Chapter

Microalgae: The Multifaceted Biomass of the 21st Century

Donald Tyoker Kukwa and Maggie Chetty

Abstract

Microalgae are unicellular, eukaryotic organisms which possess unique qualities of replication, producing biomass as a precursor for biofuels, nutraceuticals, biofertilizer, and fine chemicals including hydrocarbons. Microalgae access nitrates and phosphates in wastewater from municipalities, industries, and agricultural processes to grow. Wastewater is, therefore, culture media for microalgae, and provides the needed nutrients, micronutrients, inorganic and organic pollutants to produce microalgae biomass. Suitable strains of microalgae cultivated under mesophilic conditions in wastewater with optimized hydrodynamics, hydraulic retention time (HRT), luminous intensity, and other co-factors produce biomass of high specific growth rate, high productivity, and with high density. The hydrodynamics are determined using a range of bioreactors from raceway ponds, photobioreactors to hybrid reactors. Carbon dioxide is used in the photosynthetic process, which offers different growth stimuli in the daytime and the night-time as the microalgae cultivation technique is navigated between autotrophy, heterotrophy, and mixotrophy resulting in microalgal lipids of different compositions.

Keywords: biomass production, autotrophy, heterotrophy, mixotrophy, wastewater treatment, pollutant sequestration, microalgal lipid production, biofuels, nutraceuticals, biofertilizer, photobioreactors, hybrid reactors

1. Introduction

Algae represent a highly diverse consortium of polyphyletic, thallophytic, photosynthetic, and cryptogamic organisms. *They* are morphologically simple, chlorophyll-containing, non-flowering, and typically aquatic plants of a large family with members including seaweeds and a range of microscopic and unicellular to very large multicellular organisms [1]. Algae are either prokaryotes or eukaryotes and lack vascular tissue, leaves, true stems, and roots. The prokaryotic algae are the blue-green algae, which are also referred to as Cyanobacteria or Cyanoprokaryota and belong to the kingdom Eubacteria. The eukaryotic algae belong to the kingdom Protoctista. Cyanobacteria also derive their energy through photosynthesis but do not have a nucleus and membrane-bound organelles, like chloroplasts (see **Figures 1** and **2**) and their prokaryotic nature describes the single-stranded deoxyribonucleic acid (DNA) in their formation, which confers the bacterial identity. On the other hand, eukaryotic algae have double-stranded DNA in their makeup and are equipped with a nucleus and chloroplast. The term "algae" is therefore exclusively reserved for the eukaryotic organisms; and this chapter considers and treats the prokaryotic cyanobacteria as bacteria [1, 2].

