. Maximum cell count was achieved at 22.5 °C.

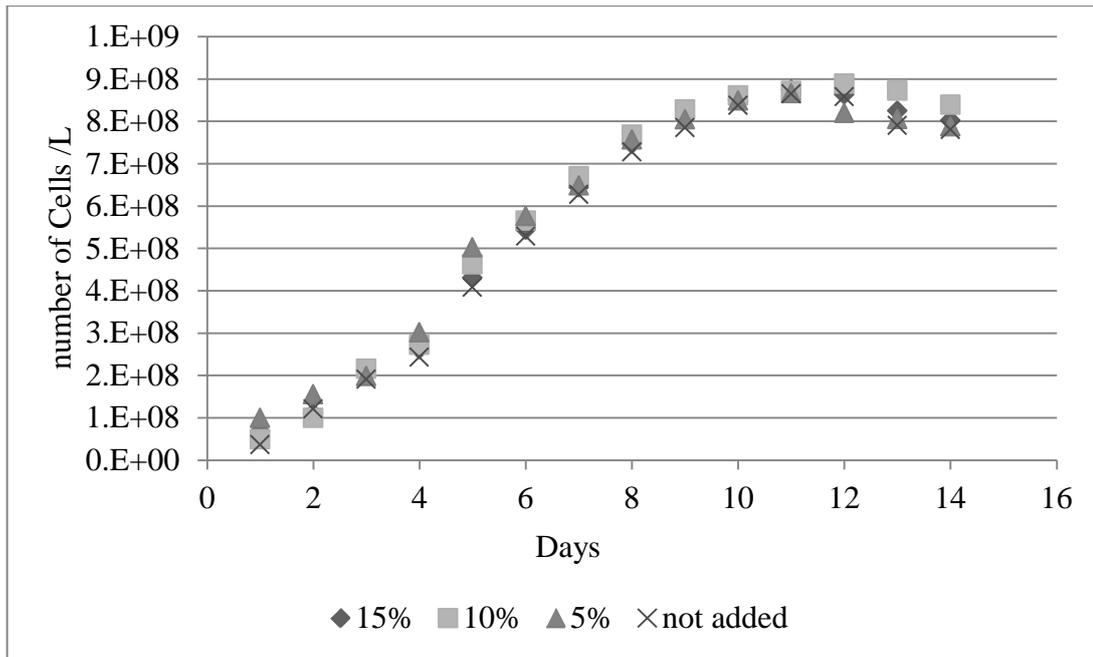


Figure 5.13. Growth curves at 22.5 °C.

5.2.3. Algal Growth at 30 °C

It was observed that the fat content obtained at 30 °C experiments was not affected by CO₂ as it is seen in Figure 5.14.

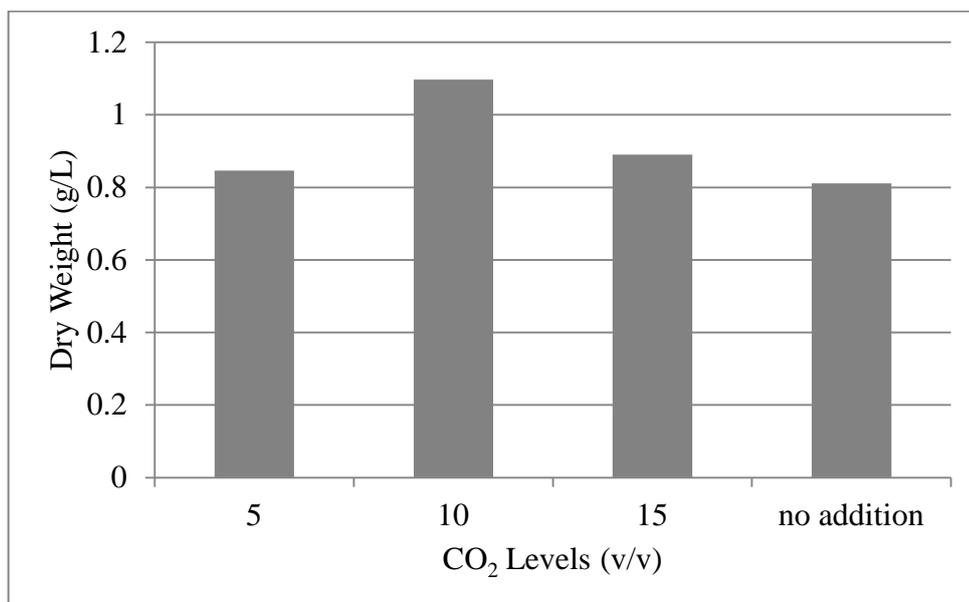


Figure 5.14. Dry weight of *Isochrysis galbana* at 30 °C for different CO₂ levels.

According to the data given before, 10% percentages CO₂ level gives the highest amount of dry weight. At this temperature dry weight was the maximum among all other temperatures. As seen in the chart, addition of CO₂ slightly increased the dry weight. With the increase of temperature, CO₂ addition was needed in order to produce higher biomass.

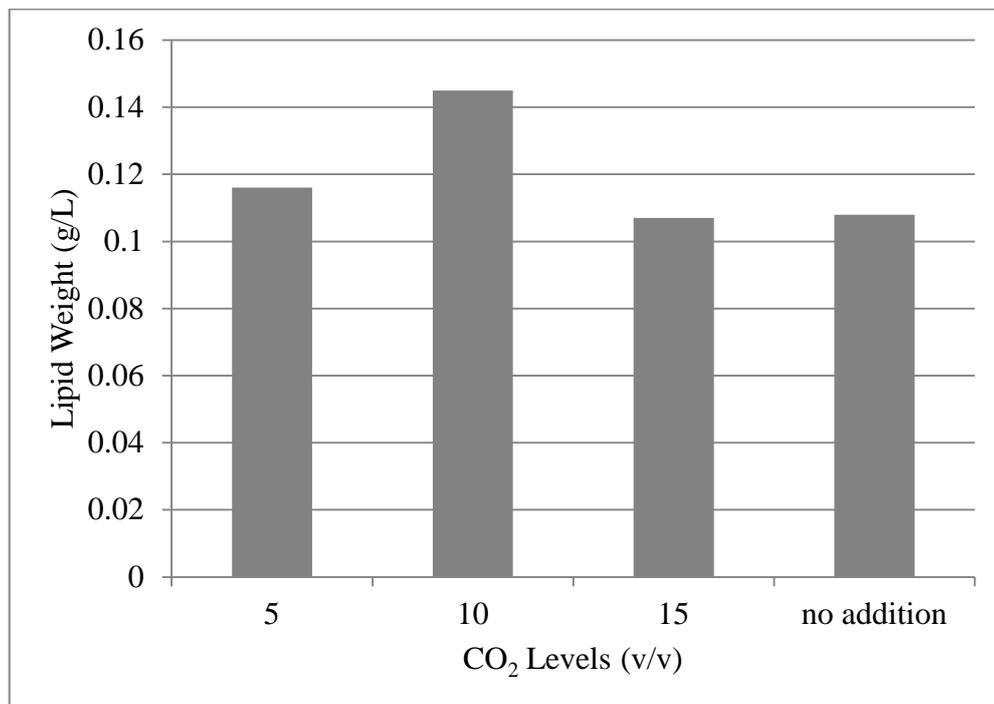


Figure 5.15. Lipid weight of *Isochrysis galbana* at 30 °C for different CO₂ levels.

Lipid weight production at this temperature increased at %10 level of carbon dioxide and this effect is shown in Figure 5.15. This result can be correlated to the dry mass amount. There are no significant differences between 15% and no addition of CO₂. When compared with 15 °C experiment it is the direct opposite.

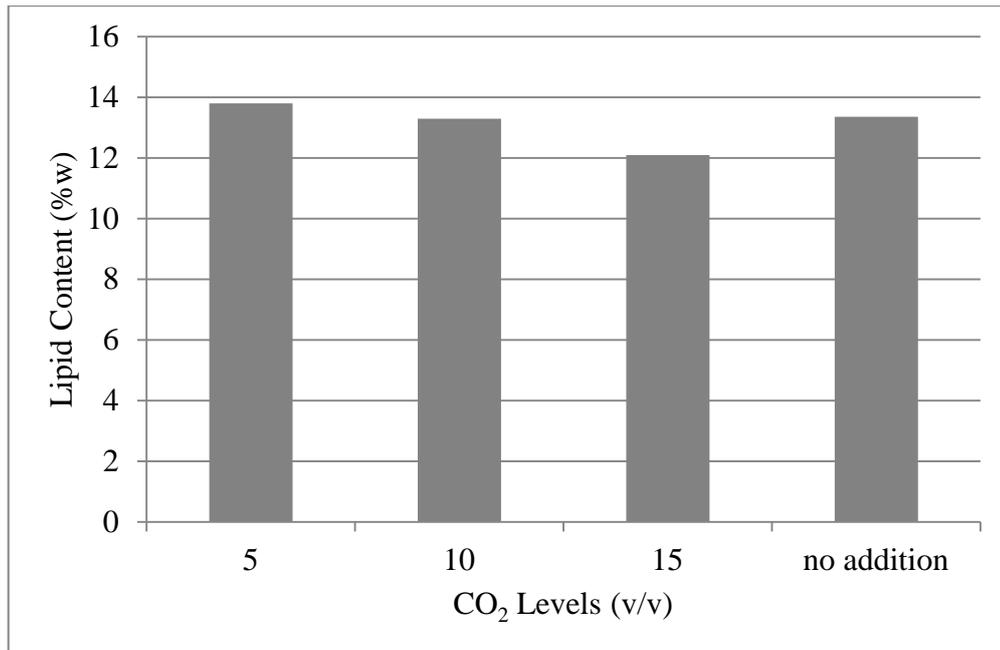


Figure 5.16. Lipid content (w%) of *Isochrysis galbana* at 30 °C for different CO₂ levels.

Lipid content was not changing significantly with levels of CO₂ at 30 °C (Figure 5.16). Yet with increased CO₂ levels decreased lipid content observed. The lowest amount was observed for %15 CO₂ at 30 °C.

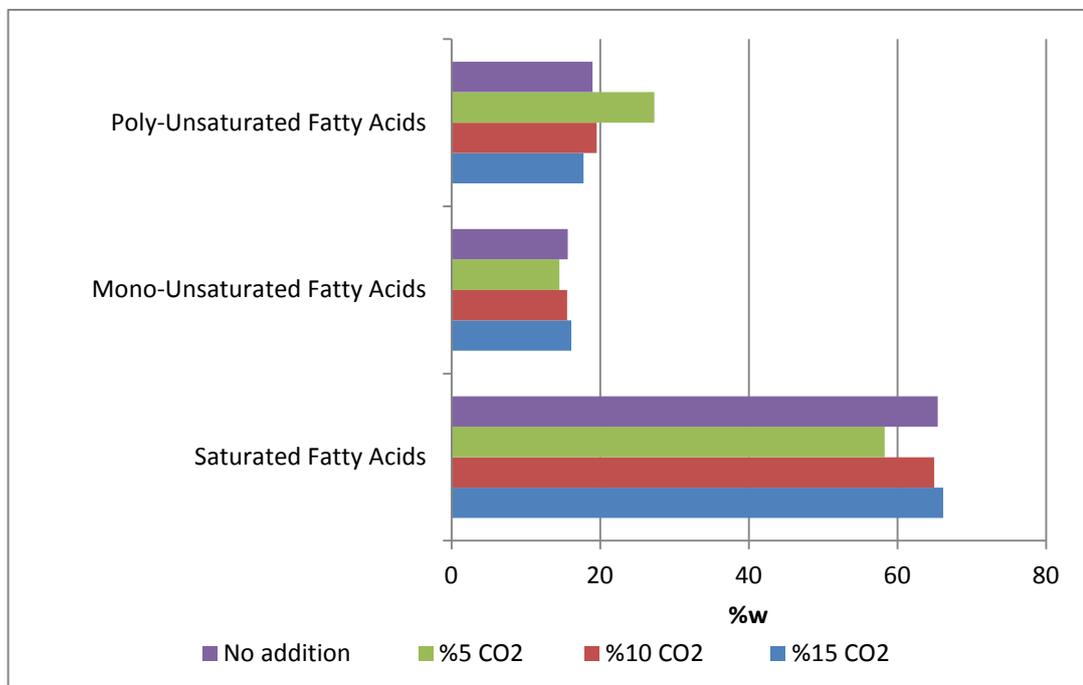


Figure 5.17. Saturated and unsaturated fatty acid distribution at 30 °C.

