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Dr. Kinel-Tahan has continued her research at the Algal Biotechnology Center at Bar Ilan University. Her main scientific interests include finding potential uses of algae as a source of valuable products such as biofuel, lipids, and natural antioxidants.

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Dr. David Iluz is a lecturer in Bar Ilan University, Ramat Gan, Israel. He obtained his Ph.D. from Bar Ilan University in 1998 *summa cum laude* and spent his post-doctoral fellowship at Hebrew University, Israel; Dr. Iluz worked on the photoacclimation of marine and freshwater phytoplankton (Lake Kinneret, Mediterranean, Red Sea). The focus of his work has been the study of bio-optical parameters in different biogeochemical provinces of Israel's water bodies and their relevance for estimating primary production. He developed algorithms to determine pigment concentration and composition from upwelling light, which can be estimated from underwater *in situ* light spectra or by satellite remote sensing.

Additional projects combining his interests in archeology and biology were the study of the chemistry and molecular biology of pigments obtained from scale aphids. He identified a species of Kermes oak coccid that grew in Israel, the aphid from which the precious Biblical scarlet dye [*tola'at shani*] was extracted, providing new insights on the ancient dye trade.

Dr. Iluz participated in several research expeditions to Red Sea coral reefs and collected and analyzed bio-optical data from the First Israel-Eritrea Joint Cruise, the GAP-IOLR cruise in the Eastern Mediterranean, and in the First Israel-Seychelles Joint Cruise to the Indian Ocean. He has published 45 scientific papers and is the recipient of several international and Israeli grants.

Dr. Iluz is a popular, enthusiastic teacher and field naturalist inspiring many students to follow in his footsteps and collaborate in his research.

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Dr. Zvy Dubinsky was born in Barcelona, 18/10/1934, emigrated to Israel in 1944 where he served in the army, was member of a Kibbutz, trained as a teacher, and worked as such for a few years. He studied at Bar Ilan University where he was offered a position at the Department of Life Sciences and progressed rapidly to full professor. His contributions revolutionized views in the related fields of the biophysics of photosynthesis, algal biotechnology, and coral reef conservation.

A characteristic of his scientific philosophy has been to develop interdisciplinary approaches and seek for synthesis and integration of results in different fields such as physics, physiology, and ecology. This has resulted in the publication of some 200 scientific papers (68 in the last 10 years) in top journals and the invitation to scores of International symposia, seminars, conferences, research cruises, workshops, all over the world, guest appointments, and sabbaticals in most prestigious institutions in the USA and Japan.

Recently, he was invited by Springer to edit a new volume covering the current views and recent developments in the field of coral and coral reef research: Coral Reefs an Ecosystem in transition (2011), with the leading authorities in the field contributing chapters. An additional volume coedited by him has likewise been published by Springer: "All Flesh Is Grass: Plant-Animal Interactions" (2011).

In 2010, the European Research Council awarded him the prestigious "Advanced Award" (€ 3,300.000, for 5 years) funding his CORALWARM project studying the effects of seawater warming and ocean acidification on corals.

His activity attracted over 60 graduate students; several of them became leaders in their fields as scientists.

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Dr. Yaron Yehoshua obtained his Ph.D. from Bar Ilan University; Ramat Gan, Israel, in 2003, and continued his studies, research, and lecturing there. Dr. Yehoshua spent his postdoctoral fellowship at University of Constance, Germany, and at University of Zurich, Switzerland, and worked on the ecophysiology role of phytoplankton and epilithic algae of lakes, changes in the algal communities, biomass, and primary production. Dr. Yehoshua's scientific interests include algal biotechnology for CO_2 sequestration, biofuel, and fine chemicals production such as polyunsaturated fatty acids and antioxidants.

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ALGAL OILS: BIOSYNTHESIS AND USES

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1. Introduction

The exponentially growing human population has led to increasing energy demands all over the world. The reported current rate of consumption of petroleum is 105 times faster than the rate that nature can create it (Netravali and Chabba, 2003), and it has more than doubled over a period of 20 years {(Diaz-Tovar et al., 2011; Oil World Annual, 2009) #3580}. The burning of any fossil fuel, gas, oil, or coal adds to atmospheric CO_2, resulting in accelerated global warming and oceanic acidification. Hence, there is growing interest in biofuel, since the plants grown to produce it absorb carbon dioxide during their growth, either from the atmosphere or in aquatic plants – from its dissolved forms in water. Biofuel is a fossil-fuel replacement that is produced from vegetable oils, recycled cooking fats, waste oils, animal fats, or microalgal lipids (Table 1).

Microalgae, like higher plants, besides the ubiquitous structural membrane lipids, produce storage lipid bodies in the form of triacylglycerols (TAGs/TGL), free fatty acids (FFA) (Wang et al., 2009), and various photosynthetic pigments, also classified as lipids.

Lipids are a loosely defined group of organic compounds. The following are the main lipid groups (Fig. 1), functions, and their main biosynthesis pathways (Fig. 2):

Triacylglycerols (TAGs) are neutral lipids that are the major component of many natural oils, such as olive oil (Khandelia et al., 2010). In mammals, TAGs are present mostly inside trafficking lipoprotein particles, which transport cholesterol and TAGs between tissues (Jackson et al., 1976), and in lipid droplets (LDs) (Fujimoto et al., 2008). LDs are also present in other eukaryotes and in some prokaryotic cells that synthesize TAGs for energy and carbon storage (Waltermann et al., 2005).

Fatty acids are the building blocks in various biosynthetic pathways leading to various lipid groups as well as products generated whenever fats are broken down. These acids are not highly soluble in water and can be used for energy by most types of cells. They may be monounsaturated, polyunsaturated, or saturated. Fatty acids are components of cell membranes, hence required for their development,

Table 1. Oil yield of sources of biodiesel (Satyanarayana et al., 2011).