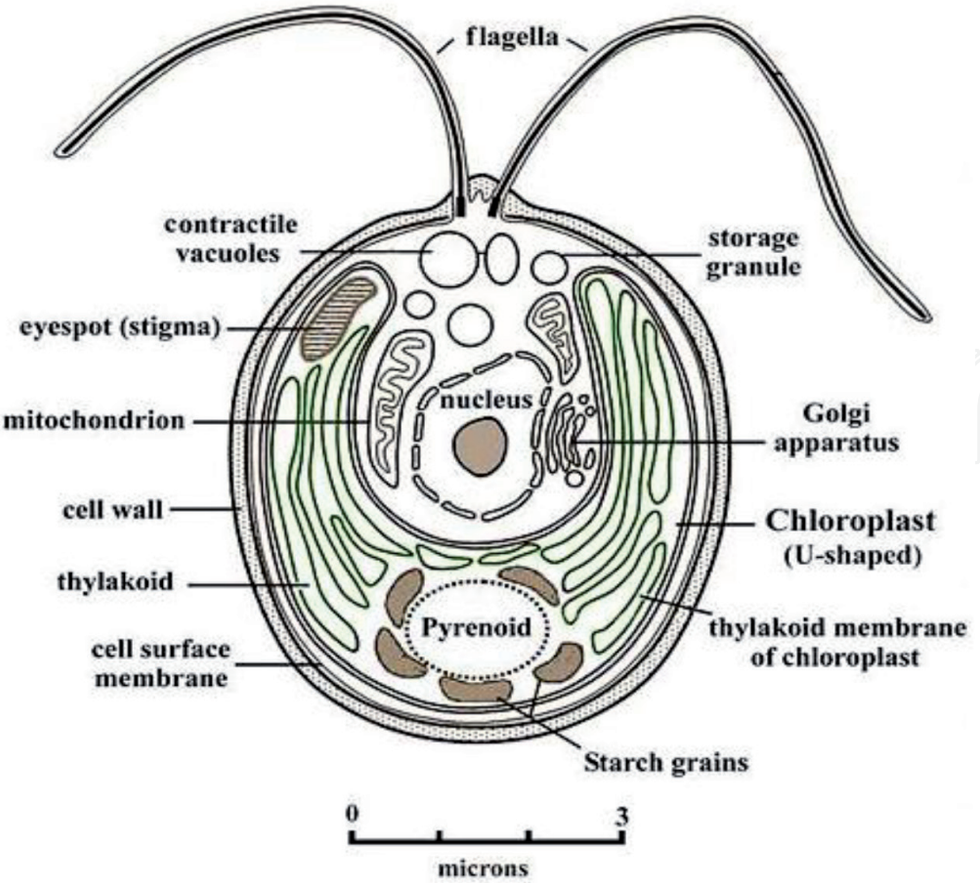

Figure 1.
The microalga Chlamydomonas reinhardtii's *cell structure [3].*

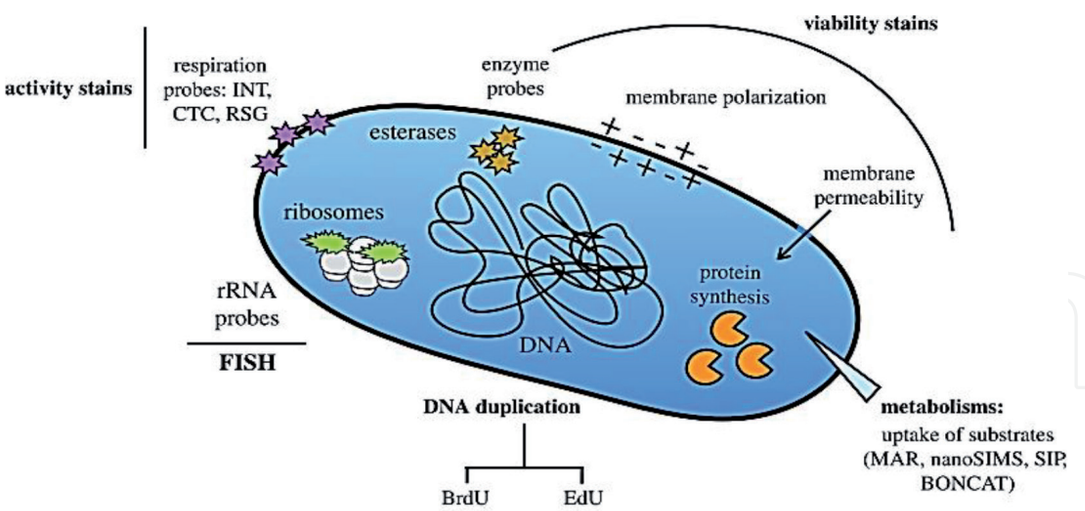

Figure 2.
Schematic of a prokaryotic cell with an indication of some of the methods used to probe cellular activity or growth [4].

Algae have six types of life cycles viz. haplontic, diplontic, isomorphic, heteromorphic, haplobiontic, and diplobiontic cycles; the exposition of these algal life cycles is discussed elsewhere [5]. The microscopic algae are the microphytes or microalgae and are typically found in freshwater and marine ecosystems at the benthic depths and in the water column. They are reported to be the chief converters of water and carbon dioxide to biomass and oxygen (see Eq. (1)) as they receive radiation from sunlight, and are therefore referred to as primary producers. Microalgae

exist either individually, or in chains or groups; and depending on the species, their sizes are typically 3–30 μm, while the cyanobacteria are as small as 0.2–2 μm [2].

$$H_2O + CO_2 \xrightarrow{h\nu} CH_2O + O_2 \quad (1)$$

Aside from producing oxygen and availing themselves as food for a large number of aquatic animals, algae are a good resource base for fine chemicals, crude oil, food supplement for humans, and some pharmaceutical products and finished goods [5].

