Production of Lipids in Photobioreactors Using Microalgae

Rajesh Ghosh, Dr. Roshan Makam, Dr V Krishnamurthy,Sounak Bhattacharjee

Abstract— Microalgae represent an exceptionally diverse but highly specialized group of micro-organisms adapted to various ecological habitats. Many microalgae have the ability to produce substantial amounts (e.g. 20–50% dry cell weight) of triacylglycerols (TAG) as a storage lipid under photo-oxidative stress or other adverse environmental conditions. Fatty acids, the building blocks for TAGs and all other cellular lipids, are synthesized in the chloroplast using a single set of enzymes, of which acetyl CoA carboxylase (ACCase) is key in regulating fatty acid synthesis rates. However, the expression of genes involved in fatty acid synthesis is poorly understood in microalgae. Synthesis and sequestration of TAG into cytosolic lipid bodies appear to be a protective mechanism by which algal cells cope with stress conditions, but little is known about regulation of TAG formation at the molecular and cellular level.Lipid production is done in Photobioreactor nowadays but before it was done in open system. Today, the production of algal oil is primarily confined to high-value specialty oils with nutritional value, rather than commodity oils for biofuel. This review provides a brief summary on production of lipid in photobioreactor using microalgae ,the difference between open system and photobioreactor and a historical perspective and path forward for microalgae-based biofuel research and commercialization.

Index Terms—biofuels, fatty acids , lipids, microalgae, open system ,photobioreactor, triacylglycerol.

1 INTRODUCTION

In recent years, global warming, world oil supply, energy demand have all played a part in the push for alternatives to petroleum-based fuels. The Inter governmental Panel on Climate Change (IPCC) affirms that during the 20th century, the Earth's average temperature increased by 0.6°C and will continue to increase anywhere from 1.5°C to 4.5°C by the year 2100 .[1] This increase in global temperature is enough to cause flooding in coastal regions and make storms like Hurricane Katrina a more common occurrence.[2] The major force in rising global temperatures is anthropogenic carbon dioxide emissions, which accounts for 80% of all greenhouse gases produced .[1]

Sustainable production of renewable energy is being debated globally since it is increasingly
understood that first generation biofuels, primarily produced from food crops and mostly oil seeds, compete for arable land, freshwater or bio diverse natural landscapes and are limited in their ability to achieve targets for biofuel production. These concerns have increased the interest in developing second and third generation biofuels such as lignocellulosics and microalgae, respectively, which potentially offer great opportunities in the longer term and do not need to compete for arable land and precious freshwater .[2,3] Due to continuous and increasing combustion of fossil carbon, the amount of greenhouse gas CO_2 has increased. As a result global warming and climate change are threatening ecological stability, food security and social welfare .[4,5] The transportation and energy sector are the two major sources, responsible for the generation of 20% and 60% of greenhouse gases (GHG) emissions, respectively, and it is expected that with the development of emerging economies the global consumption of energy will rise considerably and this will lead to more environmental damage .[6]

Photosynthesis is the only process that can convert CO_2 into organic compounds with high energy
content, and thus can provide a source for sustainable transport fuel production. There is an urgent need to develop technologies which are able to produce an additional five to six billion tons of organic carbon apart from the current harvest from agricultural crops .[4] Large-scale cultivation of microalgae may be 10–20 times more productive on a per hectare basis than other biofuel crops, are able to use a wide variety of water sources, and have a strong potential to produce biofuels without the competition for food production [3]. Al-

gae can be produced either as macrophytes via marine aquaculture [7] or in large-scale microalgae cultivation systems in open ponds or in photobioreactors .[2] Microalgae are currently considered the most promising types of algae for biofuel production, based on their high lipid contents. Recent progress in the production of microalgae has been intensively reviewed [8], and future perspectives have been presented by Stephens et al. [6] Microalgae can also be cultivated as an integrated concept with wastewater treatment to optimize the energetic and financial input for the production process .[9]

Triacylglycerides (TAGs) generally serve as energy storage in microalgae that, once extracted, can
be easily converted into biodiesel through transesterification reactions. [4,10] These neutral lipids bear a
common structure of triple esters where usually three long-chain fatty acids (FAs) are coupled to a glycerol molecule. Transesterification displaces glycerol with small alcohols (e.g., methanol).
Recently, the rise in petroleum prices and the need to reduce greenhouse gas emission has seen a renewed interest in large-scale biodiesel production. [11]
Earlier lipid production from microalgae were used to be done in open system but now it is done in photobioreactor. This review mainly provides a brief summary on production of lipid in photobioreactor using microalgae ,the difference between open system and photobioreactor and a historical perspective and path forward for microalgae-based biofuel research and commercialization.