Source	Yield of oil (L ha^{-1} year^{-1})	Required land area (Mha[a])
Corn	172	1,540
Soybean	446	594
Canola	1,190	223
Jatropha	1,892	140
Coconut	2,689	99
Oil palm	5,950	45
Microalgae[b]	70,405	7.6
Microalgae[c]	35,202	15.2

Based on Chisti (2007)
[a]To meet 50% of all transport fuel needs of USA
[b]40% oil (% dry wt) in biomass
[c]20% oil (% dry wt) in biomass

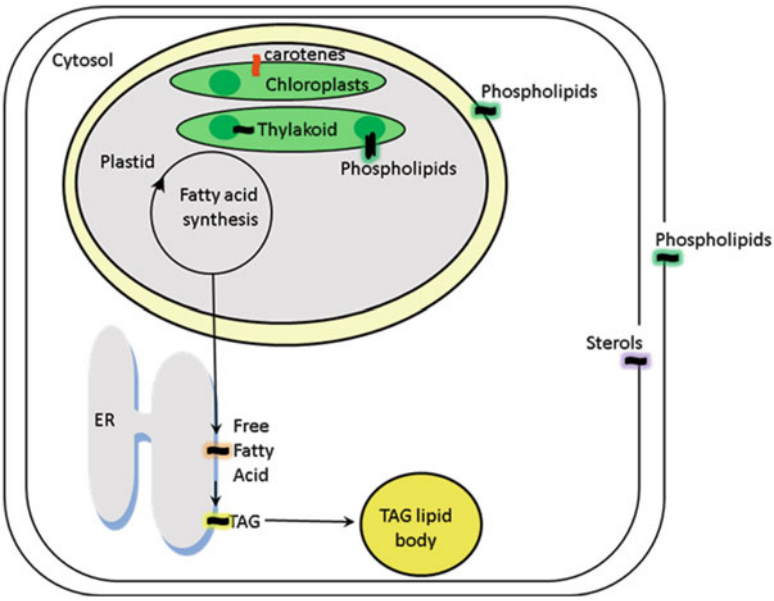

Figure 1. The location of major lipids in the algal cell (After Cowan, 2006; Nabil and Cosson, 1996; Radakovits et al., 2010).

integrity, and function. Fatty acids can be attached to other molecules, such as in triglycerides or phospholipids. When they are not attached to other molecules, they are known as "free" fatty acids (FFA) (Wang et al., 2009).

Phospholipids: This is a general term that includes all lipids containing phosphorus. However, it is a term often mistakenly equated with phosphoglycerides, the most

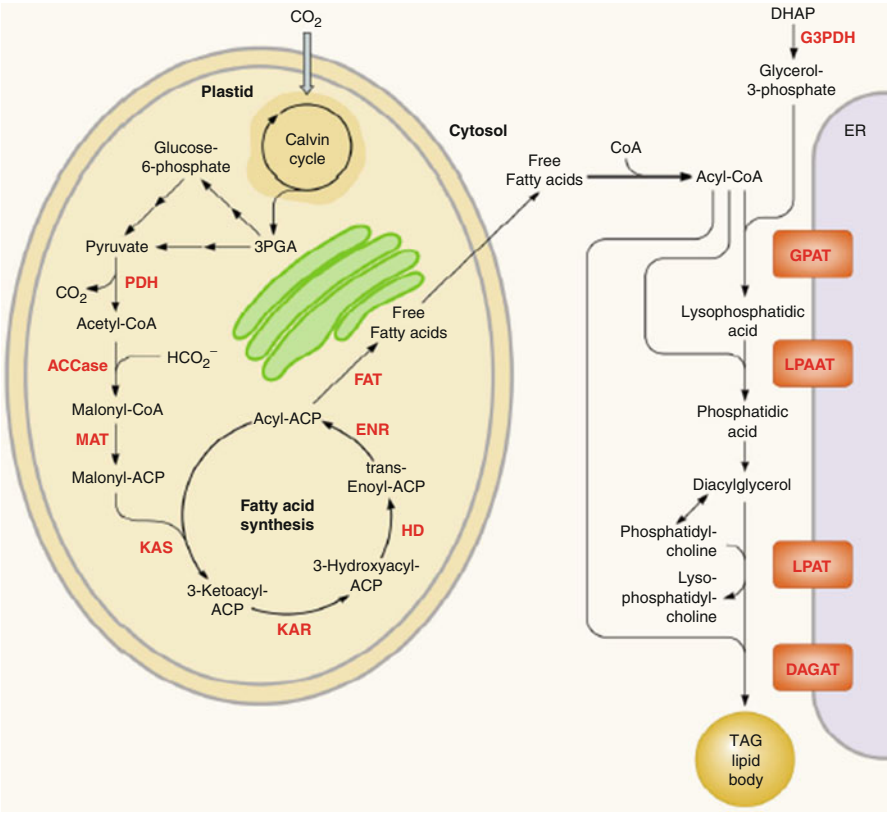

Figure 2. Simplified overview of the metabolites and main pathways in microalgal lipid biosynthesis shown in *black* and key enzymes in *red*. Free fatty acids are synthesized in the chloroplast, while TAGs may be assembled at the ER. *ACCase* acetyl-CoA carboxylase, *ACP* acyl carrier protein, *CoA* coenzyme A, *DAGAT* diacylglycerol acyltransferase, *DHAP* dihydroxyacetone phosphate, *ENR* enoyl-ACP reductase, *FAT* fatty acyl-ACP thioesterase, *G3PDH* gycerol-3-phosphate dehydrogenase, *GPAT* glycerol-3-phosphate acyltransferase, *HD* 3-hydroxyacyl-ACP dehydratase, *KAR* 3-ketoacyl-ACP reductase, *KAS* 3-ketoacyl-ACP synthase, *LPAAT* lyso-phosphatidic acid acyltransferase, *LPAT* lyso-phosphatidylcholine acyltransferase, *MAT* malonyl-CoA:ACP transacylase, *PDH* pyruvate dehydrogenase complex, *TAG* triacylglycerols (Radakovits et al., 2010).

common of the phospholipids. The major phosphoglycerides of animal tissues are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI) (Tocher et al., 2008). Phospholipids play a key role in processes such as signal transduction, cytoskeletal rearrangement, and membrane trafficking (Cowan, 2006).

Phosphoglycerolipids (e.g., phosphatidylcholine and phosphatidylethanolamine) are abundant constituents of the plasma membrane, the tonoplast, and the endoplasmic reticulum.