As seen in this Figure 5.17, saturated fatty acids are dominant compared to unsaturated fatty acids.

The 30 °C experiments had a linear growth curve. The explanation for this behavior could be that the cultivated cells due to the high temperature could adapt to the environment through the stationary phase (Thompson 1996). However, this linear increase in the production of high-biomass has failed contrary to the preliminary expectations. The development of *Isochrysis galbana* at 30 °C temperature was below that at the optimum growth temperature. The high amount of CO₂ did not result in an increase in the production of certain fatty acids. At this temperature the need for the addition of CO₂ to the growth has emerged. Due to the CO₂ depletion, the cells did not reach high density in 30 °C mediums.

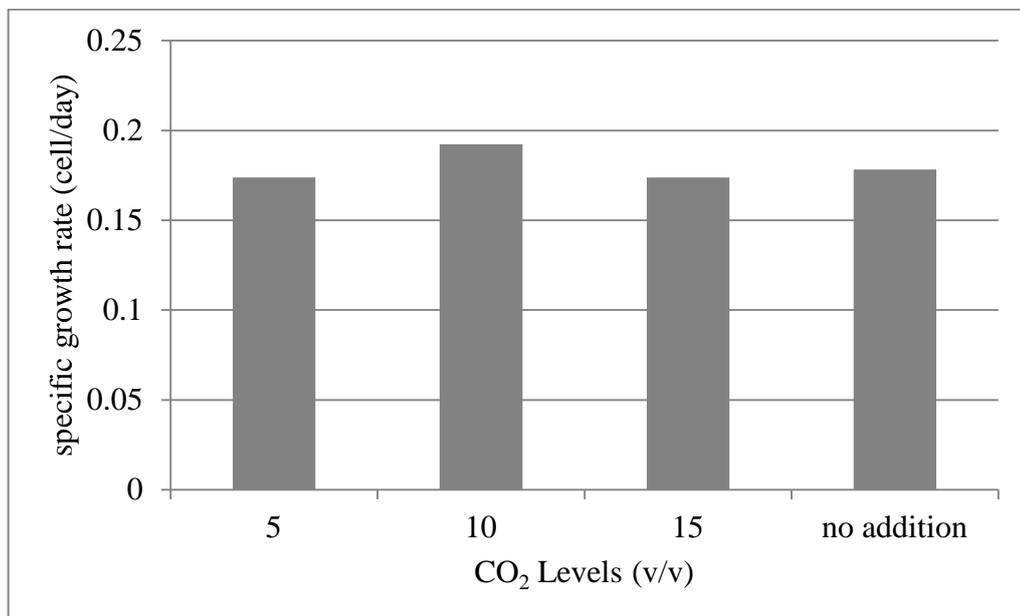


Figure 5.18. Specific growth rate of *Isochrysis galbana* at 30 °C for different CO₂ levels.

Observed specific growth rates at 30 °C were similar to each other and have the same order of magnitude. Figure 5.18. shows the specific growth rate values.

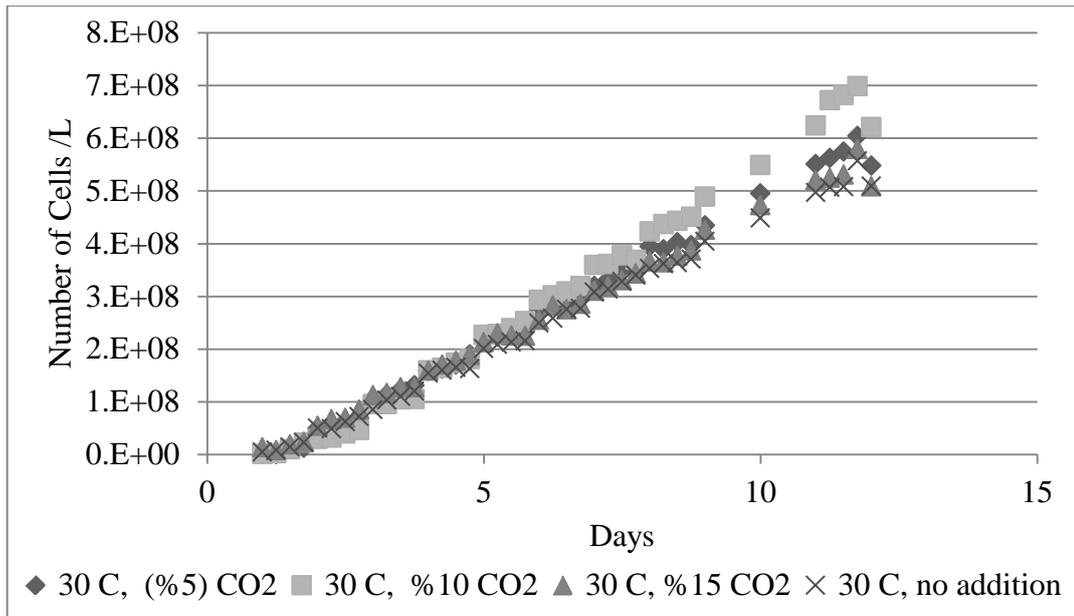


Figure 5.19. Growth curves of *Isochrysis galbana* at 30°C.

Because of the high temperature, the cells showed rapid adaptation to the growth conditions. No lag phase and stationary phase was observed (Figure 5.19). The lowest cell count was achieved at 5% and no CO₂ addition experiments. Experimental outputs of 30 °C given below in Table 5.3.

Table 5.3. Growth results of *Isochrysis galbana* at 30° C

CO ₂ Levels (v/v)	dry weight (g/L)	lipid weight (g/L)	lipid content %w/w	Specific growth rate (μ) (day ⁻¹)
5%	0.846	0.116	13.8	0.173
10%	1.097	0.145	13.3	0.192
15%	0.89	0.107	12.1	0.173

5.2.4. Calculation for the Standard Error of Response Variables

The Central experiments evaluated at the mid levels of temperature and CO₂ were used to calculate the experimental error for replicated analysis since other design points were replicated once. The standard error of the mean values (SE) was given as

standard deviation values (S) divided by square root of n, where n is the number of replicates (S/\sqrt{n}). The average and standard deviation values for central experiments are given in Table 5.4.

Table 5.4. Errors calculation in 22.5 °C experiments.

	Average	Standard deviation (S)	SE
Dry weight (g/L)	1.072	0.051	0.029
Lipid weight (g/L)	0.127	0.023	0.003
Lipid content (% w)	11.264	0.084	0.046
Specific growth rate (cell/hour)	0.163	0.017	0.005
Saturated fatty acids (% w)	61.140	0.726	0.414
Unsaturated fatty acids (% w)	38.869	0.722	0.414
Short chain fatty acids (% w)	2.710	0.842	0.487
Medium chain fatty acids (% w)	70.171	0.954	0.551
Long chain fatty acids (% w)	27.135	0.646	0.372
Saturated fatty acids (% w)	61.147	0.723	0.414
Mono-unsaturated fatty acids (% w)	15.373	0.610	0.354
Poly-unsaturated fatty acids (% w)	23.496	1.250	0.722

5.3. Discussion of the Results

According to the results given above, lipids with long chain were varying from 22.5 and 30 °C. It might be a result of changes and adaptation to the environmental conditions. Algae resist adapting to changing conditions and stresses in their environment. Various types of lipids act as structural components of the plasma membranes and organelles of the cells and signalling components in the intracellular communication pathways. As part of membranes, lipids provide barriers to separate the various compartments within the cells (Thompson 1996).

Among poly-unsaturated fatty acids highest level observed at 5% CO₂ and 30°C. There is no significant weight change due to mono-unsaturated fatty acids. This situation is similar to 22.5 cases, while at 15 °C it is completely different.

Growth at 22.5 °C and 30 °C did not show significant differences in lipid profiles. Only the amount of oil obtained per unit dry weight (%) was lower at 22.5 °C. It was found that Pandecanoic acid and Palmitic acid with 16 carbons are dominant fatty acids. Similar fatty acid profiles in 22.5 and 30 °C experiments were achieved. A representative chromatogram of these analyses is given in Figure 5.20.

In Table 5.5 nomenclature of fatty acids are given. The detailed fractions of fatty acids are given in Table 5.6.

Table 5.5. Nomenclature for Fatty Acid Methyl Ester

FAME	Number of carbons
1. Butyric Acid Methyl Ester	(C4:0)
2. Caproic Acid Methyl Ester	(C6:0)
3. Caprylic Acid Methyl Ester	(C8:0)
4. Capric Acid Methyl Ester	(C10:0)
5. Undecanoic Acid Methyl Ester	(C11:0)
6. Lauric Acid Methyl Ester	(C12:0)
7. Tridecanoic Acid Methyl Ester	(C13:0)
9. Myristoleic Acid Methyl Ester	(C14:1)
8. Myristic Acid Methyl Ester	(C14:0)
11. cis-10-Pentadecenoic Acid Methyl Ester	(C15:1)
10. Pentadecanoic Acid Methyl Ester	(C15:0)
13. Palmitoleic Acid Methyl Ester	(C16:1)
12. Palmitic Acid Methyl Ester	(C16:0)
15. cis-10-Heptadecenoic Acid Methyl Ester	(C17:1)
14. Heptadecanoic Acid Methyl Ester	(C17:0)
21. α -Linolenic Acid Methyl Ester	(C18:3n6)
19. Linoleic Acid Methyl Ester,	(C18:2n6c)
20. Linolelaidic Acid Methyl Ester	(C18:2n6t)
22. β -Linolenic Acid Methyl Ester	(C18:3n3)
17. Oleic Acid Methyl Ester	(C18:1n9c)
18. Elaidic Acid Methyl Ester	(C18:1n9t)
16. Stearic Acid Methyl Ester	(C18:0)
28. Arachidonic Acid Methyl Ester	(C20:4n6)
29. cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester	(C20:5n3)
26. cis-8,11,14-Eicosatrienoic Acid Methyl Ester	(C20:3n6)
25. cis-11,14-Eicosadienoic Acid Methyl Ester	(C20:2)
24. cis-11-Eicosenoic Acid Methyl Ester,	(C20:1n9)
27. cis-11,14,17-Eicosatrienoic Acid Methyl Ester	(C20:3n3)
23. Arachidic Acid Methyl Ester	(C20:0)
30. Heneicosanoic Acid Methyl Ester	(C21:0)
34. cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester	(C22:6n3)
33. cis-13,16-Docosadienoic Acid Methyl Ester,	(C22:2)
32. Erucic Acid Methyl Ester	(C22:1n9)
31. Behenic Acid Methyl Ester	(C22:0)
35. Tricosanoic Acid Methyl Ester	(C23:0)
37. Nervonic Acid Methyl Ester	(C24:1n9)
36. Lignoceric Acid Methyl Ester	(C24:0)
Lipids with over 24 C	

Table 5.6. Fatty acid profile at different temperature and CO₂ conditions.