2 HISTORICAL PERSPECTIVE AND RECENT ADVANCES

2.1 Mass culture of microalgae.

Prior to the establishment of the US Department of Energy's (DOE) Aquatic Species Program, very little work had been conducted on bio fuel production from lipid-accumulating algae. While the general idea of using algae for energy production has been around for over 50 years (Meier, 1955), the concept of using lipids derived from algal cells to produce liquid fuels arose more recently .[13] Historically, algae have been seen as a promising source of protein and have been actively cultured by man for centuries, mainly for food. Growing algae as a source of protein on a large scale in open ponds was first conceived by German scientists during World War II (Soeder, 1986). The first attempt in the USA to translate the biological requirements for algal growth into engineering specifications for a large-scale plant was made at the Stanford Research Institute (1948–1950). During 1951, Arthur D. Little made a further advance through the construction and operation of a Chlorella pilot plant for the Carnegie Institute (Burlew, 1953). These studies eventually provided some of the most comprehensive early information on the growth, physiology and biochemistry of algae. Therefore, the concept of using mass-cultured algae for fuel production could be traced directly back to these early efforts on using algae for food production. Microalgae as a source of energy. The concept of using algae as a fuel was first proposed by Meier (1955) for the production of methane gas from the carbohydrate fraction of cells.[13] This idea was further developed by Oswald and Golueke (1960), who introduced a conceptual techno-economic engineering analysis of digesting microalgal biomass grown in large raceway ponds to produce methane gas. In the 1970s, as the cost of conventional fuels began rising rapidly, the possibility of using algae as a fuel source received renewed attention. A more detailed design and engineering analysis of this concept was carried out by Benemann et al. (1978), who concluded that such systems could produce biogas competitively with projected fossil fuel prices.
Lipid accumulation by microalgae. Under certain growth conditions, many microalgae can produce lipids that are suitable for conversion to liquid transportation fuels. In the late 1940s, nitrogen limitation was reported to significantly influence microalgal lipid storage. Spoehr and Milner (1949) published detailed information on the effects of environmental conditions on algal composition, and described the effect of varying nitrogen supply on the lipid and chlorophyll content of Chlorella and some diatoms. Investigations by Collyer and Fogg (1955) demonstrated that the fatty acid content of most green algae was between 10 and 30% DCW [16]. Werner (1966) reported an increase in the cellular lipids of a diatom during silicon starvation.[42] Coombs et al. (1967) reported that the lipid content of the diatom Navicula pelliculosa increased by about 60% during a 14 h silicon starvation period. [15] In addition to nutrition, fatty acid and lipid composition and content were also found to be influenced by a number of other factors such as light (Constantopolous and Bloch, 1967; Nichols, 1965; Pohl and Wagner, 1972; Rosenberg and

Gouaux, 1967) [17,18] and low temperatures (Ackman et al., 1968). With the advent of the oil embargo in the early 1970s, a search for alternative energy sources set the stage for an almost twenty-year research effort devoted to biofuel production from algal lipids.

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2.2 Essence of using microalgae in lipidproduction

- Ease of growth – In lipid production microalgae as feed stock when compared to more traditional crops is that it can be grown much more easily.[20] Algae can be grown in land that would not be considered suitable for the growth of the regularly used crops.[19] In addition to this, wastewater that would normally hinder plant growth has been shown to be very effective in growing algae.[20] Because of this, algae can be grown without taking up arable land that would otherwise be used for producing food crops, and the better resources can be reserved for normal crop production. Microalgae also require fewer resources to grow and little attention is needed, allowing the growth and cultivation of algae to be a very passive process.[19]

- The per unit area yield of oil from algae is estimated to be from between 5,000 to 20,000 US gallons per acre per year, and this is 7 to 30 times greater than the next best crop, Chinese tallow. So we can see that the growth rate to land ratio for algae is much higher than other agricultural crops and biodiesel feedstock.

- Impact on food -Many traditional feed stocks for biodiesel, such as corn and palm, are also used as feed for livestock on farms, as well as a valuable source of food for humans. Because of this, using them as biofuel reduces the amount of food available for both, resulting in an increased cost for both the food and the fuel produced. Using algae as a source of biodiesel can alleviate this problem in a number of ways. First, algae is not used as a primary food source for humans, meaning that it can be used solely for fuel and there would be little impact in the food industry.[21] Second, many of the waste-product extracts produced during the processing of algae for biofuel can be used as a sufficient animal feed. This is an effective way to minimize waste and a much cheaper alternative to the more traditional corn or grain based feeds.[22]

- We know that carbon dioxide is the greenhouse gas mostly responsible for climate change problem that is released in the atmosphere by fossil fuels burning. Some latest studies have shown that the production of each gallon of oil from algae consumes 13 to 14 kilograms of the carbon dioxide.

- Different algae species can be adapted to grow in different environmental conditions. So it is possible to find best suitable local environments for different species. But this still has not been possible with other feed stocks such as soybean, sunflower, palm oil etc.

- Minimization of waste -Growing algae as a source of biofuel has also been shown to have numerous environmental benefits, and has presented itself as a much more environmentally friendly alternative to current biofuels. For one, it is able to utilize run-off, water contaminated with fertilizers and other nutrients that are a by-product of farming, as its primary source of water and nutrients.[20] Because of this, it prevents this contaminated water from mixing with the lakes and rivers that currently supply our drinking water. In addition to this, the ammonia, nitrates, and phosphates that would normally render the water unsafe actually serve as excellent nutrients for the algae, meaning that fewer resources are needed to grow the algae. [19] Many algae species used in biodiesel production are excellent bio-fixers, meaning they are able to remove carbon dioxide from the atmosphere to use as a form of energy for themselves. Because of this, they have found use in industry as a way to treat flue gases and reduce GHG emissions.[19]

- They can be grown using water unsuitable for human consumption and have the ability to easily obtain nutrient from the environment. They can be produced even using ocean and waste water.