Sterols: glycerolipids and sphingolipids constitute the major lipid classes in plants. Sterol lipids are composed of free and conjugated sterols, i.e., sterol esters, sterol glycosides, and acylated sterol glycosides. Sterol lipids play a crucial role during adaptation to abiotic stresses and plant-pathogen interactions (Wewer et al., 2011).

Sphingolipids are amphipathic molecules with varying degrees of hydrophobic and hydrophilic properties. The sphingoid base is usually 18 carbons in length. Taking into account that there are at least five different sphingoid bases present in mammalian cells, with more than 20 arrangements of fatty acids that differ in alkyl-chain length and the level of both saturation and hydroxylation, and coupled with more than 500 carbohydrate structures reported in the glycosphingolipids, the number of possible structures is considerable (Fuller, 2010).

2. Lipid Functions

2.1. STORAGE (LIQUID/SOLID)

Plant lipids are stored in the crystalline/fluid phase and the solid/gel phase.

The liquid phase is typical for young, normal cells. In this state, the membrane is flexible due to free motility of the fatty acid "tail," resulting in optimal biological functionality. This phase is abundant in unsaturated fatty acids such as linoleic, linolenic, and arachidonic acids. Lipids are also stored during periods when cell doubling is limited by shortage of nutrients such as nitrogen and phosphorus, whereas conditions for photosynthesis remain favorable (Dubinsky and Berman-Frank, 2001). Noteworthy cases are these of hydrocarbon synthesis by *Botryococcus braunii* (Fig. 3) and that of the photoprotective carotenoids β-carotene by *Dunaliella salina var bardawil* and astaxanthin by *Haematococcus pluvialis* (Fig. 9).

The solid phase is typical for senescent and defective cells characterized by tail loss and complete loss of motility. As a result, the membrane becomes rigid (Leshem, 1989).

In most plants, the common storage lipids are in the form of triacylglycerols (Murphy, 1990). There are very few examples of alternative forms of storage lipids in higher plants. Perhaps the most notable of these is the North American desert shrub, Jojoba, which stores its seed lipid in the form of liquid wax consisting of a long-chain fatty alcohol that esterifies into a fatty acid (Weiss, 1983). Another noteworthy exception is that of the green microalgae, *Botryococcus braunii*, which can store up to 75% of its dry biomass as hydrocarbons (Kalacheva et al., 2002).

2.2. STRUCTURE (IN MEMBRANES)

Plant membrane lipids are primarily composed of 16 and 18 carbon fatty acids containing up to three double bonds. Some 300 naturally occurring fatty acids have been described as found in seed oils, and it has been estimated that thousands more

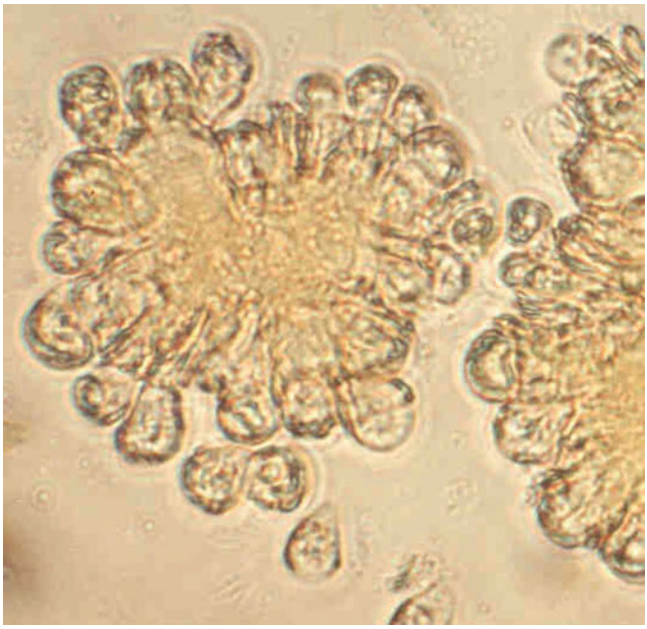

Figure 3. *Botryococcus braunii*. The live cells are seen embedded in a hydrocarbon jelly.

might be present throughout the plant kingdom. The structures of these fatty acids can vary in chain length from 8 to 24 carbons; they can have double bonds in unusual positions, or novel functional groups, such as hydroxy, epoxy, cyclic, halogen, or an acetylenic group on their acyl chain (Millar et al., 2000). Phosphoglycerides are characterized by a common backbone of phosphatidic acid (PA) (Tocher et al., 2008). The phospholipids contain a polar phosphorus head-group and a glycerol chain. In general, phospholipids fulfill structural and signaling functions in algae characterized by continued turnover of their pools (Cowan, 2006).

The thylakoid membrane of chloroplasts consists of the usual cell-membrane components and, as such, is rich in galactolipids (Yamaryo et al., 2003). The thylakoid membrane is the site of four main components of the photosynthetic apparatus, PS1, PS2, cytb6f, and ATPsynthase (Choquet and Vallon, 2000), which contain the various chlorophylls, mostly in their antennae.

2.3. PIGMENTS (SOME OF THESE ALSO BELONG TO THE LIPIDS)

Three major classes of photosynthetic pigments exist among algae and plants in general: chlorophylls, carotenoids (carotenes and xanthophylls), and phycobilins. Only the first two belong to the lipid class. Phycobilins are water-soluble protein-linked chromophores and, as such, do not belong to the lipids.

2.3.1. Chlorophylls

The basic structure of a chlorophyll molecule is a porphyrin ring, coordinated with a central magnesium atom. There are actually three main types of chlorophyll: chlorophyll a, chlorophyll b, and chlorophyll c. The first two are Mg-chlorins, which differ only slightly in the composition of a side chain ($-CH_3$ in chlorophyll a, CHO in chlorophyll b), and the last one is Mg-phytoporphyrins (Fieser and Fieser, 1956; Stryer, 1975; Zapata et al., 2006). The chlorophylls are intimately involved in all aspects of the primary events of photosynthesis: light harvesting, energy transfer, and light energy conversion. The great majority of chlorophyll molecules in the photosynthetic apparatus constitute a light-harvesting apparatus that acts as the initial photoreceptor. Electronic excitation energy that results from absorption of a photon is transferred by the light-harvesting or antenna chlorophyll to a small number of chlorophyll molecules in a photoreaction center, where the electronic excitation energy is trapped and converted to an electron (reducing capacity) and a positive hole (oxidizing capacity) (Katz et al., 1978).