Temperature	15 °C				22.5 °C				30 °C			
	5%	10%	15%	0%	5%	10%	15%	0%	5%	10%	15%	0%
Fatty Acid												
C4:0	3.71	3.07	1.77	1.23	3.49	3.50	3.69	3.44	4.61	6.24	8.48	5.70
C6:0	0.00	0.74	0.53	0.39	0.50	0.61	0.62	0.61	0.75	1.02	1.66	1.03
C8:0	0.64	0.00	0.00	0.00	0.88	0.93	0.98	0.51	0.87	0.90	0.00	0.00
C10:0	0.31	0.84	0.00	0.18	0.57	0.59	0.62	0.49	0.49	0.54	0.54	0.42
C11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C12:0	5.21	3.77	3.53	3.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C13:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C14:1	0.53	0.50	0.75	0.46	3.06	3.08	3.07	2.92	3.02	2.86	2.68	3.11
C14:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C15:1	29.56	29.35	28.72	27.30	0.36	0.36	0.36	0.32	0.30	0.36	0.00	0.00
C15:0	25.85	24.59	25.00	23.91	27.32	27.05	27.14	26.78	27.01	25.53	21.59	26.16
C16:1	1.76	0.40	0.00	0.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C16:0	0.00	0.34	0.00	0.00	23.25	23.11	23.40	22.96	22.82	21.67	18.69	22.42
C17:1	0.98	1.03	1.49	0.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C17:0	0.00	0.00	0.00	0.00	0.31	0.30	0.28	0.36	0.28	0.40	0.45	0.30
C18:3n6	16.02	15.48	17.76	15.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C18:2n6c, C18:3n3, C18:1n9c	0.65	0.65	0.78	0.00	0.93	0.89	0.93	1.09	0.96	0.92	0.73	0.83
C18:1n9t	1.76	1.60	1.20	1.04	16.47	16.16	16.33	16.96	16.34	15.43	12.53	15.79
C18:0	1.17	0.38	0.00	0.15	0.57	0.55	0.59	0.55	0.58	0.48	0.61	0.55
C20:4n6	0.78	0.42	0.53	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:5n3	5.40	3.93	2.03	1.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:3n6	0.00	0.00	0.00	0.00	1.30	1.16	1.31	1.13	1.22	1.20	1.24	1.25
C20:2	0.61	0.40	0.00	0.33	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:1n9	0.00	0.00	0.00	0.00	2.80	2.65	2.83	2.13	2.90	2.78	3.00	2.61
C20:0	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C21:0	0.00	0.00	0.00	0.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C22:6n3	0.00	0.00	1.25	1.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C22:2	1.95	1.62	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C22:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C23:0	3.13	2.42	3.11	2.85	0.22	0.23	0.24	0.28	0.00	0.00	0.00	0.24
C24:1n9	0.00	0.00	0.00	0.00	1.40	1.28	1.40	1.42	1.36	1.32	0.99	1.37
C24:0	0.00	0.38	0.00	0.00	3.10	2.95	3.00	2.91	3.12	3.04	2.36	3.09
Over C24:0	0.00	8.08	11.53	18.06	13.26	14.59	13.21	15.14	13.38	15.32	24.44	15.16
Total SFA	40.01	36.52	33.96	32.70	60.22	59.83	60.56	58.89	60.52	59.81	54.38	59.89
Total MUFA	34.58	32.88	32.15	29.91	24.10	23.53	23.99	23.75	23.92	22.75	19.20	22.87
Total PUFA	25.41	30.59	33.89	37.39	15.68	16.65	15.46	17.35	15.56	17.44	26.42	17.24

Observation showed that, lipids containing 24 or more carbons were observed slightly more at 15 °C and 10% CO₂ level (Table 5.6). Comparisons of different CO₂ levels for lipid composition are shown in Figure 5.21.

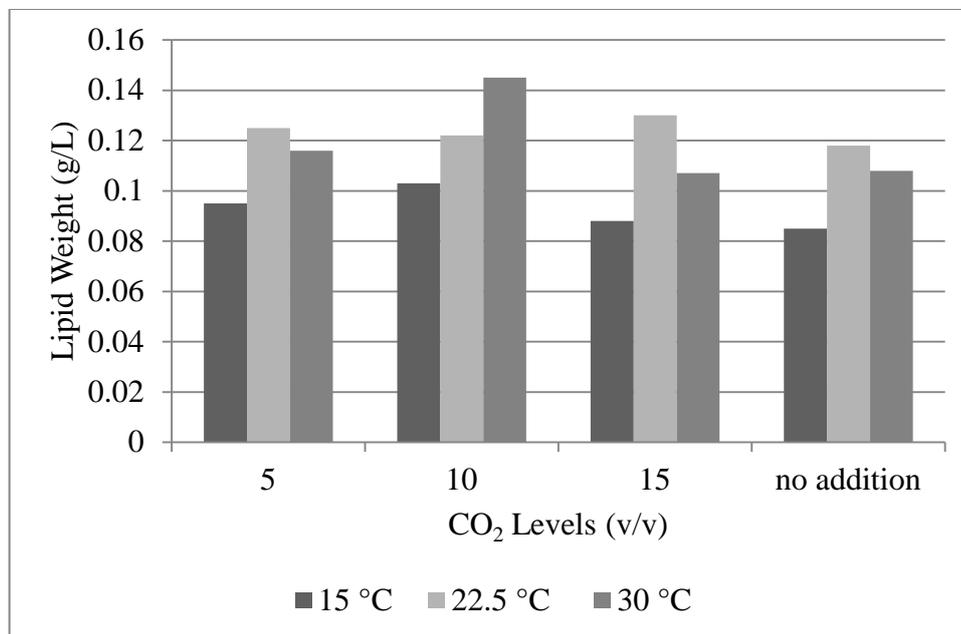


Figure 5.21. Comparison of Lipid weight at different experimental conditions.

The analysis of lipid data with respect to CO₂ and temperature is given in Table 5.7. ANOVA table shows the reduced model with only significant terms. However, this linear model does not represent the lipid content since it has very low R² values and a significant lack of fit.

Table 5.7. ANOVA results: Lipid weight (g/L) versus CO₂ and Temperature;

Term	Coef	P
Constant	-0.00216	0.96
CO ₂	0.004617	0.073
Temp	0.002878	0.09

S: 0.0278745

PRESS: 0.0170793

R-Sq: 46.15%

R-Sq(pred): 0.00%

R-Sq(adj): 34.18%

Lack-of-Fit: P: 0.015

Surface Plot of lipid weight(g/L) vs Temp; CO2

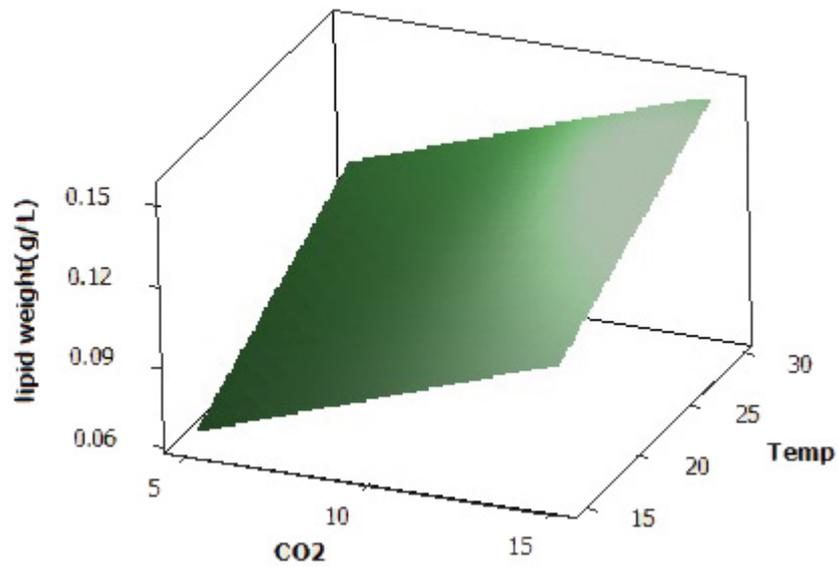


Figure 5.22. Surface plots for lipid weight of *Isochrysis galbana*.

Change in lipid weight with experimental parameters CO₂ levels and temperature is given in Figure 5.22. Lipid weight increases both with temperature and CO₂ as expected due to higher growth with higher temperature and nutrition. All types of bio fuel and a high fat content are important to meet the need for oil. Large-scale production of this species should be considered in this issue is the amount of high temperature and CO₂.

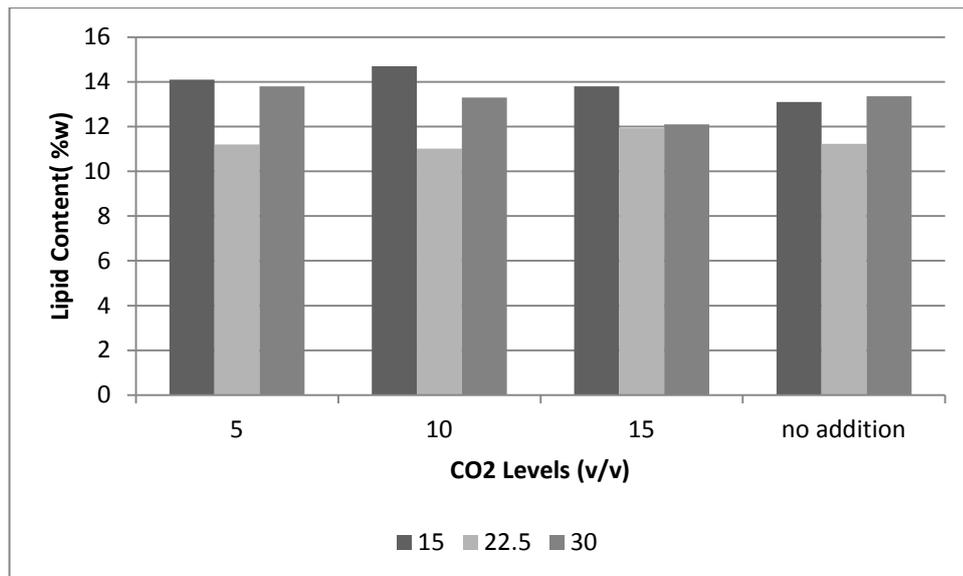


Figure 5.23. Comparison of % Lipid content at different experimental conditions.

The change in lipid content as % of dry weight is given in Figure 5.23. It can be observed that lipid production is higher at 15 C experiments. Table 5.8 shows the data analysis for lipid %. ANOVA model is quadratic with temperature and linear with CO₂ terms.

Table 5.8. ANOVA results: lipid content (%w) versus CO₂ and Temperature.

Term	Coef	P
Constant	27.3133	0
CO ₂	0.444	0.046
Temp	-1.4942	0.001
Temp*Temp	0.0358	0.001
CO ₂ *Temp	-0.0213	0.03

S: 0.590611 PRESS: 13.739

R-Sq: 88.06% R-Sq(pred):32.84% R-Sq(adj):81.24%

Lack-of-Fit: P: 0.229

Surface Plot of lipid content(%w) vs Temp; CO₂

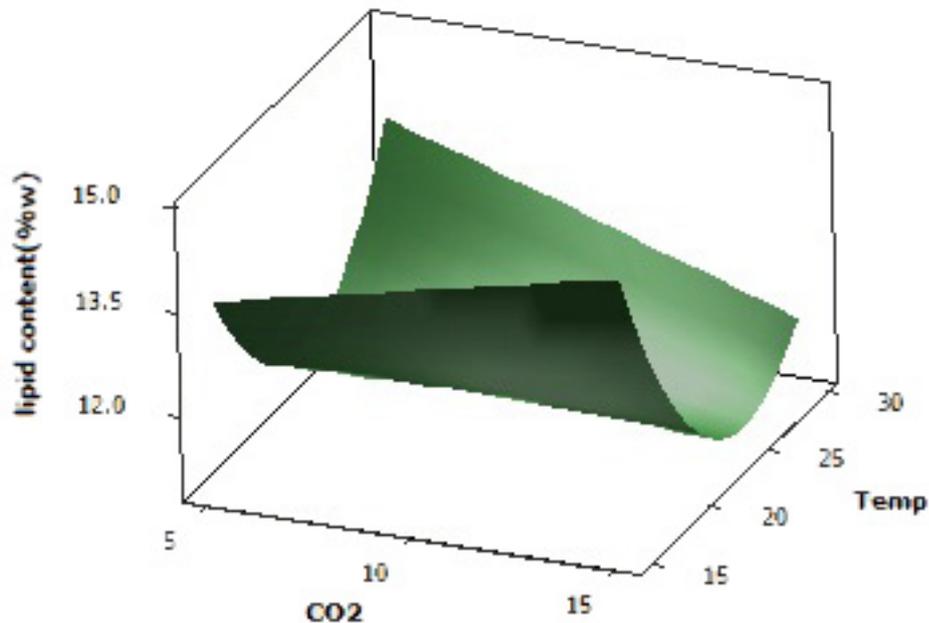


Figure 5.24. Surface plots for lipid content % weight (w/w)

Lipid % content was observed as maximum at either low CO₂ levels-high temperature or high CO₂ levels-low temperatures and lower at moderate temperature as shown in Figure 5.24. Contrary to lipid content, dry weight has maximum at moderate

temperature and high CO₂ levels as shown in Figure 5.25. It is known that *Isochrysis galbana* has optimum growth at 22.5 °C, yet it is known that algae increases lipid content under stressed conditions and this result in higher lipid content at lower and higher temperatures.