2.3 Algae Strains

Microalgae have many different species with widely varying compositions and live as single cells or colonies without any specialization. Although this makes their cultivation easier and more controllable, their small size makes subsequent harvesting more complicated. Macroalgae are less versatile, there are far fewer options of species to cultivate and there is

only one main viable technology for producing renewable energy: anaerobic digestion to produce biogas. Both groups will be considered, but there is more research, practical experience, more fuel options from microalgae, for this it take a bigger share in most research (GBEP, 2009). Biologists have categorized, microalgae in a variety of classes, mainly distinguished by their pigmentation, lifecycle and basic cellular structure, but the most important four are diatoms (Bacillariophyceae), green algae (Chlorophyceae), blue-green algae (Cyanophyceae), golden algae (Chrysophyceae). There are more than (30,000) to (100,000) kind of strain of algae, each kind includes many species (Nichols, J), but researches focused on microalgae for mass-production of oil, the preference toward microalgae is due to its less complex structure, fast growth rate, and high oil content.

Below given table summarizes the percentage lipid content by weight for some microalgae strains.

Lipid Content of some Microalgae (Chisti, Y.2007)

Microalgae	Lipid Content (%dry wt)
Botryococcus braunii	25-75
Chlorella sp	28-32
Crypthecodinum cohnii	20
Cylindrotheca sp	16-37
Dunaliella primolecta	23
Isochrysis sp	25-33
Monallanthus salina	>20
Nannochloris sp	20-35
Nannochloropsis sp	31-68
Neochloris oleoabundans	35-54
Nitzschia sp	45-47
Phaeodactylum tricornutum	20-30
Schizochytrium sp	50-77
Tetraselmis sueica	15-23

2.4 Selection of Algal Strain

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Ideally, algal strains are selected based on the following criteria :

Ability to capture large quantities of carbon dioxide

Lipid content/capability to produce high levels of algal oil

Resistance to contamination

Adaptability to temperature extremes

Specificity to the type of industry, source of CO2 and to local water conditions in growth ponds.

3 LIPIDS IN MICROALGAE

Lipids produced by microalgae generally include neutral lipids, polar lipids, wax esters, sterols and hydrocarbons, as well as prenyl derivatives such as tocopherols, carotenoids, terpenes, quinines and pyrrole derivatives such as the chlorophylls. Lipids produced by microalgae can be grouped into two categories, storage lipids (non-polar lipids) and structural lipids (polar lipids). Storage lipids are mainly
in the form of TAG made of predominately saturated FAs and some unsaturated FAs which can be transesterified to produce biodiesel. Structural lipids typically have a high content of polyunsaturated
fatty acids (PUFAs), which are also essential nutrients for aquatic animals and humans. Polar lipids (phospholipids) and sterols are important structural components of cell membranes which act as a selective permeable barrier for cells and organelles. These lipids maintain specific membrane
functions, providing the matrix for a wide variety of metabolic processes and participate directly in membrane fusion events. In addition to a structural function, some polar lipids may act as key
intermediates (or precursors of intermediates) in cell signaling pathways (e.g., inositol lipids,
sphingolipids, oxidative products) and play a role in responding to changes in the environment.

Of the non-polar lipids, TAGs are abundant storage products, which can be easily catabolized to provide metabolic energy [23]. In general, TAGs are mostly synthesized in the light, stored in cytosolic lipid bodies, and then reutilized for polar lipid synthesis in the dark [24]. Microalgal TAGs are generally characterized by both, saturated and monounsaturated FAs. However, some oil-rich species have demonstrated a capacity to accumulate high levels of long-chain polyunsaturated fatty acids (PUFA) as TAG [25,26]. A detailed study on both accumulation of TAG in the green microalga Parietochloris incisa and storage into chloroplastic lipids (following recovery from nitrogen starvation) led to the conclusion that TAGs may play an additional role beyond being an energy storage product in this alga [25,27]. Hence, PUFA-rich TAGs are metabolically active and are suggested to act as a reservoir for specific fatty acids. In response to a sudden change in the environmental condition, when the de novo synthesis of PUFA may be slower, PUFA-rich TAG may donate specific acyl groups to monogalactosyldiacylglycerol (MGDG) and other polar lipids to enable rapid adaptive membrane reorganization [27,28].

- *Author name is currently pursuing masters degree program in electric power engineering in University, Country, PH-01123456789. E-mail: author_name@mail.com*
- *Co-Author name is currently pursuing masters degree program in electric power engineering in University, Country, PH-01123456789. E-mail: author_name@mail.com*
(*This information is optional; change it according to your need.*)

4 TAG

A **triglyceride** (**TG**, **triacylglycerol**, **TAG**, or **triacylglyceride**) is an ester derived from glycerol and three fatty acids. [31] Alcohols have a hydroxyl (HO-) group. Organic acids have a carboxyl (-COOH) group. Alcohols and organic acids join to form esters. The glycerol molecule has three hydroxyl (HO-) groups. Each fatty acid has a carboxyl group (-COOH). In triglycerides, the hydroxyl groups of the glycerol join the carboxyl groups of the fatty acid to form ester bonds:
$HOCH_2CH(OH)CH_2OH + RCO_2H + R'CO_2H + R''CO_2H \rightarrow RCO_2CH_2CH(O_2CR')CH_2CO_2R'' + 3H_2O$