2.3.2. Carotenoids

The photoacclimative plasticity of algal cell, especially that of planktonic species, which are routinely exposed to fast changes in the light intensity to which they are exposed in the course of the vertical mixing of natural water bodies, or the forced mixing in culture ponds or photobioreactors induces dramatic changes in the kind and cellular content of these pigments. In terms of their function in algal cells, they may be divided into two groups having opposite – but complementary – roles. Light-harvesting carotenoids such as peridinin and fucoxanthin expand the light harvesting of algal cells as they efficiently absorb the green wavelengths not absorbed by the chlorophylls. Photoprotective carotenoids include β-carotene and astaxanthin and protect the photosynthetic apparatus from harmful, excessively high light. Most carotenoids contain a linear C_{40} hydrocarbon backbone that includes between 3 and 15 conjugated double bonds (1, 5, 6). The number of double bonds largely determines the spectral properties of a given carotenoid (Armstrong and Hearst, 1996). Carotenoids are found in specific locations and orientations in subcellular structures, and their chemical and physical properties are strongly influenced by other molecules in their vicinity, especially proteins and membrane lipids. In turn, the carotenoids influence the properties of these subcellular structures. Structural features, such as size, shape, and polarity, are essential determinants of the ability of a cartenoid to fit correctly into its molecular environment, which is essential for it to function normally (Britton, 1995).

3. Uses of Lipids

At present, the use of microalgae in aquaculture is increasing, mostly as food for aquatic organisms, such as oysters, shrimp, and fish in artificial food chains, and for direct human consumption as “health food” additives.

Table 2. Lipid compounds and their industrial applications.

Lipid compound	Use/function
Triacylglycerides	Main constituents of edible oil[a]
Diacylglycerides	Food additive: Beneficial effects on obesity and weight-related disorders[a]
Monoacylglycerides	Emulsifiers in food industry[a]
Fatty acids	Pharmaceutical and food industries[a]
Fatty esters	Main constituents of biodiesel[a]
Tocopherols	Main constituents of vitamin E[a]
Phospholipids	Emulsifiers, lubricants, and surfactant[a]
Sterols	Starting materials for synthesis of steroids[a]
Hydrocarbons	Gas and oil[b]
Waxes	Cosmetics, pharmaceuticals, packaging, and plastics[c]
Carotenes	Antioxidants and natural coloring materials[a]

[a]D'iaz-Tovar et al. (2011)
[b]Valero (2010)
[c]Tennant (2004)

Table 3. Oil content in some microalgae (Satyanarayana et al., 2011).

Species	Oil content (% dry wt)
Botryococcus braunii	25–75
Chlorella sp.	28–32
Chlorella emersonii	63
Chlorella minutissima	57
Chlorella protothecoides	23
Chlorella sorokiniana	22
Chlorella vulgaris	40,56.6
Cylindrotheca	16–37
Crypthecodinium	20
Dunalielia primolecta	23
Isochrysis sp.	25–33
Monodus subterraneus	39.3
Monallanthus salina	>20
Nitzschia laevis	69.1
Nannochloris sp.	20–35
Nitzchia sp.	45–47
Parietochloris incisa	62
Phaeodactylum tricornutum	20–30
Schizochytrium sp.	50–77
Tetraselmis suecica	15–23

The algal biomass can be extracted as a source of chemicals for industry, toxins, glycerol, carotene, vitamins, lipids, amino acids, carbohydrates, volatile substances, and the high protein residue fed to poultry or consumed as dried whole cells (Dubinsky and Aaronson, 1982; Pulz and Gross, 2004).

In this review, the emphasis is on lipids, showing the different lipid compounds and their industrial applications (Table 2), and oil content in some major microalgae (Table 3).

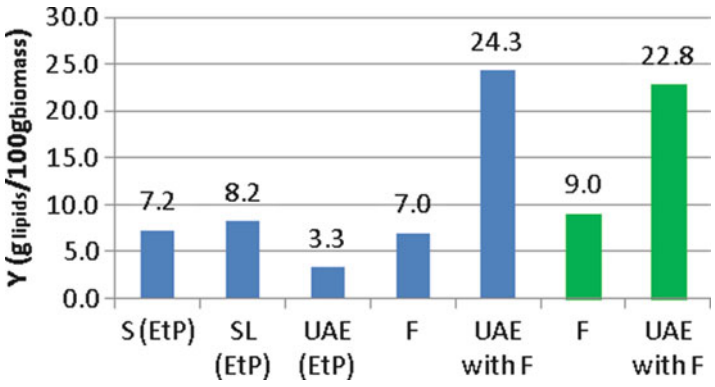

Figure 4. Lipid yield (g_{lipids}/100 g_{mass}) obtained by different techniques of extraction from *Nannochloropsis oculata* grown at 20 °C, 70 µE m^{-2} s^{-1}, and 0.3 g L-1 $NaNO_3$. (■) Wet biomass; (■) dry biomass; S (classic extraction); SL (Soxhlet); UAE (ultrasound-assisted extraction); and F (Folch method).

Vegetable oils and fats play an important role in human nutrition as a source of energy, polyunsaturated fatty acids (PUFA), and fat-soluble vitamins. Chemical industries have focused on the production of renewable sources of energy, notably biodiesel. World production of fats and oils has been growing rapidly over the past few decades and has more than doubled itself from 79.2 million tons in 1990 (Diaz-Tovar et al., 2011) to nearly 165 million tons in the year 2009 {Annual, 2009 #3580}.

3.1. EXTRACTION METHODS

There are a few lipid extraction methods: (a) lipid extraction in solution (Bligh and Dyer, 1959); (b) with ultrasonic bath (Widjaja et al., 2009); (c) Soxhlet (Virot et al., 2007); (d) with petroleum ether (EtP), using ultrasonic bath; and (e) Folch method with ultrasonic bath (Fig. 4) (Converti et al., 2009).