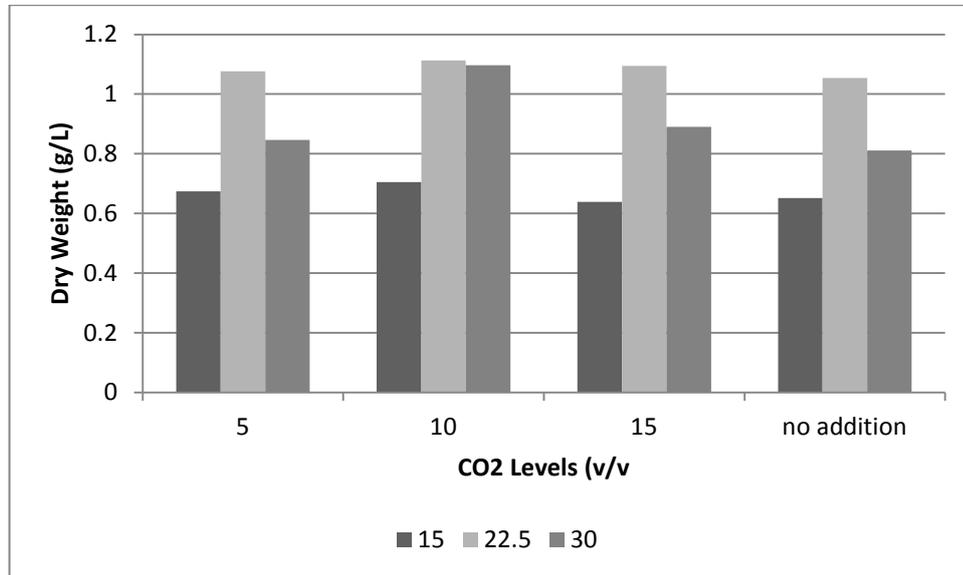


Figure 5.25. Comparison of dry weight of *Isochrysis galbana* at different experimental conditions.

Table 5.9. ANOVA results: Dry weight versus CO₂ and Temperature;

Term	Coef	T
Constant	-1.31693	0.103
CO ₂	0.015	0.224
Temp	0.18434	0.022
Temp*Temp	-0.00381	0.029

S: 0.139294 PRESS: 0.438653

R-Sq: 59.57% R-Sq(pred): 0.00% R-Sq(adj): 44.41%

Lack-of-Fit: P: 0.247

Surface Plot of dry weight (g/L) vs Temp; CO2

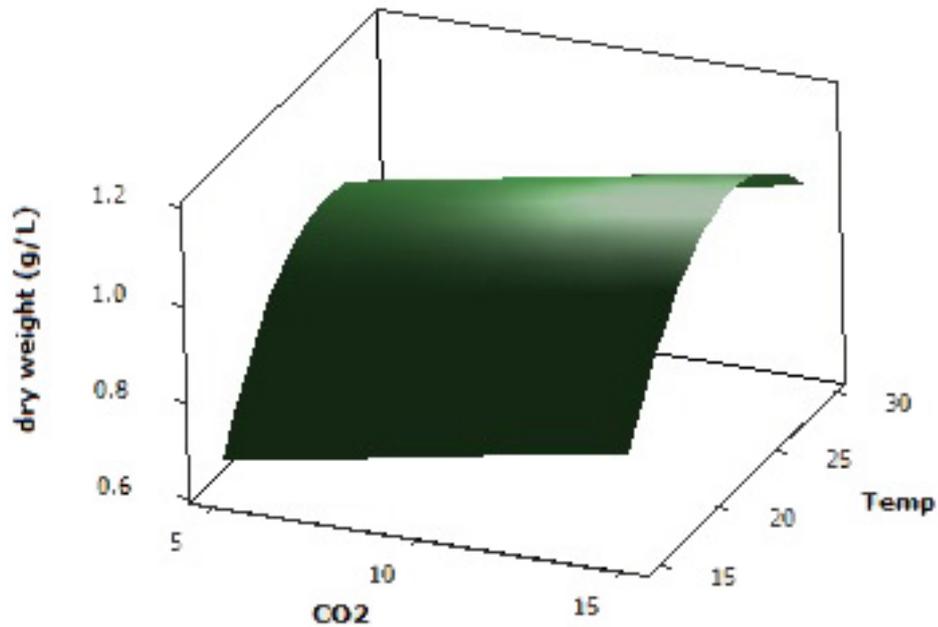


Figure 5.26. Surface plot of dry weight (g/L) *Isochrosis galbana*.

High dry mass production, particularly in the food industry can be used in many fields such as fertilizer or fish or animal feed. The main points to be considered for these types of productions are the high amount of CO₂ and temperature around 26 °C (Figure 5.26).

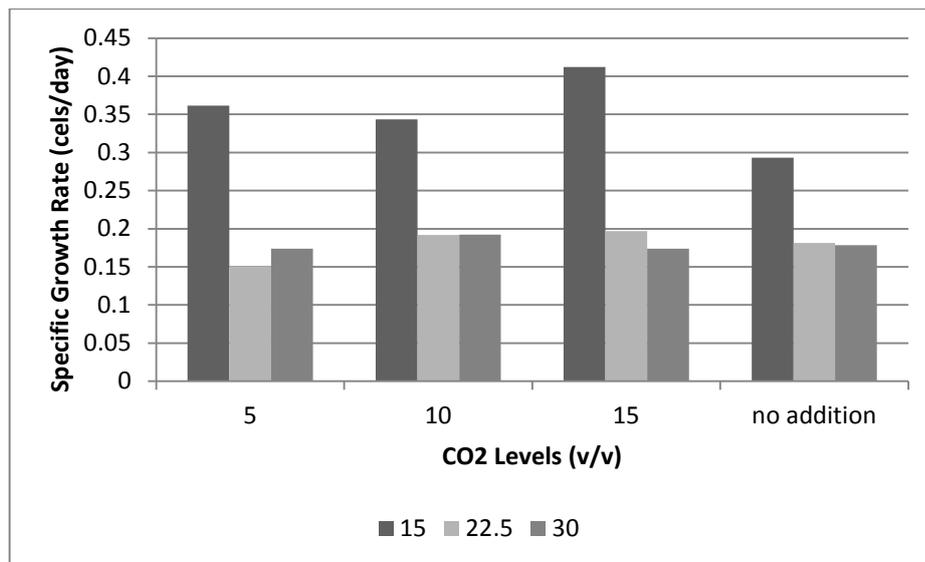


Figure 5.27. Specific growth rate of *Isochrosis galbana* at 30 °C for different CO₂ levels.

Table 5.10. ANOVA results: Specific growth versus CO₂ and Temperature;

Term	Coef	P
Constant	0.902743	0.03
CO ₂	-0.03841	0.261
Temp	-0.04334	0.163
CO ₂ *CO ₂	0.00329	0.046
Temp*Temp	0.001263	0.073
CO ₂ *Temp	-0.00162	0.063

S: 0.0533897 PRESS: 0.148744

R-Sq: 81.83% R-Sq(pred): 0.00% R-Sq(adj): 66.69%

Lack-of-Fit: P: 0.004

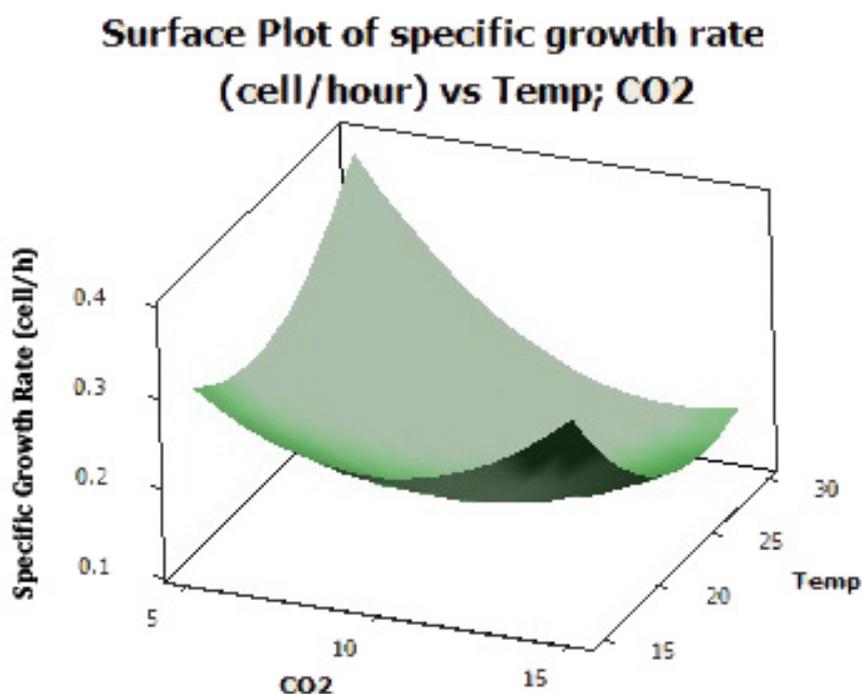


Figure 5.28. Surface plot of specific growth rate (day⁻¹) of *Isochrosis galbana*.

Surface plot of specific growth rate shows that a local minimum observed at moderate temperature and CO₂ levels. Higher algal growth at maximum and minimum values of parameters was observed (Figure 5.28).

Table 5.11. Fatty acid distribution at each experimental case.

	Saturated Fatty Acids	Unsaturated Fatty Acids	Short Chain FFA	Medium Chain FFA	Long Chain FFA	Saturated Fatty Acids	Mono-Unsaturated Fatty Acids	Poly-Unsaturated Fatty Acids
%15 CO ₂ at 15°C	45.51	54.49	5.50	60.70	33.80	45.51	18.17	36.32
%10 CO ₂ at 15°C	41.13	58.87	3.57	63.17	33.26	41.13	16.56	42.31
%5 CO ₂ at 15°C	37.92	62.08	2.49	66.81	30.70	37.92	16.86	45.22
no addition at 15°C	37.34	62.66	1.74	55.95	42.31	37.34	15.94	46.72
%15 CO ₂ at 22.5°C	66.38	33.62	2.77	73.39	23.84	66.38	15.98	17.64
%10 CO ₂ at 22.5°C	66.04	33.96	2.95	72.64	24.41	66.04	15.59	18.36
%5 CO ₂ at 22.5°C	66.63	33.37	3.04	73.44	23.52	66.63	15.93	17.43
No addition at 22.5°C	65.06	34.94	2.41	73.49	24.11	65.06	15.12	19.82
%15 CO ₂ at 30°C	66.16	33.84	3.26	72.76	23.99	66.16	16.09	17.76
%10 CO ₂ at 30°C	64.91	35.09	4.04	70.02	25.94	64.91	15.56	19.53
%5 CO ₂ at 30°C	58.24	41.76	4.25	60.76	34.99	58.24	14.50	27.26
No addition at 30°C	65.42	34.58	2.70	71.58	25.71	65.42	15.62	18.96
central experiments %10 CO ₂ at 22.5°C average	61.16	38.84	2.73	70.35	26.92	61.16	15.38	23.45

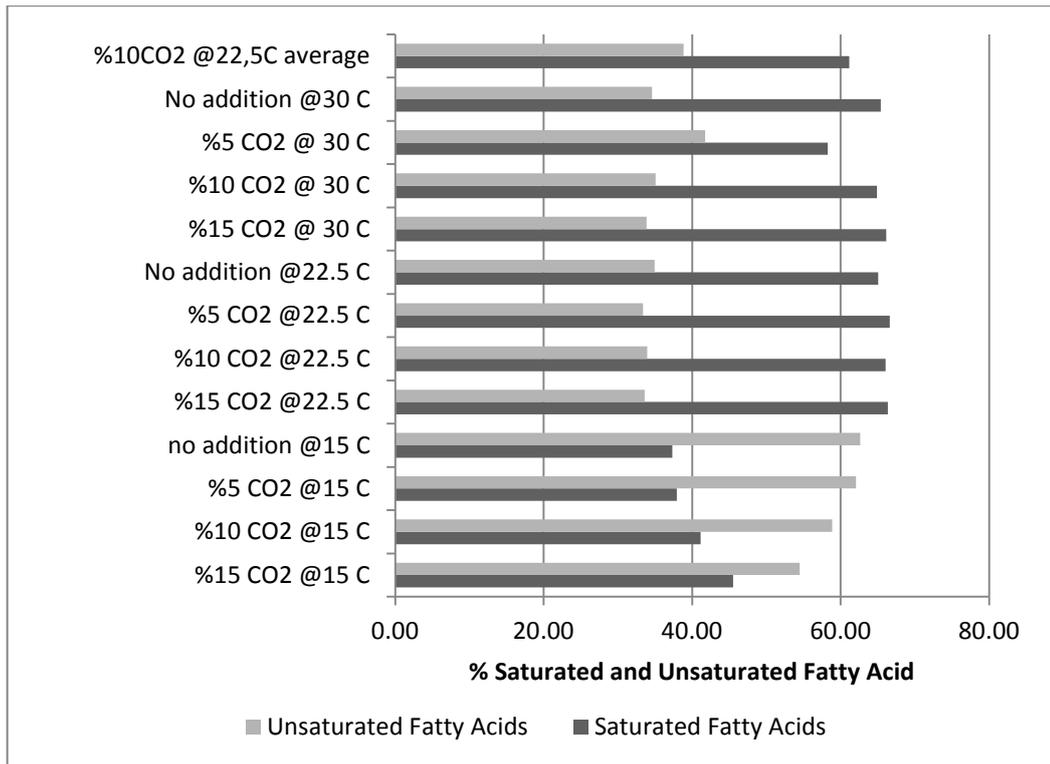


Figure 5.29. Saturated-unsaturated fatty acid percentages for each experimental condition.