As a blood lipid, they help enable the bidirectional transference of adipose fat and blood glucose from the liver. There are many triglycerides: depending on the oil source, some are highly unsaturated, some less so.
Saturated compounds are "saturated" with hydrogen — all available places where hydrogen atoms could be bonded to carbon atoms are occupied. Unsaturated compounds have double bonds (C=C) between carbon atoms, reducing the number of places where hydrogen atoms can bond to carbon atoms. Saturated compounds have single bonds (C-C) between the carbon atoms, and the other bond is bound to hydrogen atoms (for example =CH-CH=, $-CH_2-CH_2-$, etc.).
Unsaturated fats have a lower melting point and are more likely to be liquid. Saturated fats have a higher melting point and are more likely to be solid at room temperature.
Triglycerides are the main constituents of vegetable oil (typically more unsaturated) and animal fats (typically more saturated). [33]Triglycerides are a major component of human skin oils.[34]

5 FACTORS AFFECTING TRIACYLGLYCEROL ACCUMULATION AND FATTY ACID COMPOSITION

Although the occurrence and the extent to which TAG is produced appear to be species/strain-specific, and are ultimately controlled by the genetic make-up of individual organisms, oleaginous algae produce only small quantities of TAG under

optimal growth or favorable environmental conditions (Hu, 2004).[53] Synthesis and accumulation of large amounts of TAG accompanied by considerable alterations in lipid and fatty acid composition occur in the cell when oleaginous algae are placed under stress conditions imposed by chemical or physical environmental stimuli, either acting individually or in combination. The major chemical stimuli are nutrient starvation, salinity and growth-medium pH. The major physical stimuli are temperature and light intensity. In addition to chemical and physical factors, growth phase or aging of the culture also affects TAG content and fatty acid composition.

- Nutrients -Of all the nutrients evaluated, nitrogen limitation is the single most critical nutrient affecting lipid metabolism in algae. A general trend towards accumulation of lipids, particularly TAG, in response to nitrogen deficiency has been observed in numerous species or strains of various algal taxa. In diatoms, silicon is an equally important nutrient that affects cellular lipid metabolism. For example, silicon-deficient Cyclotella cryptica cells had higher levels of neutral lipids (primarily TAG) and higher proportions of saturated and mono-unsaturated fatty acids than silicon-replete cells(Roessler, 1988).[29] Other types of nutrient deficiency that promote lipid accumulation include phosphate limitation and sulfate limitation. Phosphorus limitation resulted in increased lipid content, mainly TAG, in Monodus subterraneu, P. tricornutum and Chaetoceros sp. (Bacillariophyceae), and I. galbana and Pavlova lutheri (Prymnesiophyceae), but decreased lipid content in Nannochloris atomus (Chlorophyceae) and Tetraselmis sp. (Prasinophyceae) (Reitan et al., 1994). Of marine species examined (Reitan et al., 1994), increasing phosphorus deprivation was found to result in a higher relative content of 16:0 and 18:1 and a lower relative content of 18:4x3, 20:5x3 and 22:6x3. Studies have also shown that sulfur deprivation enhanced the total lipid content in the green algae Chlorella sp. (Otsuka, 1961) and C. reinhardtii (Sato et al., 2000). Cyanobacteria appear to react to nutrient deficiency differently to eukaryotic algae. Piorreck and Pohl (1984) investigated the effects of nitrogen deprivation on the lipid metabolism of the cyanobacteria Anacystis nidulans, Microcystis aeruginosa, Oscillatoria rubescens and Spirulina platensis, and reported that either lipid content or fatty acid composition of these organisms was changed significantly under nitrogen-deprivation conditions.[30] When changes in fatty acid composition occur in an individual species or strain in response to nutrient deficiency, the C18:2 fatty acid levels decreased, whereas those of both C16:0 and C18:1 fatty acids increased, similar to what occurs in eukaryotic algae (Olson and Ingram, 1975). In some cases, nitrogen starvation resulted in reduced synthesis of lipids and fatty acids (Saha et al., 2003).

- Temperature -Temperature has been found to have a major effect on the fatty acid composition of algae. A general trend towards increasing fatty acid unsaturation with decreasing temperature and increasing saturated fatty acids with increasing temperature has been observed in many algae and cyanobacteria (Lynch and Thompson, 1982; Murata et al., 1975;Raison, 1986; Renaud et al., 2002; Sato and Murata, 1980).[54,55,56,57] It has been generally speculated that the ability of algae to alter the physical properties and thermal responses of membrane lipids represents a strategy for enhancing physiological acclimatization over a range of temperatures, although the underlying regulatory mechanism is unknown(Somerville, 1995). Temperature also affects the total lipid content in algae. For example, the lipid content in the chrysophytan Ochromonas danica (Aaronson, 1973) and the eustigmatophyte Nannochloropsis salina (Boussiba et al., 1987) increases with increasing temperature. In contrast, no significant change in the lipid content was observed in Chlorella sorokiniana grown at various temperatures (Patterson, 1970). As only a limited amount of information is available on this subject, a general trend cannot be established.
- Light intensity- Algae grown at various light intensities exhibit remarkable changes in their gross chemical composition, pigment content and photosynthetic activity (Falkowski and Owens,1980; Post et al., 1985; Richardson et al., 1983; Sukenik et al.,1987). Typically, low light intensity induces the formation of polar lipids, particularly the membrane polar lipids associated with the chloroplast, whereas high light intensity decreases total polar lipid content with a concomitant increase in the amount of neutral storage lipids, mainly TAGs (Brown et al.,1996; Khotimchenko and Yakovleva, 2005;Napolitano, 1994; Orcutt and Patterson, 1974; Spoehr and Milner, 1949; Sukenik et al., 1989).[58,59,60]The degree of fatty acid saturation can also be altered by light intensity. In Nannochloropsis sp., for example, the percentage of the major PUFA C20:5x3 remained fairly stable (approximately 35% of the total fatty acids) under light-limited conditions. However, it decreased approximately threefold under light-saturated conditions, concomitant with an increase in the proportion of saturated and mono-unsaturated fatty acids (i.e. C14, C16:0 and C16:1x7) (Fabregas et al., 2004). Based upon the algal species/strains examined (Orcutt and Patterson, 1974; Sukenik et al., 1993), it appears, with a few

exceptions, that low light favors the formation of PUFAs, which in turn are incorporated into membrane structures. On the other hand, high light alters fatty acid synthesis to produce more of the saturated and mono-unsaturated fatty acids that mainly make up neutral lipids.