The use of a lipid such as diesel fuel requires a transesterification process. This is costly, and various methods are being explored in order to improve the process's economics. One such method is the production of biodiesel directly from crude dried solid microalgae mixed with methanol-chloroform and a strontium oxide (SrO) catalyst using microwave irradiation (Koberg et al., 2011).

One of the few methods available to analyze lipids according to their different constituent types is by using the Iatroscan TH-10 TLC-FID analyzer. This method – not used routinely today, involves separate analyses of two samples of total lipids in solvents designed to separate neutral and polar lipid classes, together with calibration by a composite standard similar in composition to the sample under analysis. This method does not depend on the degree of unsaturation of the fatty acids present, is rapid, and compares well in accuracy with conventional

combined gravimetric, colorimetric, and densitometric procedures (Fraser et al., 1985). The most widely currently used method is gas chromatography (GC) analysis, in which the sample is dissolved in ethyl acetate and then injected into GC-17A device equipped with a nonpolar column and a flame ionization detector (Widjaja et al., 2009).

4. Factors That Affect Algal Lipid Production

4.1. TEMPERATURE

The effect of temperature on lipid composition in microalgae as well as in bacteria is related to their melting points. The more a lipid is saturated, the higher its melting point, hence at low temperatures, unsaturated lipids are advantageous, and specialized desaturases are activated in several organisms when they encounter low ambient temperatures (Harwood and Guschina, 2009). The effect of growth temperature on lipid content was investigated in the microalgae *Nannochloropsis oculata* and *Chlorella vulgaris*. Both species showed a change in lipid content when temperature was altered. *C. vulgaris* had the highest lipid productivity when the temperature was 25°C (20.22 mg_{lipid}/L^{-1} day^{-1}). When temperature increased, productivity decreased to 8.21 mg_{lipid}/L^{-1} day^{-1} (at 35°C) (Converti et al., 2009). For *N. oculata*, there was a small change in lipid content when the temperature was altered beyond the optimal growth temperature of 20°C (10.01 mg_{lipid}/L^{-1} day^{-1}), and at 15 and 25°C, it was 9.11 and 10.1 mg_{lipid}/L^{-1} day^{-1}, respectively (Converti et al., 2009).

Growth and total lipid content of *Spirulina platensis* (UTEX 1928) were affected by changes in growth temperature from 25 to 38°C. With increased growth rate, total lipid content increased (Fig. 5) (Tedesco and Duerr, 1989).

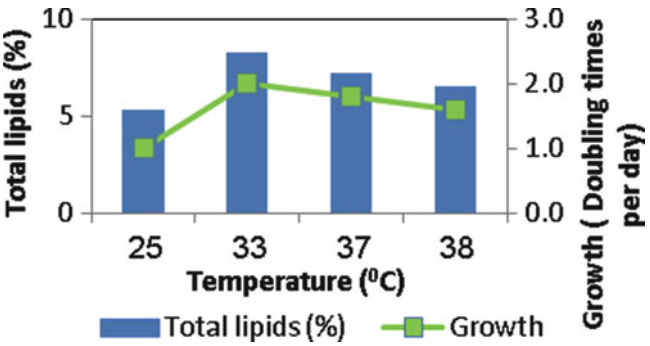

Figure 5. Comparison of the total lipid content and doubling time of *S. platensis* (UTEX 1928) at different temperatures.

Table 4. Comparison of lipids and fatty acids of *S. platensis* (UTEX 1928) at three culture temperatures.

Temp (°C)	Growth	Total lipid	Total fatty acids	Unsaturated/ saturated fatty acids	Major fatty acids (% of total fatty acids)					
					16:0	17:0	18:0	18:1	18:2	18:3
25	1.0	5.3(1.8)	1.8(0.2)	0.81	52.7(0.6)	1.9(0.1)	1.3(0.1)	1.6(0.2)	7.7(0.3)	35.0(0.9)
33	2.0	8.3(1.4)	2.0(0.1)	0.79	54.5(0.5)	*	1.2(0.2)	2.3(0.2)	7.0(0.5)	34.7(0.6)
37	1.8	7.2(0.2)	2.2(0.2)	0.78	54.4(1.0)	*	1.8(0.2)	4.4(0.7)	7.7(1.4)	31.7(1.7)
38	1.6	6.5(5.1)	1.4(0.1)	0.68	56.6(1.1)	*	2.9(0.6)	5.5(0.2)	6.9(0.3)	28.3(1.1)

Growth was measured as number of doublings per day. Total lipid and total fatty-acid levels are listed as % of dry weight. All numbers are the mean of four replicate cultures (except at 33 °C, where $n = 3$). The values in parentheses denote the standard deviation. Trace amounts of fatty acids are indicated with an asterisk *

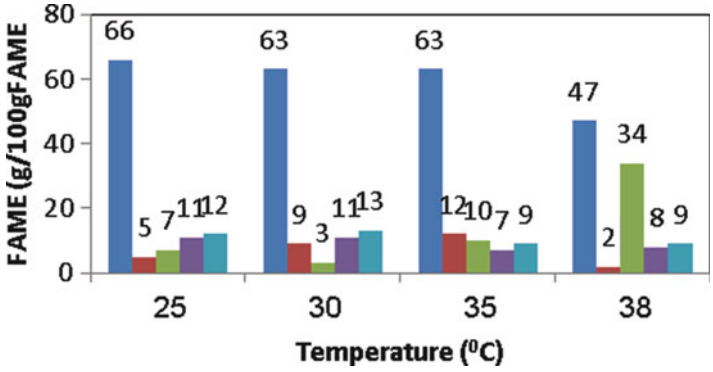

Figure 6. Percentages of individual fatty-acid methyl esters (FAMEs) on the total FAMEs (g/100 gFAME) in *C. vulgaris* at different temperatures.

The lipid composition of *Spirulina platensis* (UTEX 1928) and *C. vulgaris* was investigated and the results given in Table 4 (Tedesco and Duerr, 1989) and Fig. 6 (Converti et al., 2009), respectively.