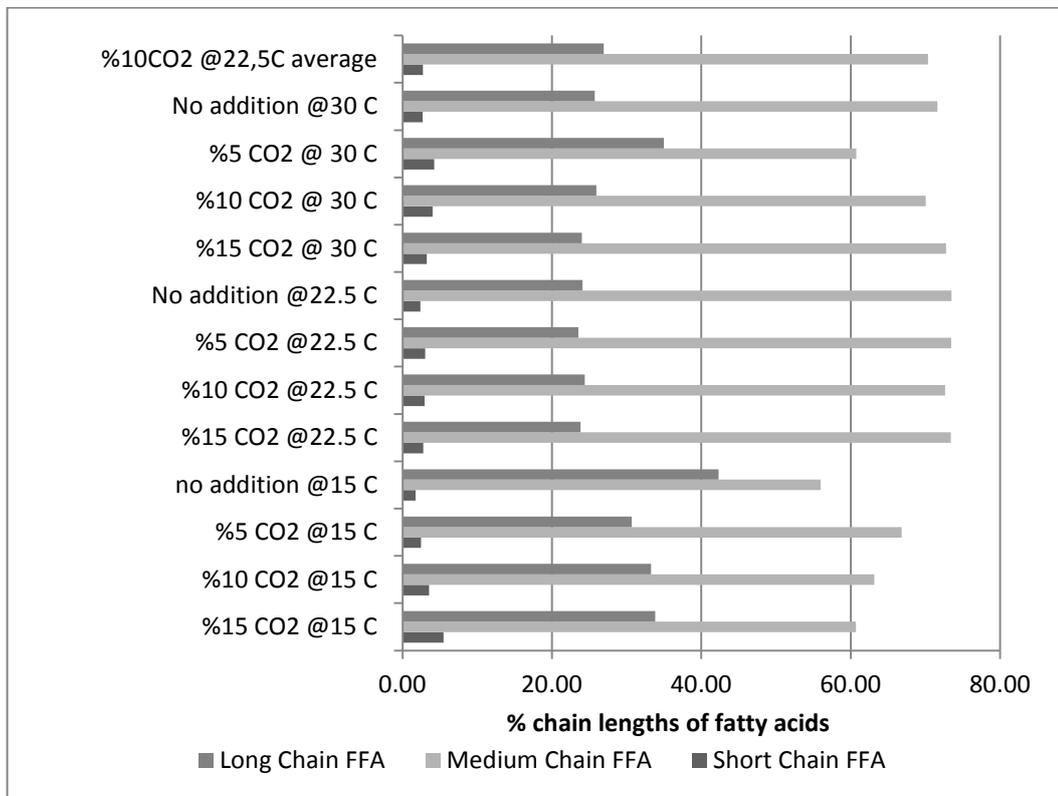


Figure 5.30. Long (longer than 18 C), medium (10C-18C) and short (lower than 10 C) fatty acid distributions for each experimental condition.

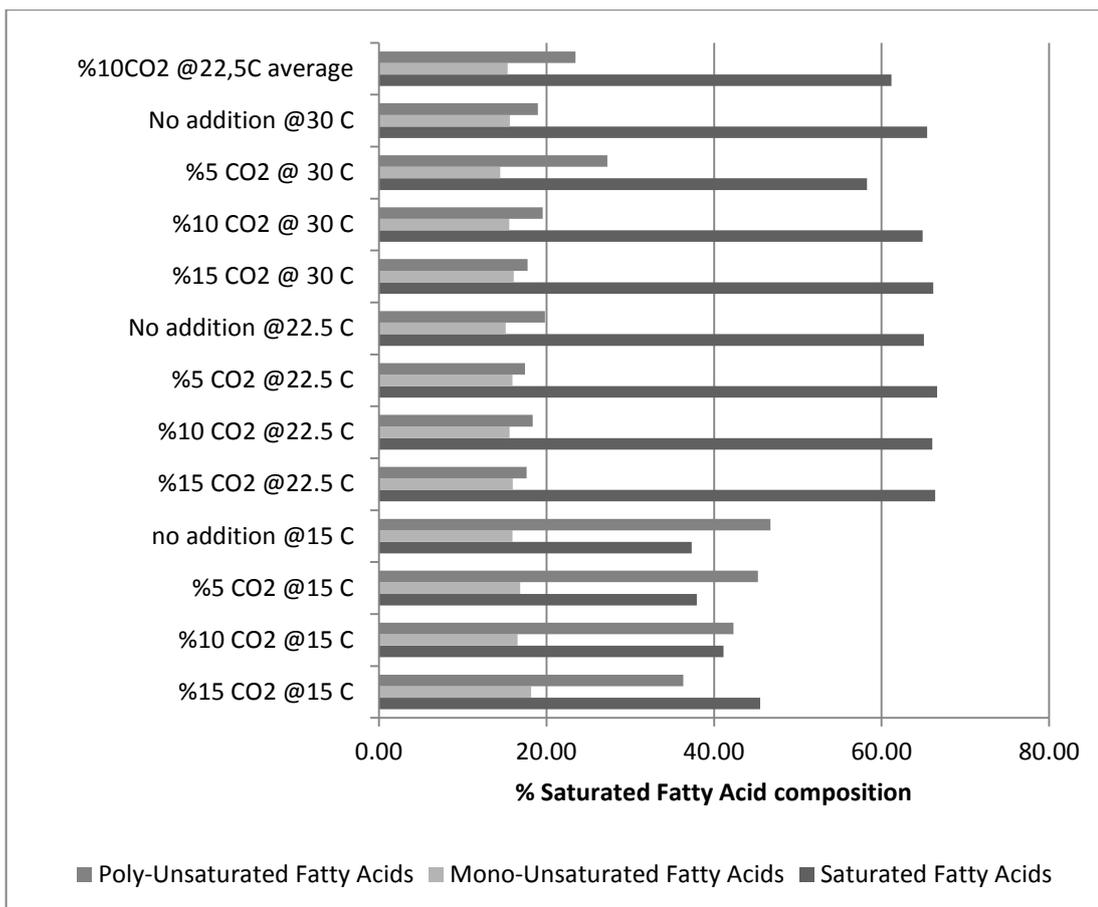


Figure 5.31. Fatty acid distributions for each experimental condition.

Outputs of fatty acid distribution at each case summarized in Table 5.11. Concurrently, lipid chain lengths, composition of saturated, mono-unsaturated and poly-unsaturated fatty acid can be seen in Figure 5.29, Figure 5.30 and Figure 5.31.

Table 5.12. ANOVA results. Saturated Fatty Acid versus CO₂ and Temperature;

Term	Coef	P
Constant	-76.6232	0.001
CO ₂	0.5081	0.066
Temp	10.587	0
Temp*Temp	-0.2033	0

S: 2.92418 PRESS: 147.828

R-Sq: 94.29% R-Sq(pred): 87.66% R-Sq(adj): 92.15%

Lack-of-Fit: P: 0.260

Surface Plot of Saturated Fatty Acids vs Temp; CO2

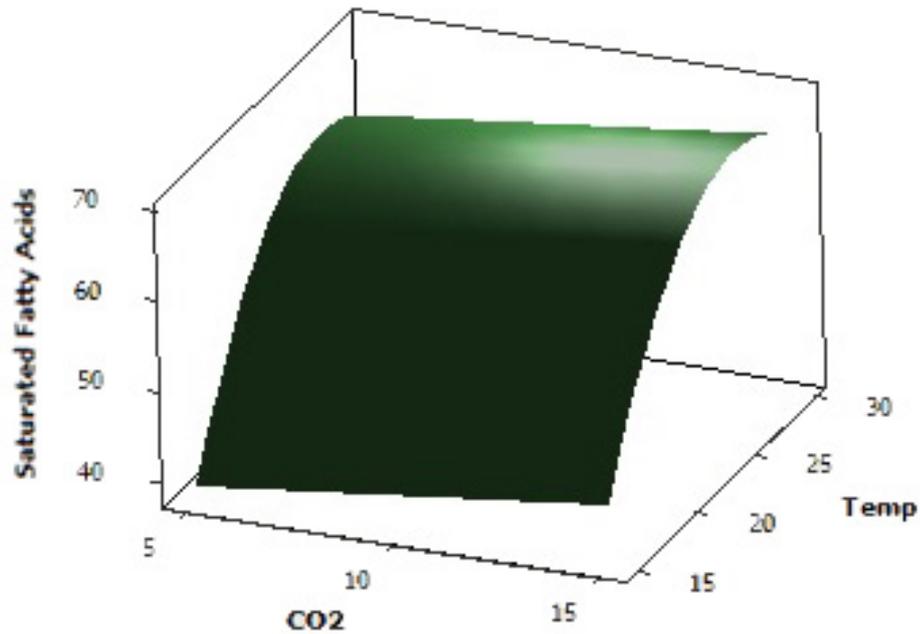


Figure 5.32. Surface plots for saturated fatty acids.

Data analysis shows that lower levels of saturated fatty acids was observed at low temperatures and slightly decreased at higher temperatures as seen in Figure 5.32, and the levels of mono and poly-unsaturated acids decreases with increasing medium temperature as seen in Figures 5.33 and 5.34.

Table 5.13. ANOVA for CCD. Mono-Unsaturated Fatty Acids versus CO₂ and Temperature;

Term	Coef	P
Constant	23.5258	0
CO ₂	0.0982	0.064
Temp	-0.6708	0.033
Temp*Temp	0.0122	0.066

S : 0.559875 PRESS: 5.69721

R-Sq: 75.68% R-Sq(pred): 44.74% R-Sq(adj): 66.56%

Lack-of-Fit: P: 0.442

Surface Plot of Mono-Unsaturated Fatty Acids vs Temp; CO2

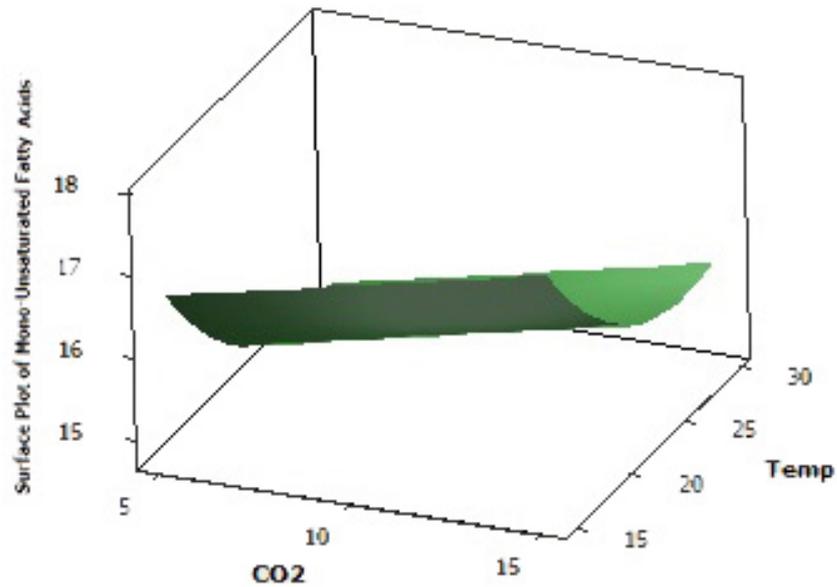


Figure 5.33. Surface plots for Mono-unsaturated fatty acids.