- Growth phase and physiological status-Lipid content and fatty acid composition are also subject to variability during the growth cycle. In many algal species examined, an increase in TAGs is often observed during stationary phase. For example, in the chlorophyte Parietochloris incise, TAGs increased from 43% (total fatty acids) in the logarithmic phase to 77% in the stationary phase (Bigognoet al., 2002), and in the marine dinoflagellate Gymnodinium sp., the proportion of TAGs increased from 8% during the logarithmic growth phase to 30% during the stationary phase(Mansour et al., 2003).[31] Coincident increases in the relative proportions of both saturated and mono-unsaturated 16:0 and 18:1 fatty acids and decreases in the proportion of PUFAs in total lipid were also associated with growth-phase transition from the logarithmic to the stationary phase. In contrast to these decreases in PUFAs, however, the PUFA arachidonic acid (C20:4x6) is the major constituent of TAG produced in Parietochloris incise cells (Bigogno et al., 2002),[62] while docosahexaenoic acid (22:6x3) and eicosapentaenoic acid (20:5x3) are partitioned to TAG in the Eustigmatophyceae N. oculata, the diatoms P. tricornutum and T. pseudonana, and the haptophyte Pavlova lutheri (Tonon et al., 2002). Culture aging or senescence also affects lipid and fatty acid content and composition. The total lipid content of cells increased with age in the green alga Chlorococcum macrostigma (Collins and Kalnins, 1969), and the diatoms Nitzschia palea (von Denffer, 1949), Thalassiosira fluviatillis (Conover, 1975) and Coscinodiscus eccentricus (Pugh,1971). An exception to this was reported in the diatom P. tricornutum, where culture age had almost no influence on the total fatty acid content, although TAGs were accumulated and the polar lipid content was reduced (Alonsoet al., 2000). Analysis of fatty acid composition in the diatoms P. tricornutum and Chaetoceros muelleri revealed a marked increase in the levels of saturated and monounsaturated fatty acids (e.g. 16: 0, 16:1x7 and 18:1x9), with a concomitant decrease in the levels of PUFAs (e.g. 16:3x4 and 20:5x3) with increasing culture age (Liang et al., 2006).[63]Most studies on algal lipid metabolism have been carried out in a batch culture mode. Therefore, the age of a given culture may or may not be associated with nutrient depletion, making it difficult to separate true aging effects from nutrient deficiency-induced effects on lipid metabolism.

- Physiological roles of triacylglycerol accumulation-Synthesis of TAG and deposition of TAG into cytosolic lipid bodies may be, with few exceptions, the default pathway in algae under environmental stress conditions. In addition to the obvious physiological role of TAG serving as carbon and energy storage, particularly in aged algal cells or under stress, the TAG synthesis pathway may play more active and diverse roles in the stress response. The de novo TAG synthesis pathway serves as an electron sink under photo-oxidative stress. Under stress, excess electrons that accumulate in the photosynthetic electron transport chain may induce overproduction of reactive oxygen species, which may in turn cause inhibition of photosynthesis and damage to membrane lipids, proteins and other macromolecules. The formation of a C18 fatty acid consumes approximately 24 NADPH derived from the electron transport chain, which is twice that required for synthesis of a carbohydrate or protein molecule of the same mass, and thus relaxes the over-reduced electron transport chain under high light or other stress conditions. The TAG synthesis pathway is usually coordinated with secondary carotenoid synthesis in algae(Rabbani et al., 1998; Zhekisheva et al., 2002). The molecules (e.g. b-carotene, lutein or astaxanthin) produced in the carotenoid pathway are esterified with TAG and sequestered into cytosolic lipid bodies. The peripheral distribution of carotenoid-rich lipid bodies serve as a 'sunscreen' to prevent or reduce excess light striking the chloroplast under stress. TAG synthesis may also utilize PC, PE and galactolipids or toxic fatty acids excluded from the membrane system as acyl donors, thereby serving as a mechanism todetoxify membrane lipids and deposit them in the form of TAG.