Lipid composition was investigated at various growth temperatures in the cyanobacterium *Anacystis nidulans*. When growth temperature was changed from 38 to 22 °C, the content of digalactosyldiglyceride decreased, and the contents of monogalactosyl- and sulfoquinovosyldiglycerides increased, while the content of phosphatidylglycerol remained constant (Sato et al., 1979). A similar change in lipid composition with growth temperature was reported in a unicellular alga, *Cyanidium caldarium* (Kleinschmidt and McMahon, 1970).

4.1.1. Light Intensity

The effect of light on total lipid content was investigated in *Spirulina platensis* (UTEX 1928) at several intensities, ranging from 170 to 1,400 µmol photons m^{-2} s^{-1}. It was shown that light intensity affected growth rate and total lipid content, while

Table 5. Total lipid and fatty-acid content (% dry weight) and composition of *S. platensis* (UTEX 1928) under various light intensities.

Light	Growth	Total lipid	Total fatty acids	Unsaturated/ saturated fatty acids	Major fatty acids (% of total fatty acids)				
					16:0	18:0	18:1	18:2	18:3
170	0.9	11.8(2.7)	2.3(2.7)	0.76	55.5(1.1)	1.4(0.7)	3.8(0.9)	6.5(0.5)	33.2(1.7)
330	1.3	8.0(1.0)	1.7(0.1)	0.83	52.5(2.9)	2.2(0.8)	6.3(1.0)	7.8(1.1)	31.0(1.7)
600	1.8	7.2(0.2)	2.2(0.2)	0.78	54.4(1.0)	1.8(0.2)	4.4(0.7)	7.7(1.4)	31.7(1.7)
860	2.1	3.2(0.8)	1.5(0.3)	0.73	54.7(1.6)	3.0(0.5)	3.5(1.0)	7.8(1.0)	31.1(1.1)
1,400	2.2	5.8(1.7)	2.3(0.1)	0.86	52.2(0.8)	1.7(0.2)	2.1(0.1)	8.0(0.3)	36.0(0.8)

The standard light condition was 600 µmole photons m^{-2} s^{-1}. Growth was measured as the number of doublings per day. All numbers are the means of four replicate cultures. The values in parentheses give the standard deviation (Tedesco and Duerr, 1989)

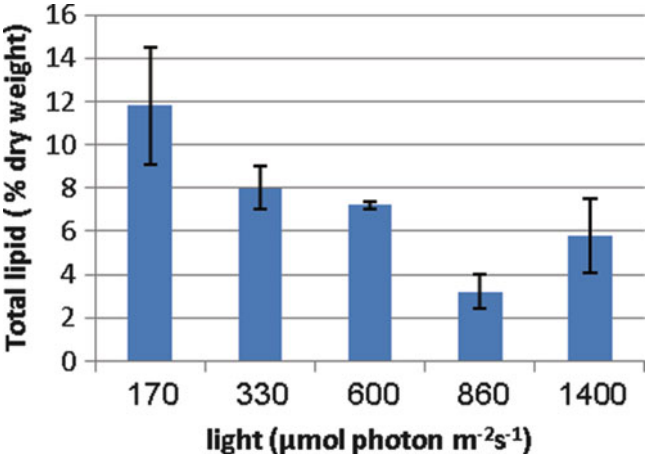

Figure 7 Total lipid (% dry weight) content of *S. platensis* (UTEX 1928) under various light intensities (Tedesco and Duerr, 1989).

growth rates declined with the increasing culture density. Total lipid as a percentage of dry weight decreased as light intensity increased, except at the highest irradiance (Table 5; Fig. 7) (Tedesco and Duerr, 1989).

Another experiment on light intensity was conducted with six strains of marine diatoms: *Cylindrotheca fusiformis* (B211), *Phaeodactylum tricornutum* (B114, B118, and B221), *Nitzschia closterium* (B222), and *Chaetoceros gracilis* (B13). The number of total lipids of B13, B114, and B211 grown at 5,000 lx was lower than those grown at 1,500 Ix. No evident changes were observed in Bl18, B221, and B222 (Table 6; Fig. 8) (Liang et al., 2001).

In Fig. 9, it is shown that different irradiance levels can affect the pigment expression in the green algae *Haematococcus pluvialis.* Above a minimal irradiance

Table 6. The final biomass (dry weight) and total lipids of 6 diatoms strains (Liang et al., 2001).

	DW (g/L)		Total lipids (% DW)	
Microalgal strains	**1,500 lx**	**5,000 lx**	**1,500 lx**	**5,000 lx**
B13	0.35 ± 0.03	0.31 ± 0.22	10.78 ± 2.69	6.97 ± 2.93
B118	0.25 ± 0.00	0.54 ± 0.18	3.64 ± 0.01	4.32 ± 2.33
B221	0.30 ± 0.01	0.46 ± 0.02	5.93 ± 1.03	5.90 ± 4.04
B114	0.40 ± 0.06	0.65 ± 0.13	13.38 ± 1.80	5.18 ± 1.83
B211	0.45 ± 0.39	0.68 ± 0.09	15.93 ± 0.91	13.00 ± 0.28
B222	0.49 ± 0.05	0.72 ± 0.02	4.14 ± 4.00	5.38 ± 1.79

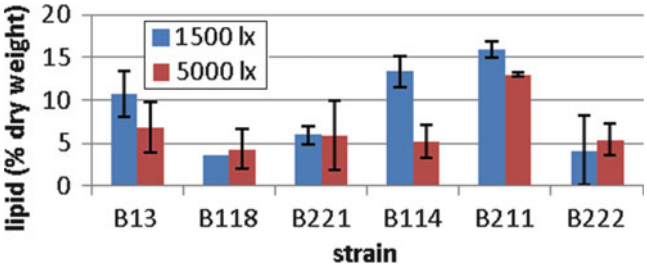

Figure 8. Total lipids (% dry weight) of six diatom strains under various light intensities (Liang et al., 2001).