Table 5.14. ANOVA results: Poly-Unsaturated Fatty Acids versus CO₂ and Temperature;

Term	Coef	P
Constant	153.097	0
CO ₂	-0.606	0.057
Temp	-9.916	0
Temp*Temp	0.191	0.001

S: 3.33737 PRESS: 195.866

R-Sq: 91.73% R-Sq(pred): 81.81% R-Sq(adj): 88.62%

Lack-of-Fit: P: 0.344

Surface Plot of Poly-Unsaturated Fatty Acids vs Temp; CO₂

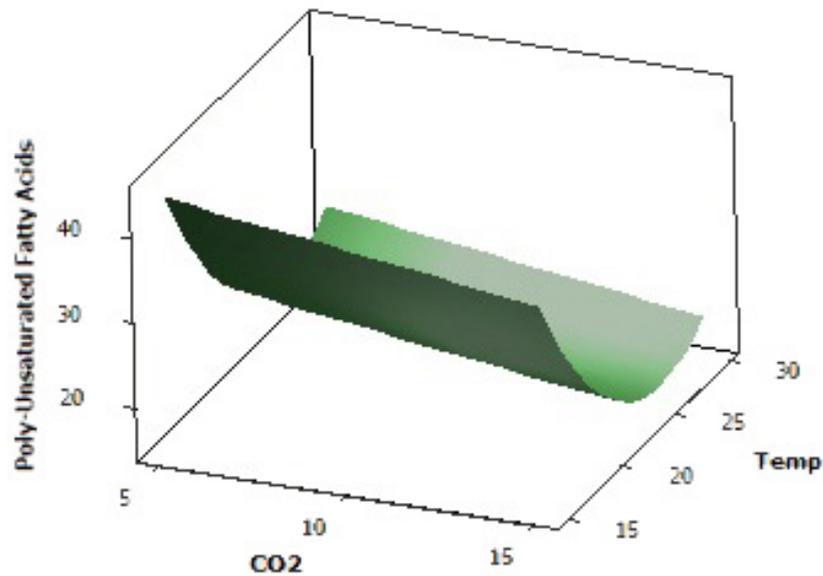


Figure 5.34. Surface plots for poly-unsaturated fatty acids.

Lipid composition is highly important for usability in biodiesel production. This figure (Figure 5.34) relates the degree of saturation of biodiesel from different sources and experimental results. Chemical composition of these sources determines the final fuel properties. Both cloud point and fuel viscosity increases with the degree of saturation, measured as iodine value, of lipid source. Yet increase in saturation increases cetane number of the fuel. Another factor that increases cetane number is chain length. Chain length of lipid is proportional of cetane number of the fuel produced (Knothe 2005). Another important effect of saturation extent is the stability of FAME molecules. Biodiesel with low polyunsaturated fat levels should also emit lower levels of nitrogen oxides.

Thus a balanced composition of these properties should be selected for biodiesel production. Through this study alga produces lipids in two groups;

- Lipids with low polyunsaturation and high saturation at higher temperatures than 15 °C and
- Lipids with high polysaturation and low saturation at low temperatures such as 15 °C.

Especially lipids produced at higher temperatures have monounsaturated fatty acid content similar to palm oil as 45%, thus these lipid compositions are in suitable

ranges for use as a feedstock to produce biodiesel (Knothe 2005). Further targeted fuel properties can be achieved by changing growth parameters and this way changing lipid composition of *Isochrysis galbana* for improved cold flow, improved ignition quality (CN), and presumably reduced nitrogen oxide emissions.

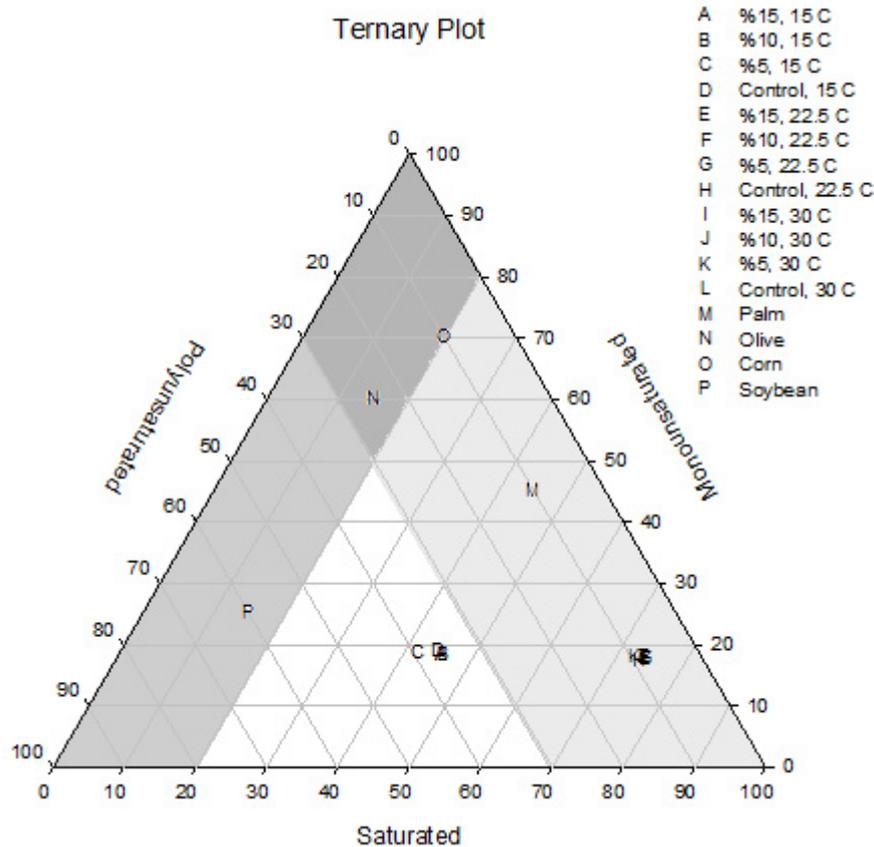


Figure 5.35. Degree of saturation of fatty acids on cold filter plug point (CFPP, bimedium grey) and cetane number as well as iodine value (light grey).

One of the most significant results due to these experiments was decreasing amount of CO₂ and oil. Algal lipids are composed of fats, phospholipids, steroids which are secondary metabolites important for nutrition. Figure 5.35 represents the fatty acid comparisons. As seen in Table 5.15, *Isochrysis galbana* has high levels of omega-3 Fatty acids, which are C 18:2,(n-3) (α -Linolenic acid-ALA), C C 20:5 (n-3) (Eicosapentaenoic Acid-EPA) and C 22:6 (n-3) (Docosahexaenoic acid - DHA), have a concentration up to 23.39 at 15 °C experiments. The key omega -6 Fatty acids, C 18:2 (n-6) (Linoleic acid) and C 20:4 (n-6) (Arachidonic acid) amounts were reported in Table 5.15. Other than their nutritional properties these fatty acids have pharmaceutical uses like rheumatoid arthritis and multiple sclerosis therapy (Bishop-Weston 2008).

Table 5.15. % Omega acids for each experimental case.

Temperature (°C)	CO ₂ Concentration (v%)	Omega-3	Omega-6
15	15	23.39	1.60
	10	18.63	1.29
	5	21.49	1.37
	no addition	20.39	0.45
22.5	15	0.00	0.93
	10	0.00	0.89
	5	0.00	0.93
	no addition	0.00	1.09
30	15	0.00	0.96
	10	0.00	0.92
	5	0.00	0.73
	no addition	0.00	0.83

5.4. Results of Validation Experiments at 15 °C and 26 °C

It was observed that the unsaturated fatty acid contents were higher at lower temperatures. On the other hand, the amount of lipid contents were observed higher at growth mediums around optimum or higher temperature conditions for *Isochrysis galbana*. For these reasons, some experiments were performed at 15 °C and 26 °C at exactly the same CO₂ conditions (5%, 10%, 15%). The experimental conditions were repeated or changed to see whether the determined equations at the end of CCD could be used to estimate the response variables observed at different conditions. The results of the validation experiments were given in Table 5.16.

Table 5.16. Experimental results of validation experiments.

CO ₂ Levels (v/v)	Temperature (°C)	dry weight (g/L)	lipid weight (g/L)	lipid content (%w)	specific growth rate
5	15	0.661	0.094	14.3	0.581
10	15	0.681	0.101	14.9	0.586
15	15	0.652	0.089	13.7	0.458
no addition	15	0.658	0.094	14.3	0.347
5	26	1.076	0.136	12.7	0.161
10	26	1.152	0.146	12.1	0.151
15	26	1.170	0.145	12.4	0.194
no addition	26	1.044	0.13	12.5	0.176

The observed values were compared with the estimated values, which were determined by the regression equations of central composite design. The observed, predicted and error values with the model constants for experimental parameters (linear, interaction and quadratic) for all responses are given in Table 5.17. It was seen that models for dry weight and lipid contents fits well in the range with low error %.

Table 5.17. Calculations for 15 C and 26 C validation experiments at 5%-10%-15% CO₂ levels.

	Biomass (μ)	Dry weight g/L	Lipid weight g/L	Lipid content %	SFA	MUFA	PUFA
B₀	0.902743	-1.31693	-0.00216	27.3133	-76.6232	23.5258	153.097
B₁	-0.04334	0.18434	0.002878	-1.4942	10.587	-0.6708	-9.916
B₂	-0.03841	0.015	0.004617	0.444	0.5081	0.0982	-0.606
B₁₁	0.001263	-0.00381	0	0.0358	-0.2033	0.0122	0.191
B₂₂	0.00329	0	0	0	0	0	0
B₁₂	-0.00162	0	0	-0.0213	0	0	0
R2-adj	0.67	0.44	0.34	0.81	0.92	0.67	0.89
R2-pred	0,62	0,75	0,14	0.33	0.88	0.45	0.82
LOF-p	0.00	0.25	0.02	0.23	0.26	0.44	0.34
Y observed	0.581	0.661	0.094	14.300	33.956	32.154	33.890
	0.586	0.681	0.101	14.900	36.523	32.883	30.594
	0.458	0.652	0.089	13.700	36.324	31.389	32.287
	0.161	1.076	0.136	12.700	50.281	33.343	16.376
	0.151	1.152	0.146	12.100	49.617	32.998	17.385
	0.194	1.170	0.145	12.400	52.432	32.521	15.047
Y predicted	0.306	0.666	0.064	13.578	38.980	16.700	44.302
	0.239	0.741	0.087	14.200	41.520	17.191	41.272
	0.336	0.816	0.110	14.823	44.061	17.682	38.242
	0.309	0.975	0.096	12.116	63.749	14.823	21.367
	0.153	1.050	0.119	11.567	66.289	15.314	18.337
	0.162	1.125	0.142	11.018	68.830	15.805	15.307
% Error	47.415	0.740	31.814	5.050	14.795	48.063	30.723
	59.263	8.732	13.683	4.696	13.683	47.722	34.904
	26.546	25.139	23.893	8.196	21.300	43.669	18.443
	92.106	9.354	29.593	4.599	26.784	55.543	30.480
	1.583	8.824	18.604	4.406	33.602	53.591	5.476
	16.499	3.816	2.122	11.146	31.274	51.400	1.727

CHAPTER 6

CONCLUSION

The growth and lipid production of *Isocrysis galbana* was studied at different CO₂ levels and growth medium temperatures. It was observed that the highest dry weight achieved at 22 °C, which is the optimal temperature. Lipid composition of algae greatly changed at 15 °C and high ratios of lipids with carbon chains of 24 or over were observed. At 15 °C medium temperature, growth takes longer time to achieve high concentrations of biomass and lipid compared to other temperatures studied. The effects of temperature on growth were clearly observed. At temperatures 22.5 °C and 30 °C a similar fatty acid profile was observed as high saturated fatty acids and low unsaturated fatty acids.