2. Photosynthetic pigments

Pigments are chemical compounds that reflect and transmit only certain wavelengths of visible light. This makes them appear as the colors perceived. More important than their reflection of light is the ability of pigments to absorb light of certain wavelengths. A photosynthetic pigment (accessory pigment; chloroplast pigment; antenna pigment) is a pigment that is present in chloroplasts of algae and other photosynthetic organisms and captures the light energy necessary for photosynthesis. The reaction of each pigment is associated with only a narrow range of the spectrum, and it is necessary to produce several kinds of pigments with different colors to capture more of the sun's energy. Five important pigments found in algae are (i) chlorophyll (ii) xanthophyll (iii) fucoxanthin (iv) phycocyanin and (v) phycoerythrin [6].

2.1 Chlorophyll

Algae and plants have chloroplasts in which the light-capturing chlorophyll is located, while in cyanobacteria the main light-capturing complex protein molecular assemblies are the phycobilisomes, which are located on the surface of thylakoid membranes [7]. Both chlorophyll and phycobilisomes absorb light most strongly between the high-frequency, high-energy wavelengths of 450 and 495 nm, which happen to be the blue region of the electromagnetic spectrum. Also, the photosynthetic pigments absorb the low-frequency, low-energy wavelengths between 620 and 750 nm, which is the red region of the electromagnetic spectrum. The chlorophyll pigment comes in different forms, and the structure of each type of Chlorophyll pigment is anchored on a chlorin ring with a magnesium ion at the centre. The side chain of each chlorophyll pigment type is different and they are so identified (see **Figure 3** and **Tables 1** and **2**) [7, 8].

Chlorophyll a with the molecular formula $C_{55}H_{72}O_5N_4Mg$ is the most common type of Chlorophyll. It is a green pigment with a chlorin ring having magnesium at the centre (see **Figure 3**). Chlorin is a tetrapyrrole pigment, which is partially hydrogenated porphyrin. The ring-shaped molecule is stable with electrons freely migrating around it to establish resonance structures [9]. It also has side chains and a hydrocarbon trail and contains only $-CH_3$ groups as side chains. The long hydrophobic tail anchors the molecule to other hydrophobic proteins on the surface of the thylakoid membrane. The chemical structural layout of chlorophyll shows a porphyrin ring attached to a protein backbone (see **Figure 3**). By substituting functional groups at positions C2, C3, C7, C8, and the C17-C18 bond, one can identify the structure of the desired chlorophyll (see **Tables 1** and **2**). Chlorophyll captures and absorbs blue, violet, and red light from the spectrum to transmit or reflect green, which is the color that the green algae exhibit [9, 10]. Oxygenic photosynthesis uses chlorophyll a to furnish electrons in the electron-transport chain. Photosystems I and II harbor many pigments that help to capture light energy.

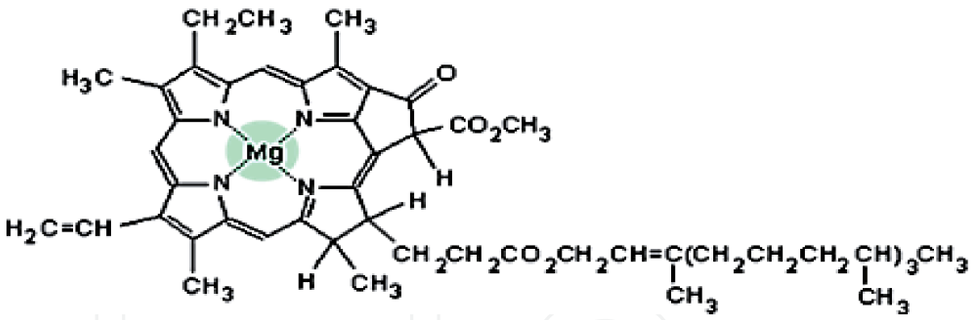

Figure 3.
Chlorophyll - a porphyrin ring structure attached to a protein backbone. The porphyrin is built up of pyrrole molecules – 5 membered aromatic rings which are made of four carbons and one nitrogen atom. This ring system acts as a polydentate ligand and has a magnesium cation at its Centre [8].