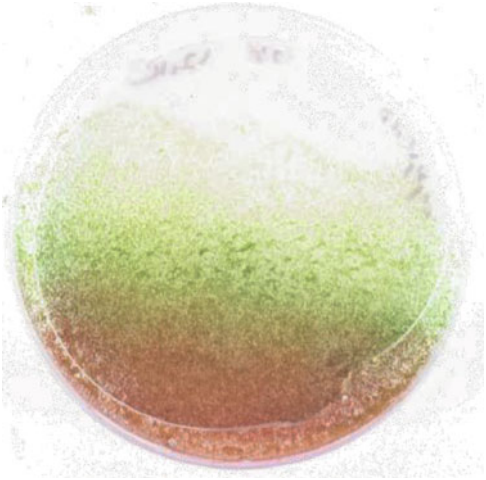

Figure 9. The effect of irradiance level on pigmentation in *Haematococcus pluvialis*. The light gradient went from darkness at the top towards ~1,000 μmol photons m^{-2} s^{-1}. The *red* pigment is astaxanthin.

level, the light-harvesting green chlorophylls (a and b) predominate, whereas under increasing light intensity, their cellular content decreases and the photoprotective carotenoid astaxanthin becomes visible, masking the green color. High light stress is used in the biotechnological production of astaxanthin, mostly as a component in fish feed pellets required in the culture of salmon.

4.1.2. Salinity

The effect of salinity on total lipid content and triacylglycerides (TGs) was investigated in *Dunaliella* cells. An increase of the initial NaCl concentration from 0.5 (equal to seawater) to 1.0 M resulted in higher intracellular lipid content (67.8% are lipids while – of them – 57% are TGs in comparison with 60% being lipids and 41% of the lipids are TG, respectively) for a salt concentration of 0.5 M. The addition of 0.5 or 1.0 M NaCl at the mid-log phase or at the end of the log phase during cultivation with the initial NaCl concentration of 1.0 M further increased the lipid content (71% of the dry weight were lipids, 34% of which are TGs, in comparison with 70% lipids and 32% of them TGs, respectively) (Takagi et al., 2006).

The effect of salinity on *Botryococcus braunii (LB 572)* was investigated. Two-week-old culture of *B. braunii LB 572* grown in modified Chu 13 medium was used as an inoculum at 20% (V/V); sodium chloride was added to the flasks in the range of 17–85 mM and inoculated. The total fat content of the alga grown at different salinities varied in the range of 24–28% (w/w), whereas in the control, it was 20% (Rao et al., 2007).

4.1.3. Nitrogen (N) Starvation

Under nutrient-sufficient conditions, cells synthesize mainly proteins to support growth and division (Myers, 1980). However, when a culture is deprived of an essential nutrient, cell division is stopped, and the fraction of carbon allocated to lipids and carbohydrates can be greatly increased at the expense of protein synthesis (Sukenik and Wahnon, 1991).

The effect of nitrogen starvation on lipid content was investigated in cyanobacterium *Spirulina platensis*. It was shown that the lipids decreased slightly over the first 40 h of N-starvation then increased for the next 40 h. The fatty acids decreased rapidly from approximately 2–1.2% of dry weight with N-starvation (Tedesco and Duerr, 1989).

A green microalga, *Chlorella vulgaris*, was tested for N-starvation effect. It was found that the lipid content was higher with longer incubation time, which led to less nitrogen concentration in the medium. It was also shown that longer time of nitrogen starvation resulted in higher accumulation of lipids inside the cells (Fig. 10) (Widjaja et al., 2009) and different composition (Fig. 11) (Converti et al., 2009).

The effect of N-starvation on lipid content was investigated in *Chlorella* sp. and *Phaeodactylum tricornutum*, which were grown for 7 days in rich media (control) and then harvested and transferred to media without nitrogen.

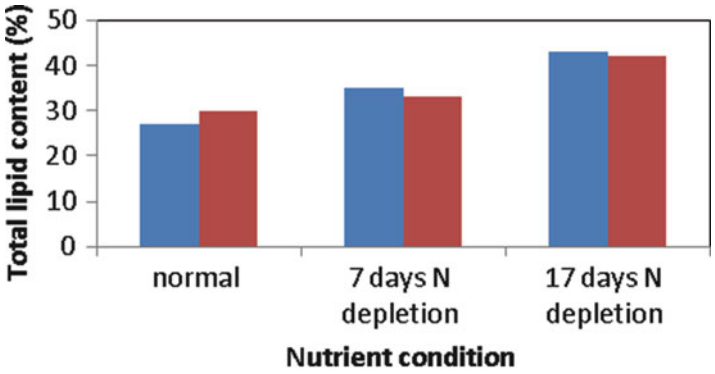

Figure 10. Comparison of total lipid content during normal nutrition and nitrogen starvation at CO_2 flow rate of 20 mL/min. Incubation time under normal nutrition was conducted for 15 days ( ) and 20 days ( ). After normal nutrition, the medium was changed to a nitrogen-depleted one and growth continued for 7 and 17 days. Total lipid content was calculated as the w/w ratio of the chloroform/methanol soluble fraction to dried algal sample. Data were expressed as mean values ($n = 3$) (Widjaja et al., 2009).

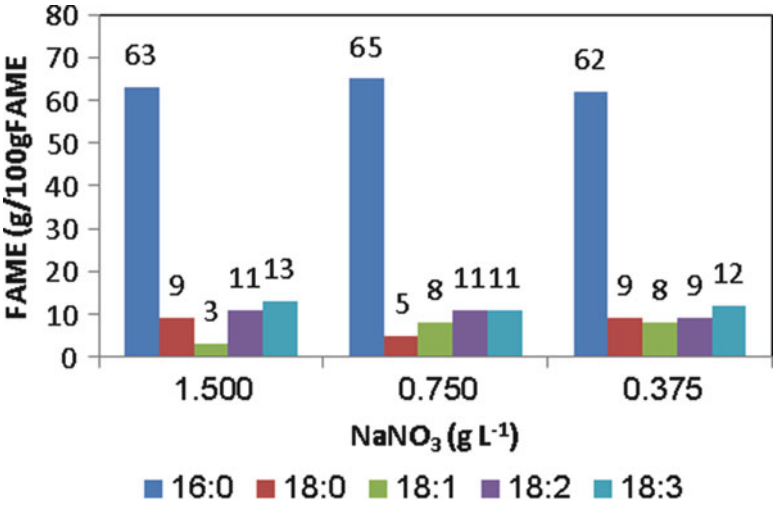

Figure 11. Percentages of individual fatty-acid methyl esters (FAMEs) on the total FAMEs ($g/100g_{FAME}$) in *C. vulgaris* at different concentrations of $NaNO_3$ in the growth medium (Converti et al., 2009).