High molecular weight lipid production was promoted at 15 °C growth mediums. At 22.5 °C and 30°C growth conditions there was no significant change in lipid composition in terms of chain length and saturation levels. As a result of data analysis, temperature was shown to be more significant parameter than CO₂ in all response variables. Experimental studies showed that growth medium conditions could be arranged for different type of algal products. Lipid profile and amount of biomass of *Isocrysis galbana* can be controlled by changing temperature and CO₂ levels to satisfy various needs such as source of omega fatty acids, biodiesel production and animal feed.

REFERENCES

- Andersen Robert Arthur, "Algal Culturing Techniques", Academic Press, US, pg. 248-266, 2005
- Arad, Shoshana; Spharim, Ishai, "Production of Valuable Products from Microalgae: An Emerging Agroindustry", CRC Press. p. 638, 1998
- Atay, D., "Denizyosunları ve Değerlendirme Olanakları", Başbakanlık Basımevi, Ankara 1982
- Atay, D. and S. Bekcan, "Marine Fish and Production Technique" Ankara University, Ankara, Turkey, ISBN: 975-482-517-3, 2000
- Barbosa M., Hans Wolkers, Dorinde M.M. Kleinegriss, Rouke Bosma, René H. Wijffels "Microalgae: the green gold of the future?", Wageningen Ur, Nl., pg . 16 – 22, 2011
- Barsanti, L., Paolo Gualtieri, "Algae: Anatomy, Biochemistry, and Biotechnology", Taylor & Francis, 2006
- Becker, E.W., "Microalgae Biotechnology and Microbiology", Cambridge University Press, 1995
- Bigogno, C., I Khozin-Goldberg, S Boussiba, A Vonshak; Z Cohen., "Lipid and fatty acid composition of the green oleaginous alga *Parietochloris incisa*, the richest plant source of arachidonic acid". *Phytochemistry* 60 (5): 497–503. doi:10.1016/S0031-9422(02)00100-0.PMID 12052516, 2002
- Bishop-Weston Yvonne, "Plant based sources of vegan & vegetarian Docosahexaenoic acid - DHA and Eicosapentaenoic acid EPA & Essential Fats", 2008
- Borowitzka, M.A., "Pharmaceuticals and Agrochemicals from Microalgae". In: *Chemicals From Microalgae*, Cohen, Z. (Ed.). Taylor and Francis, London, ISBN-13:9780748405152, pp: 313-352, 1999
- Brown, M. R., Jeffrey, S.W., Volkman, J.K. and Dunstan, G.A., "Nutritional properties of microalgae for mariculture", *Aquaculture*, 151; 315-331, 1997

- Chiu, S. Y., Kao, C. Y., Tsai, M. T., Ong, S. C., Chen, C. H. and Lin, C. S., "Lipid accumulation and CO₂ utilization of *Nannochloropsis oculata* in response to CO₂ aeration." *Bioresour. Technol.* 100(2): 833-838, 2009
- Christi, Y., "Biodiesel from Microalgae," *Biotechnology Advances*, Vol. 25, pp. 294-306, 2007
- Cirik, S. ve Gökpinar, Ş., "Plankton Bilgisi ve Kültürü". Ege Üniversitesi Su Ürünleri Fakültesi Yayınları No:47, 274 s., İzmir, 1993.
- Cohen P., "The ASME Handbook on Water Technology for Thermal Power Systems", The American Society of Mechanical Engineers, 442, 1989
- Coutteau, P., Manual on the production and use of live food for aquaculture. In: Lavens, P. and Sorgeloos, P. (Editors). page 7-49. FAO publications, Roma, Italy, 1996
- Emdadi. D., Berland, B., "Variation in lipid class composition during batch growth of *Nannochloropsis salina* and *Pavlova lutheri*, *Mar. Chem.* 26: 215-225, 1989
- FAO, "Production and Use of Live Food for Aquaculture", e-book, issn 0429-934, 2008
- Gökpinar, Ş., "Observations on the culture of a marine diatom *Phaeodactylum*", Ege Ü. Su ürünleri Fakültesi yayınları, 1983
- Gökpinar, Ş., "Mikroalg Kültürleri I-Uygulama ve Kullanım Alanları", Ege Ü. Su Ürünleri Derg., 7; 46-56, 1990.
- Grima, E.M., Fernandez, F.G.A., Camacho, F.G., Chisiti, Y., "Photobioreactors: Light Regime, Mass Transfer, and Scaleup", *Journal of Biotechnology*, 70, 231-247, 1998
- Guillard, R.R.L., "Culture of phytoplankton for feeding marine invertebrates", In Smith W.L. and Chanley M.H (Eds.) *Culture of Marine Invertebrate Animals*. Plenum Press, New York, USA, 26-60, 1975
- Guillard, R.R.L., "Counting slides. In: Sournia, A. (Ed.) *Phytoplankton Manua*",. Unesco Publ., Paris, pg. 182-190, 1978

- Guillard, R.R.L. and Ryther, J.H., "Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. Can", *J. Microbiol.* 8: 229-239, 1962.
- Hori T. J. C. Green., "The ultrastructure of mitosis in *Isochrysis galbana* Parke (Prymnesiophyceae)", *Protoplasma* 125: 140-151, 1985
- Knothe G., Jon Van Gerpen, Furgun Khral, "The Biodiesel Handbook", AOCS Press, 2005
- Lee W., James E. Graham, Wilcox, Linda E. Graham, "Algae", 2nd ed., Pearson College Division, 2011
- Lewis, J G; Stanley, N F; Guist, G G,. "9 Commercial production of algal hydrocolloides". In Lembi, C.A.; Waaland, J.R.. *Algae and Human Affairs*. Cambridge: Cambridge University Press. ISBN 978-0-521-32115-0.), London, pg. 226, 1988
- Liu, C.P., and Lin, L.P., "Ultrastructural study and lipid formation of *Isochrysis* sp. CMP1324", *Botanical Bulletin Academia Sinica*, 42, pg. 207-214, 2001
- Marchetti A, Schruth D. M, Durkin C. A, Parker M. S, Kodner R, Berthiaume C. T, Morales R, Allen A. E, Armbrust E. V., "Comparative metatranscriptomics identifies molecular bases for the physiological responses of phytoplankton to varying iron availability", *Proceedings of the National Academy of Science*. 109: E317-E325, 2012
- Marchetti, J., Bougaran, G., Jauffrais, T., Lefebvre, S., Rouxel, C., Saint-Jean, B., Lukomska, E., Robert, R. & Cadoret, J.P.. "Effects of blue light on the biochemical composition and photosynthetic activity of *Isochrysis* sp. (T-iso)", *Journal of Applied Phycology*, 10.1007/s10811-012-9844-y, 2012
- Martinez B., Chen, P., M. Min, Y. Chen, L. Wang, Y. Li, Q. Chen, C. Wang, Y. Wan, X. Wang, Y. Cheng, S. Deng, K. Hennessy, X. Lin, Y. Liu, Y. Wang, R. Ruan. "Review of biological and engineering aspects of algae to fuel approach", *International Journal of Agricultural and Biological Engineering* 2(4):1-30, 2010.
- McHugh, Dennis J.. "9, Other Uses of Seaweeds". *A Guide to the Seaweed Industry: FAO Fisheries Technical Paper 441*. Rome: Fisheries and Aquaculture Department, Food and Agriculture Organization (FAO) of the United Nations. ISBN 92-5-104958-0, 2003

- Miron Sanchez A, Ceron Garcia MC, Contreras Gomez A, Garcia Camacho F, Molina Grima E, Chisti Y., “Shear stress tolerance and biochemical characterization of *Phaeodactylum tricornutum* in quasi steady-state continuous culture in outdoor photobioreactors”, *Biochem. Eng. J.* 16: 287-297, 2003
- Mohanty, P., Madhulika, S., Krishna, B.K., “The photosynthetic apparatus of *Spirulina*: physiology, cell-biology, and biotechnology”, London ; Bristol, PA : Taylor & Francis, pg. 140 – 230, 1997
- Mondragon, J., “Seaweeds of the Pacific Coast. Monterey”, California: Sea Challengers Publications. ISBN 0-930118-29-4. Ireland, dulce; Chile, cochayuyo. "*Durvillaea antarctica* (Chamisso) Hariot". *AlgaeBase*, 2003
- Morais, M., and J. Costa., “Carbon dioxide fixation by *Chlorella kessleri*, *C. vulgaris*, *Scenedesmus obliquus* and *Spirulina* sp. cultivated in flasks and vertical tubular photobioreactors” *Biotechnology Letters* 29(9):1349-1352, 2007
- Moretti, A., Fernandez-Criado, M.P., Cittolin, G. and Guidastri, R.. “Manual on Hatchery Production of Seabass and Gilthead Seabream”, FAO, 194 s., Rome, 1999
- Morton, Steve L. "Modern Uses of Cultivated Algae". *Ethnobotanical Leaflets*. Southern Illinois University Carbondale. Archived from the original on 2008-12-23. Retrieved 2008
- Negoro, M., Shioji, N., Miyamoto, K. and Miura, Y.. “Growth of Microalgae in High CO₂ Gas and Effects of SO_x and NO_x”, *Applied Biochemistry and Biotechnology*, 28-29, 877-886, 1991
- Olaizola M., “Commercial development of microalgal biotechnology: from the test tube to the marketplace”, *Biomol Eng.*, 20: 459–66, 2003
- Pal D, Khozin-Goldberg I, Cohen Z, Boussiba S.,”The effect of light, salinity, and nitrogen availability on lipid production by *Nannochloropsis* sp.”, *Appl Microbiol Biotechnol.*, 90(4):1429-41. doi: 10.1007/s00253-011-3170-1, 2011
- Patringenaru, I., “Powering the Future with Pond Scum New Center Aims to Bring About Algae-Powered Green Revolution” *UCSD University Communications*, 2009

- Pirt, S. J., Lee, Y. K., Walach, M. R., Bayuzi, H. H. M., Bazin, M. J. "A tubular photobioreactor for photosynthetic production of biomass from CO₂: Design and performance", *J. Chem. Technol. Biotechnol.*, 33b: 35–38, 1983
- Pulz O., "Photobioreactors: production systems for phototrophic microorganisms", *Applied Microbiology and Biotechnology*, Volume 57, Issue 3, pg 287-293, 2001
- Roessler, P., "Environmental control of glycerolipid metabolism in microalgae: commercial implications and future research directions", *J. Phycol.* 26, 393–399, 1977, 1990
- Simionato D., Giorgio Mario Giacometti, Alberto Bertucco, Tomas Morosinotto, Eleonora Sforza, "Adjusted Light and Dark Cycles Can Optimize Photosynthetic Efficiency in Algae Growing in Photobioreactors", *PLoS One.* 7(6): e38975, 2012
- Simoons, Frederick J., "6, Seaweeds and Other Algae". *Food in China: A Cultural and Historical Inquiry.* CRC Press. pp. 179–190. ISBN 0-936923-29-6., 1991
- Smith, L.L., Fox, J.M. and Granvil, D.R., "Intensive algae culture techniques. In: *CRC Handbook of mariculture. Volume 1. Crustacean Aquaculture*", 2nd Edition. McVey J.P. (Ed.). CRC Press, Inc., Boca Raton, Florida, USA, pp 3-13, 1993
- Tanaka H., Ogbonna, J.C., H. Yada, "Light supply coefficient: A new engineering parameter for photobioreactor design", *J. Ferment. Bioeng.*, 2: 369-376, 1995.
- Thompson Jr., G.A., "Lipids and membrane function in green algae", *Biochim. Biophys. Acta* 1302, 17–45, 1996
- Timur, G. "Plankton Bilgisi ve Plankton Kültürü", *Akdeniz Üniv. Yay. No: 40*, 1992
- Torzillo G., A. Vonshak, "Environmental stress physiology" A. Richmond (Ed.), *Handbook of Microalgal Culture*, Blackwell Publishers, Oxford (, pp. 57–82, 2004
- Torzillo, G., A. Sacchi, R. Materassi and A. Richmond. Effect of temperature on yield and night biomass loss in *Spirulina platensis* grown outdoors in tubular photobioreactors. *J. Appl. Phycol.*, 3: 103-109. 1991