6 CULTIVATION OF ALGAE

6.1 Like plants, algae use the sunlight for the process of photosynthesis. Photosynthesis is an important biochemical process in which plants, algae, and some bacteria convert the energy of sunlight to chemical energy. Algae capture light energy through photosynthesis and convert organic substances into simple sugars using the captured energy. There are two main methods of cultivation

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1. Open Pond
2. Photobioreactors (PBR)

- **Open Pond Cultivation System**

Open cultivation system uses ponds or lakes with added mechanical equipment to grow microalgae. Open ponds were the first cultivation technology for mass cultivation of microalgae. In this system water levels are kept no less than 15 cm, and algae are cultured under conditions identical to their natural environment. The pond is designed in a raceway structure, as shown in Fig 3.1, in which a paddlewheel circulates and mixes the algal cells and nutrients. The raceways are typically made from poured concrete or they are simply dug into the earth and lined with a plastic liner to prevent the ground from soaking up the liquid. Baffles in the channel guide the flow around the bends in order to minimize space. The system is often operated in a continuous mode, where the fresh feed (containing nutrients including nitrogen phosphorus and inorganic salts) is added in front of the paddlewheel, and algal broth is harvested behind the paddlewheel after it has circulated through the loop. Depending on the nutrients required by algal species, several sources of wastewater can be used for algal culture. For some marine-type microalgae, seawater or water with high salinity can be used. Although open ponds cost less to build and operate than closed systems using Photobioreactors, this culture system has its disadvantages. The ponds can be built on any type of land but need large land areas for considerable biomass yield. Because they are in the open air, the water levels are affected from evaporation and rainfall. Natural CO 2 levels in the atmosphere (0.03%-0.06%) are not enough for continuous mass growth of microalgae. Biomass productivity is also limited by contamination with unwanted algal species, 8-14 organisms that feed on algae or other poisonous particles. Only few species can be grown in normal conditions. Other types of construction use: 1) circular ponds where circulation is provided by rotating arms; 2) inclined systems where mixing is achieved through pumping and gravity flow.

- **Photobioreactor (PBR)**

Photobioreactor is a closed system which provides a controlled environment and enables high productivity of algae. All growth requirements of algae are introduced into the system and controlled according to the requirements. Fig 3.2 shows a PBR system that facilitates better control of culture environment such as carbon dioxide supply, makeup water supply, optimal temperature, efficient exposure to light, culture density, pH levels, gas supply rate, mixing regime, etc. From the feeding vessel, the flow progresses to the diaphragm pump which moderates the flow of the algae into the actual tube. PBR is used to promote biological growth by controlling environmental parameters including light. The tubes are made of acrylic/glass and are designed to have light and dark intervals to enhance the growth rate. PBR should have a cleaning system that cleans the inner sides of tubes without stopping the production. After the algae have completed the flow through PBR, it passes back to the feeding vessel. As it progresses through the hoses, the oxygen sensors determine how much oxygen has built up in the plant and this oxygen is released in the feeding vessel itself. It is also at this stage that the optical cell density sensor determines the harvesting rate. When the algae are ready for harvesting, they pass through the connected filtering system.

- 6.2 **Comparison between open pond and photobioreactors**

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Culture systems for microalgae	Closed system (PBRs)	Open system (Ponds)
Contamination control	Easy	Difficult
Contamination risk	Reduced	High
Sterility	Available	None
Process control	Easy	Difficult
Species control	Easy	Difficult
Mixing	Uniform	Very poor
Operation regime	Batch or semi continuous	Same
Space required	A matter of productivity	Same as PBRs
Area/Volume ratio	High (20-200 m-1)	Low (5-10 m-1)
Algal cell density	High	Low
Investment	High	Low
Operation cost	High	Low
Light utilization efficiency	High	Poor
Temperature control	More uniform temperature	Difficult
Productivity	3-5 more productive	Difficult
Water losses	Depend upon cooling design	Not specific
Hydrodynamic stress on algae	Low-High	Very low
Evaporation of growth medium	Low	High
Gas transfer control	High	Low
C02 losses	Depend on pH, alkalinity etc	Same as PBRs
02 inhibition	Greater problem in PBRs	PBRs>Ponds
Biomass concentration	3-5 times in PBRs	PBRs>Ponds
Scale up	Difficult	Difficult

7 OIL EXTRACTION

Algae lipid content is not homogenous. Algal lipids generally comprise mixtures of non-polar components such as mono- di- and tri- glycerides, carotenoids, waxes and sterols, as well as slightly polar free fatty acids and xanthophylls, and more polar phospholipids, sphingolipids, and glycolipids (W. E. Becker 1994).[52] For total lipid extraction from algae, ideally the solvent or solvent mixture used must be adequately polar to extract polar lipids and disrupt lipid associations with cell membranes and cell components but also not too polar so as to ensure that the solvent readily dissolves nonpolar lipids (Johnson 1983). In addition to solvent polarity the following considerations must be made when selecting a solvent: 1) separation of the solvent from the oil must be relatively easy, and solvent recoverability for recycle must be high, 2) the solvent should be characterized by low specific heat, heat of vaporization and density in order to reduce costs associated with the energy requirement for solvent recycle and transport through piping, 3) the solvent should be environmentally friendly and preferably renewable so as not to render the renewable fuel production process futile, and 4) the solvent must be safe for human handling (Johnson 1983).

Generally solvents used for lipid extraction can be classified as one of the following: chlorinated hydrocarbons, petroleum hydrocarbons, or alcohols. Chlorinated hydrocarbons such as dichloromethane and chloroform are effective lipid solvents; however they are expensive and highly toxic (Johnson 1983). Hexane, the most commonly used solvent for large scale lipid extraction, is a petroleum hydrocarbon. The use of a solvent that is a petroleum distillate for the production of renewable diesel may seem unreasonable, but renewable hexane can be produced as a light distillate byproduct of the algal oil hydrotreatment and cracking process (Singh 2010). The advantages of using hexane include its low latent heat of vaporization and hence recoverability, its high stability, and its
non-corrosive nature (Johnson 1983). Hexane has been found to be very effective at extracting non-polar lipids, but it only partially extracts polar lipids (Johnson 1983).[51]