Samples were stained with Nile red (Doan and Obbard, 2010) and examined in FACS (Gallios Flow Cytometer, Beckman Coulter) (Fig. 12; Table 7). In *chlorella* sp. samples, the mean fluorescence values in the nitrogen-starved samples were more than five times higher than in the control samples. Chemical tests (Koberg et al., 2011) showed a lipid yield of 11% in the control samples compare to 54%

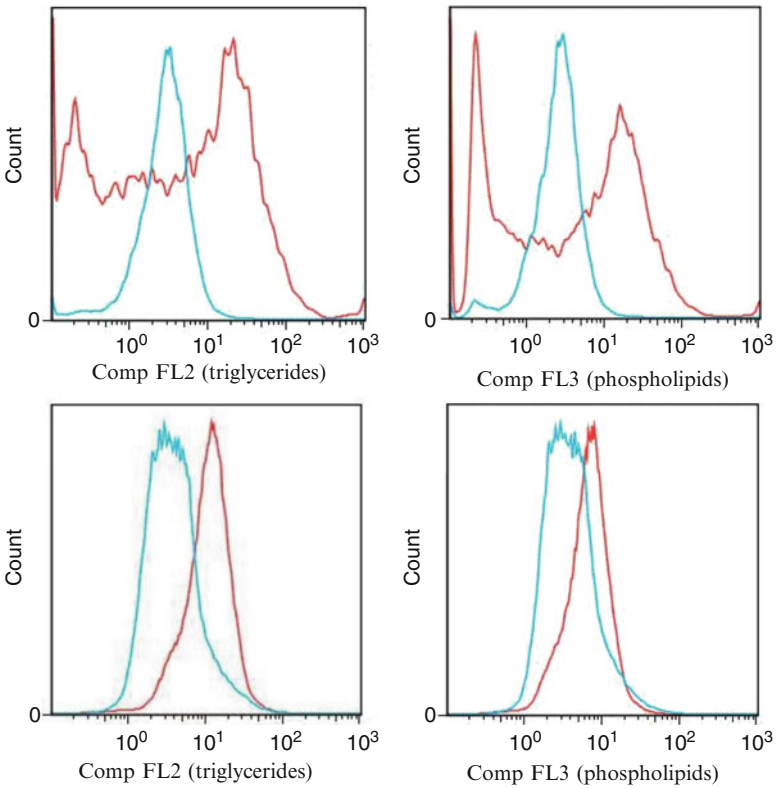

Figure 12. *Nile red* staining of *Chlorella* sp. (**a**, **b**) and *Phaeodactylum tricornutum* (**c**, **d**), as obtained by FACS. Comparison between fluorescence histograms of algal cultures grown under nitrogen starvation (*red*) and control cultures grown in rich media (*blue*). Excitation was at 488 nm; emission in channel FL-2: 575 ± 20 nm for triglycerides; in channel FL3: 620 ± 15 nm for phospholipids (note that the *x*-axis is a $\log^{10}$ scale).

Table 7. Mean fluorescence values of *Chlorella* sp. (a, b) and *P. tricornutum* (c, d).

		Growth medium	Mean FL2-A	Mean FL3-A	Cell no.
a, b		Bristol medium	3.74	3.26	16568
		Bristol medium w/o nitrogen	19.5	17.7	11288
c, d		Sludge water	5.67	5.63	119742
		F2 + Si medium w/o nitrogen	12.9	7.78	137242

Samples stained by Nile red

in microalgae grown under nitrogen-starvation conditions. In the *P. tricornutum* samples, mean fluorescence values of the triglycerides and the phospholipids in the nitrogen-starved samples were about 2.3 and 1.4 times higher than in the control, respectively (Topf and Dubinsky, unpublished).

5. Summary

Microalgae have the potential to be the next "hot item" in many areas, from energy crops to health-food sources. Their growth rates exceed by orders of magnitude those of any high plant "energy crop," combined with unique biochemical plasticity. These properties allow for maximal areal lipid yields and product quality control according to biodiesel specifications. Future lipid production from microalgae are likely to depend on the choice of appropriate algae from among the many not-yet explored and exploited species found in Nature.

Maximization of yields depends on maintaining the right balance between high quantum efficiencies and high photosynthetic rates.

High lipid yields require redirecting biosynthesis away from cell doubling towards lipid accumulation favored by nutrient limitation.

Algae agriculture is a relatively new area that has only emerged on a significant scale during the last century; therefore, we still need to learn the many novel aspects of this field.

Microalgae can be an ideal source of biofuel by using wastewater effluent as a source of essential nutrients while absorbing CO_2 from power stations and industrial smokestacks. Culturing algae on seawater in barren desert areas ensures it will not compete with agricultural food production resources.

The economic feasibility of biodiesel production based on microalgae depends on additional income from wastewater treatment, CO_2 sequestration, and extraction of valuable products from extracted residues.

Algal-oil production costs can be minimized since while generating biodiesel, we also produce valuable fine chemicals, such as vitamins, omega 3, polyunsaturated fatty acids (arachidonic and linoleic), carotenoids (e.g., astaxanthin and β-carotene), vitamin E (alpha-tocopherol), and water- and lipid-soluble antioxidants, besides the proteinaceous extracted meal. These valuable byproducts have great potential in the food, cosmetics, and pharmaceutical industries, while the extracted meal is a high protein component suitable for incorporation in animal feed.

We should not forget that the yield is a product of content and growth rate; hence, finding the best growth rate with the right conditions is an important consideration to keep in mind when aiming at minimizing expenditures while maximizing product yield.

It should be kept in mind that the technology of today will not be the technology of the future, and the algae species will probably be different from what we know nowadays.

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