- Torzillo, G., Pushparaj, B., Masojidek, J. and Vonshak, A., "Biological constraints in algal biotechnology". *Biotechnology and Bioprocess Engineering*, 8:338-348, 2003.
- Van Ness, H. C., Joseph Mauk Smith, Michael M. Abbott, "Introduction to Chemical Engineering Thermodynamics", Mc Graw Hill 7th ed, 2005
- Vonshak, A. (Ed.), "Cell biology and biotechnology", Taylor and Francis publishers, Vol:1, 1986
- Yongmanitchai, W., Ward, O.P., "Omega-3 fatty acids: alternative sources of production", *Process Biochem.* 24, 117–125, 1989
- Yoshioka Miwa, Takahide Yago, Yumiko Yoshie-Stark, Hisayuki Arakawa, Tsutomu Morinaga, "Effect of high frequency of intermittent light on the growth and fatty acid profile of *Isochrysis galbana*", *Aquaculture* 338-341 111–117, 2012
- Zeng Xianhai, Michael K. Danquah, Shiduo Zhang, Xia Zhang, Mengyang Wu, Xiao Dong Chen, I-Son Ng, Keju Jing, Yinghua Lua, "Autotrophic cultivation of *Spirulina platensis* for CO₂ fixation and phycocyanin Production", *Chemical Engineering Journal* 183 192– 197, 2012
- Zhu, C.J., Lee, Y.K., & Chao, T.M., "Effects of temperature and growth phase on lipid and biochemical composition of *Isochrysis galbana* TK1", *Journal of Applied Phycology*, 9, 451-457, 1997

APPENDIX A

CALIBRATION EQUATIONS FOR INDIVIDUAL FATTY ACIDS

Lipid	Formula	R2
Butyric Acid Methyl Ester (C4:0)	$y = 1E-07x + 6E-07$	0.8442
Caproic Acid Methyl Ester (C6:0)	$y = 3E-07x + 5E-08$	0.9713
Caprylic Acid Methyl Ester (C8:0)	$y = 5E-07x + 6E-08$	0.9708
Capric Acid Methyl Ester (C10:0)	$y = 2E-07x + 8E-08$	0.9712
Undecanoic Acid Methyl Ester (C11:0)	$y = 2E-07x + 2E-08$	0.9713
Lauric Acid Methyl Ester (C12:0)	$y = 2E-07x + 8E-08$	0.9712
Tridecanoic Acid Methyl Ester (C13:0)	$y = 2E-07x + 5E-08$	0.9713
Myristoleic Acid Methyl Ester (C14:1)	$y = 3E-07x + 3E-08$	0.9713
Myristic Acid Methyl Ester (C14:0)	$y = 5E-07x + 1E-07$	0.9709
cis-10-Pentadecenoic Acid Methyl Ester (C15:1)	$y = 8E-08x + 5E-08$	0.9712
Pentadecanoic Acid Methyl Ester (C15:0)	$y = 2E-07x + 3E-07$	0.9575
Palmitoleic Acid Methyl Ester (C16:1)	$y = 3E-07x + 2E-09$	0.9704
Palmitic Acid Methyl Ester (C16:0)	$y = 7E-07x + 3E-07$	0.9682
cis-10-Heptadecenoic Acid Methyl Ester (C17:1)	$y = 1E-07x + 2E-07$	0.9667
Heptadecanoic Acid Methyl Ester (C17:0)	$y = 3E-07x - 1E-07$	0.9671
Linolenic Acid Methyl Ester (C18:3n6)	$y = 1E-07x + 2E-07$	0.966
Linoleic Acid Methyl Ester (C18:2n6c)	$y = 2E-06x - 2E-06$	0.9459
Elaidic Acid Methyl Ester (C18:1n9t)	$y = 2E-07x + 7E-08$	0.9695
Stearic Acid Methyl Ester (C18:0)	$y = 6E-07x - 4E-07$	0.9709
Arachidonic Acid Methyl Ester (C20:4n6)	$y = 1E-07x + 1E-07$	0.9678
cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester (C20:5n3)	$y = 3E-07x - 5E-08$	0.9684
cis-8,11,14-Eicosatrienoic Acid Methyl Ester (C20:3n6)	$y = 3E-07x + 4E-08$	0.9712
cis-11,14-Eicosadienoic Acid Methyl Ester (C20:2)	$y = 3E-07x + 1E-07$	0.9701
cis-11-Eicosenoic Acid Methyl Ester (C20:1n9)	$y = 6E-07x - 8E-08$	0.9385
Arachidic Acid Methyl Ester (C20:0)	$y = 5E-07x + 3E-07$	0.9711
Heneicosanoic Acid Methyl Ester (C21:0)	$y = 1E-07x + 2E-07$	0.971
cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester (C22:6n3)	$y = 1E-07x + 1E-07$	0.9708
cis-13,16-Docosadienoic Acid Methyl Ester (C22:2)	$y = 5E-07x + 3E-07$	0.9712
Behenic Acid Methyl Ester (C22:0)	$y = 6E-07x + 3E-07$	0.9711
Tricosanoic Acid Methyl Ester (C23:0)	$y = 1E-07x + 1E-07$	0.9705
Nervonic Acid Methyl Ester (C24:1n9)	$y = 1E-07x + 2E-07$	0.9693
Lignoceric Acid Methyl Ester (C24:0)	$y = 6E-07x + 4E-07$	0.9697
Lipids with over 24 Carbons	$y = 3E-07x + 4E-06$	0.9849

APPENDIX B

CALIBRATION CURVES FOR GROWTH STUDIES

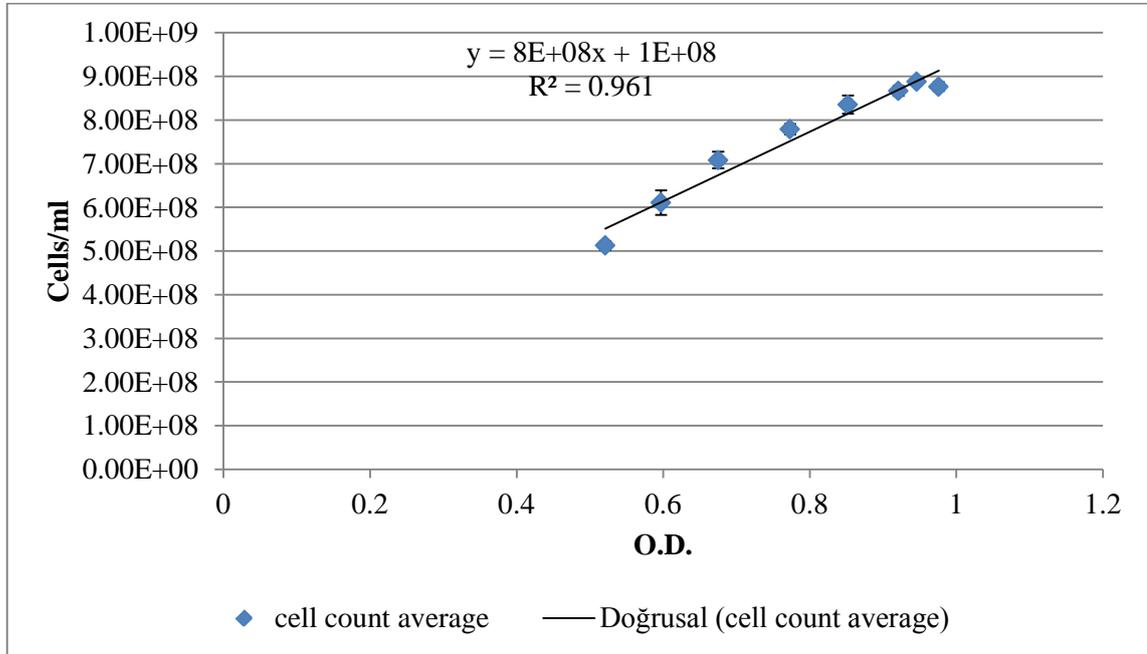


Figure B.1. Calibration Curves For Cell Count Versus Optical Density Studies.

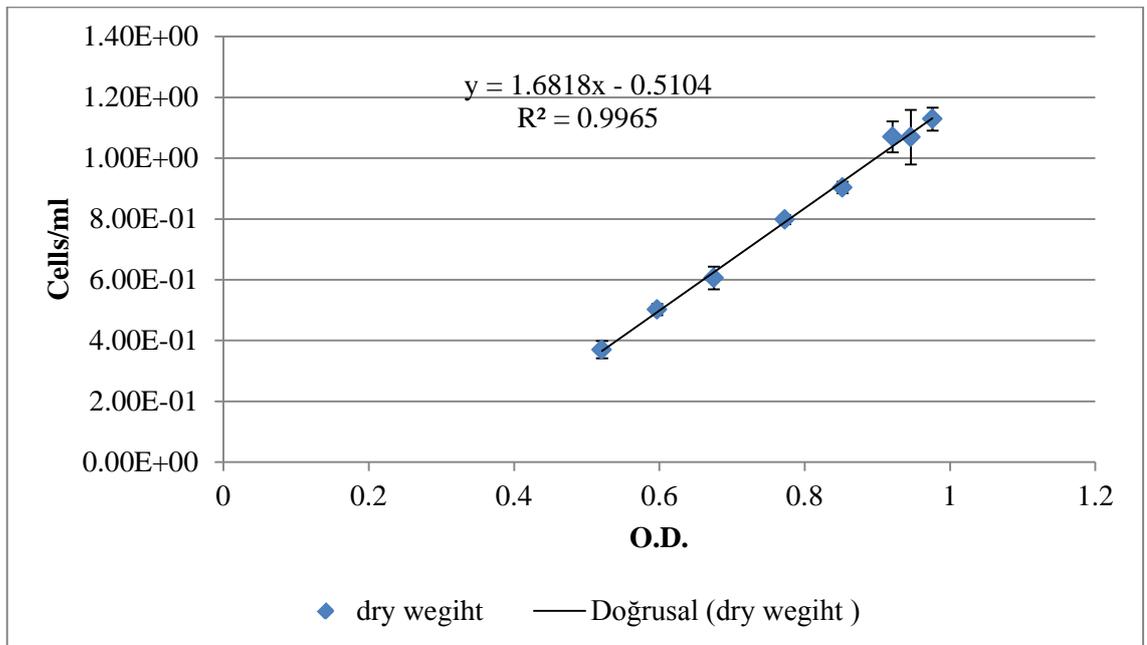


Figure B.2. Calibration Curves For Dry Weight Versus Optical Density Studies.

APPENDIX C

EXPERIMENTAL OUTPUTS AT EACH CASE

CO ₂ Levels (v/v)	Temperature (°C)	dry weight (g/L)	lipid weight (g/L)	lipid content (%w)	specific growth rate (cell/hour)	Time (days)	dry weight/day (g)	lipid weight/day (g)
5	15	0.674	0.095	14.1	0.361	18.5	0.036	0.005
10	15	0.705	0.103	14.7	0.343	18.5	0.038	0.005
15	15	0.639	0.088	13.8	0.412	18.5	0.034	0.004
no addition	15	0.652	0.085	13.09	0.293	18.5	0.035	0.004
5	22.5	1.076	0.125	11.2	0.15	14	0.076	0.008
10	22.5	1.113	0.122	11.01	0.191	14	0.079	0.008
15	22.5	1.095	0.13	11.93	0.196	14	0.078	0.009
no addition	22.5	1.054	0.118	11.23	0.181	14	0.075	0.008
5	30	0.846	0.116	13.8	0.173	12	0.0705	0.009
10	30	1.097	0.145	13.3	0.192	12	0.091	0.012
15	30	0.89	0.107	12.1	0.173	12	0.074	0.008
no addition	30	0.811	0.108	13.36	0.178	12	0.067	0.009
10	22.5	1.130	0.124	11.25	0.149	13	0.086	0.009
10	22.5	1.062	0.120	11.34	0.167	13	0.081	0.009
10	22.5	1.031	0.115	11.18	0.163	13	0.079	0.008
5	15	0.661	0.094	14.3	0.581	14	0.047	0.006
10	15	0.681	0.101	14.9	0.586	14	0.048	0.007
15	15	0.652	0.089	13.7	0.458	14	0.046	0.006
no addition	15 °C	0.6575	0.094	14.3	0.347	14	0.046	0.006
5	26 °C	1.076	0.136	12.7	0.161	11	0.097	0.012
10	26 °C	1.152	0.146	12.1	0.151	12	0.096	0.012
15	26 °C	1.17	0.145	12.4	0.194	12	0.097	0.012
no addition	26 °C	1.044	0.13	12.5	0.176	11	0.094	0.011