Depending on the conditions under which the Chlorella vulgaris was cultivated and the time of harvest, Chlorella vulgaris lipid content may be predominately polar, and hence depending on the composition of the Chlorella vulgaris used, hexane may or may not be a suitable solvent for total oil extraction from the algal biomass used in this study (W. E. Becker 1994). Alcohols such as ethanol, isopropanol, n-propanol, n-butanol, and isobutanol are also effective solvents for oil extraction. Compared to other alcohols, ethanol has a relatively low latent heat of vaporization and ethanol's polarity is most suitable for the uniform extraction of both polar and nonpolar lipids (Johnson 1983). Extraction with ethanol is an attractive option since ethanol is renewable; it is readily produced from agricultural residues. Another advantage of using ethanol over hexane is that biomass remaining post ethanol based lipid extraction, a protein rich cake known as the remnant, is higher quality than remnant remaining post hexane based lipid extraction. Remnant that has been extracted with ethanol is even suitable for use in the food industry (Johnson 1983). In a study by Ramirez Fajardo et al. a two-step ethanol based extraction process was developed to extract oil from the algal species Phaeodactylum tricornutum, and the process resulted in 96.1% lipid recovery (2007). However, the higher latent heat of vaporization of ethanol in comparison to hexane makes ethanol solvent recovery through distillation an expensive process. Non-distillation methods for solvent recovery such as chilling and
recovering the extracted miscella from the alcohol may be used to reduce costs and energy requirement for ethanol recycle; by chilling the solvent up to 30% less energy is required for ethanol recovery than would be for hexane recovery by distillation (Johnson 1983). Although common, lipid extraction using single component solvents at ambient pressures is not the only option; solvent mixtures and pressurized extractions are also options for lipid extraction.

Over the years researchers have experimented with using mixtures of solvents in various ratios to fine tune solvent polarity and enhance oil extraction properties. The current standard protocol for total oil extraction from biomass for quantitative purposes is known as the "Bligh and Dyer method" and involves utilizing a 1:2 v/v chloroform to methanol solution for oil extraction (Smedes and Askland 1999; Bligh and Dyer 1959). However a study done by Iverson et al. revealed that lipid content in samples containing more than 2% lipids were greatly underestimated using the Bligh and Dyer method (2001). Iverson et al. concluded that using the "Floch method" for oil extraction from samples containing relatively high lipid content provides a more representative quantification. The Floch
method involves using a 2:1 v/v chloroform to methanol solvent ratio for oil extraction (Folch, Lees and Sloane-Stanley 1957).[50] Multiple other modified analytical extraction procedures have been introduced over the years, and in preliminary work in the Sustainable Environmental Technologies Laboratories multiple analytical methods for oil extraction were compared. It was concluded that the Floch method is most suitable for the extraction of representative total lipids. For this study, the Floch method was assumed to extract total lipids. Multiple component solvents tend to be required for lipid extraction from wet samples.Lipid extraction from wet biomass is a challenge due to the high polarity of water. The immiscibility of strictly nonpolar solvents such as hexane with the aqueous suspension disrupts the extraction capabilities of nonpolar solvents. On the other hand, the interaction of more polar solvents, such as ethanol, with water molecules enhances the extraction of polar lipids but reduces the solvent's ability to extract nonpolar lipids. Multiple component solvents can be used to fine tune solvent polarity and enhance extraction capability in aqueous media, and multiple researchers have developed appropriate mixed solvents. Smedes and Askland introduced a procedure for oil extraction from wet aquatic biomass that utilizes a 11:8:10 v/v/v ratio of water to isopropanol to hexane, and Molina Grima et al. introduced a

procedure for lipid extraction from algae that utilizes a 6:77:17 w/w/w ratio of water to ethanol to hexane (Smedes and Askland 1999; Molina Grima, Acien Fernandez, et al. 2009). At the ratios specified by Smedes and Askland and Molina Grima et al., these alcohol hexane mixtures form a single phase with the aqueous media and exhibit the necessary degree of polarity to extract lipids from the suspended biomass.[48,49] In pressurized extractors, room temperature gases such as propane, butane, carbon dioxide and dimethyl ether have been successfully used as solvents for oil extraction purposes (Johnson 1983; Catchpole, Tallon, et al. 2007). The principle advantage of utilizing this class of solvents is that they are easily recoverable from the extracted oil by reducing pressure or applying slight heat. At high pressures these solvents are either in their liquid or supercritical fluid state. Supercritical fluids are fluids that are maintained at temperatures and pressures above their critical point, they have properties characteristic of both gases and liquids, and their density varies depending on the specific pressure and temperature conditions. Supercritical carbon dioxide is the most widely used pressurized solvent for lipid extraction.

Carbon dioxide is of interest because it has a relatively mild critical point (31°C and 7.4 MPa), and it is nontoxic, inexpensive, available in high purity, and nonflammable (Valderrama, Perrut and Majewski 2003).[47] Using carbon dioxide for lipid extraction also does not introduce residual organics in the remnant, which is an advantage if the remnant is to be further processed into animal feed or into synthetic fuels through thermo chemical means (Valderrama, Perrut and Majewski 2003). Supercritical carbon dioxide can be used to selectively extract particular compounds of interest. The solvation properties of carbon dioxide can be controlled by manipulating operating pressure and temperature during extraction; increased pressure and decreased temperature lead to decreased solvent diffusivity within the biomass matrix but increased solvent density or oil solvating power and vice versa (Marcias Sanchez, et al. 2010). Supercritical fluid extraction does not require prior cell wall disruption due to the high operating pressures, and energy costs associated with reaching the supercritical state for carbon dioxide have been shown to be less than the energy costs associated with solvent distillation (Kioschwitz and Howe-Grant 1991).[46] However carbon dioxide cannot extract complex lipids without the use of an organic co-solvent such as acetone, methanol or hexane, and carbon dioxide is immiscible in water making it a poor solvent for lipid extraction from aqueous media (Catchpole, Tallon, et al. 2007).[37]

Liquefied dimethyl ether has emerged as an alternative to supercritical carbon dioxide for pressurized oil extraction from aqueous media. Dimethyl ether is a non-toxic environmentally friendly solvent with a boiling point of -25°C. Dimethyl ether is partially miscible in water and has been previously used to dry coal, sediment and various porous media (Catchpole, J, et al. 2008; Oshitaa, et al. 2010; Kanda, Makino and Miyahara 2008).[38] In a study by the Central Research Institute of Electric Power Industry (CRIEPI) in Japan, liquefied dimethyl ether was successfully used to extract oil from cyanobacteria at moderate conditions, 20°C and 0.5MPa (2010).In this study, the efficiency of oil extraction from both wet and dry Chlorella vulgaris will be compared for different solvents to determine a feasible solvent for large-scale extraction. For freeze dried Chlorella vulgaris feedstock the extraction efficiencies of hexane, ethanol, liquid dimethyl ether, supercritical carbon dioxide and supercritical carbon dioxide with acetone, methanol and hexane co-solvents will be compared. For wet Chlorella vulgaris feedstock the extraction efficiencies of dimethyl ether, ethanol, a water to isopropanol to hexane mixture (11:8:10 v/v/v), and an ethanol to hexane to water mixture (77:17:6 w/w/w) will be compared.

8 OIL PURIFICATION

Post total lipid extraction, the extract needs to be processed to eliminate lipid components that cannot be upgraded to green diesel and that may disrupt the hydrotreating process. If total oil extraction is achieved, the crude algal oil extract contains mono, di and triglycerides, free fatty acids, waxes, sterols,tocopherols, pigments such as carotenoids and chlorophyll, phospholipids, glycolipids, and sphingolipids (Robles Medina, Molina Grima, et al. 1998).[43] Long chain hydrocarbons, alcohols, and fatty acids are ideal structures for hydrotreatment and upgrade to renewable diesel, and of these, chlorella vulgaris produces fatty acids in greatest quantities (W. E. Becker 1994). Phosphate functional groups present in phospholipids, amino groups and polar nitrogenous head groups in sphingo lipids, metal
ligand containing nitrogenous chlorophyll chlorin rings, carbohydrate groups in glycoproteins, and complex sterols are all oil components that are counterproductive to renewable diesel production. Most of these structures, however, are generally bonded to fatty acid groups that are valuable for renewable diesel .

A study by Ibnez Gonzalez et al. presents a three step method for the isolation of fatty acids from their linkages with these various groups. Ibnez Gonzalez et al. were interested in the isolation of eicosapentaenoic acid, a pharmaceutically valuable 20 carbon chain length fatty acid, from the algae Phaeodactylum tricornutum (1998). It is proposed that the process developed by Ibnez Gonzalez et al. can be adapted for the isolation of the fatty acids present in the Chlorella vulgaris oil extract; the purified fatty acids can then be processed to renewable diesel. The first step of the process proposed by Ibnez Gonzalez et al. is saponification.[45] Saponification hydrolyzes the fatty acids freeing them from their bonds with the various lipid structures and reducing them to water soluble fatty acid salts. Unsaponifiables, or lipids that are not hydrolyzed during saponification, remain nonpolar, insoluble in water, and soluble in hexane. Performing a hexane wash post-saponification
isolates the unsaponifiables. Unsaponifiables include carotenoids, sterols, and tocopherols which are all very valuable in the nutraceutical industry (Ceron, et al. 2008; Robles Medina, Molina Grima, et al. 1998).[43,44] These oil components can be isolated and processed separately as value added products to render the renewable diesel production process more economically viable. To purify the fatty acids from the aqueous phase, which also contains polar amino groups, phosphates, etc., the media is acidified to protonate the fatty acid salts. Post-protonation, fatty acids are insoluble in water and can be separated from the aqueous impurities by means of a hexane wash. Purified fatty acids are hence isolated through this
method. In this study, the oil fractionation process developed by Ibanez Gonzalez et al. is adopted to fractionate oil extracted using the different solvents described in the previous section to determine how effective these solvents are at extracting fatty acid rich lipid components.[45] Modifications of the process developed by Ibanez Gonzalez et al. are tested to render the process more feasible for use on a large-scale basis. It was also hypothesized that the aqueous waste stream produced through this process can be recycled as algal growth medium since it should contain nutrients isolated from algae, including nitrogenous groups, phosphates and metals. This hypothesis is also tested.

99 CONCLUSION

Algae are more promising feed stocks to their wide spread availability and higher oil yields. Depending on species, microalgae produce many different kinds of lipids, hydrocarbons and other complex oils.[36] It is clear that different microalgae species react to different stresses by producing different fatty acids or by altering their composition of fatty acids. Oil is made from the lipid which is produced from microalgae in photobioreator system, before photobioreactor system open system was used to produce oil. From the review we get to know that photobioreactor is much better system than open system.

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