	Chlorophyll			
	a	**b**	**c1**	**c2**
Molecular Formula	$C_{55}H_{72}O_5N_4Mg$	$C_{55}H_{70}O_6N_4Mg$	$C_{35}H_{30}O_5N_4Mg$	$C_{35}H_{28}O_5N_4Mg$
C2 group	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$
C3 group	$-CH=CH_2$	$-CH=CH_2$	$-CH=CH_2$	$-CH=CH_3$
C7 group	$-CH_3$	-CHO	$-CH_3$	$-CH_3$
C8 group	$-CH_2CH_3$	$-CH_2CH_3$	$-CH_2CH_3$	$-CH_2CH_3$
C17 group	$-CH_2CH_2COO-$ Phytyl	$-CH_2CH_2COO-$ Phytyl	$-CH_=CHCOOH$	$-CH_=CHCOOH$
C17-C18 bond	Single (chlorin)	Single (chlorin)	Double (porphyrin)	Double (porphyrin)
Occurrence	Universal	Plants	Algae	Algae

Table 1.
Chemical structure of chlorophyll.

	Chlorophyll	
	d	**f**
Molecular formula	$C_{54}H_{70}O_6N_4Mg$	$C_{55}H_{70}O_6N_4Mg$
C2 group	$-CH_3$	-CHO
C3 group	-CHO	$-CH=CH_2$
C7 group	$-CH_3$	$-CH_3$
C8 group	$-CH_2CH_3$	$-CH_2CH_3$
C17 group	$-CH_2CH_2COO-$ Phytyl	$-CH_2CH_2COO-$ Phytyl
C17-C18 bond	Single (Chlorin)	Single (chlorin)
Occurrence	Cyanobacteria	Cyanobacteria

Table 2.
Chlorophyll structural formulae.

A unique pair of pigment molecules are located at the reaction site of each photosystem. For photosystem I the unique pair is referred to as P700, while for photosystem II it is identified as P680. These reaction sites receive resonance energy released from chlorophyll a to sustain the redox reactions [10].

Chlorophyll b is found only in the green algae and in plants, and it absorbs most effectively at 470 nm (blue) but also at 430 nm and 640 nm. Molecular formula - $C_{55}H_{70}O_6N_4Mg$. It is an accessory photosynthetic pigment. The molecular structure consists of a chlorin ring with Mg centre. It also has side chains and a phytol tail. Pyrrole ring II contains an aldehyde group (– CHO). Chlorophyll b absorbs energy that chlorophyll a does not absorb. It has a light-harvesting antenna in Photosystem I [11].

2.2 Xanthophyll

Xanthophyll is one of the two major groups of the carotenoids group. Generally, it is a C_{40} terpenoid compound formed by condensation of isoprene units. Xanthophyll, with the formula $C_{40}H_{56}O_2$, contains oxygen atoms in the form of hydroxyl groups or as epoxides. Xanthophyll acts as an accessory light-harvesting pigment. They have a critical structural and functional role in the photosynthesis of algae and plants. They also serve to absorb and dissipate excess light energy or work as antioxidants. Xanthophyll may be involved in inhibiting lipid peroxidation [12].

2.3 Fucoxanthin

Fucoxanthin, with the formula $C_{42}H_{58}O_6$, is a xanthophyll carotenoid, being an accessory pigment that drives limited photosynthetic reactions in brown algae (phaeophytes) and other stramenopiles. It renders the brown or olive-green color to these seaweeds. Fucoxanthin captures the red light of the spectrum for photosynthetic activities. Some edible brown algae produce this pigment in abundance, and typical candidates in this category include *Sargassum incisifolium*, *Sargassum fulvellum, Undaria pinnatifida, Laminalia japonica,* and others. The alga *Sargassum incisifolium* has been used as a source of Fucoxanthin as a nutraceutical for its antiobesity effects and as much as 0.45 mg/g has been reported [12, 13]. Another rich source of Fucoxanthin is the South African brown alga *Zonaria subarticulata* and extracts as high as 0.50 mg/g have been reported, leading to preparations such as FucoThin™ [13]. The concentration of Fucoxanthin in any algal species may depend on geographical location, seasonal variations, life-cycle, and other factors.

2.4 Phycocyanin (PC)

Phycocyanin is a protein-pigment complex found in cyanobacteria as an accessory pigment to phycobilisomes. As a phycobiliprotein, phycocyanin is identified by the color it bears as blue phycocyanin. Depending on the cyanobacterial species, this can be phycocyanin, showing maximum absorbance at 620 nm and identified as C-PC, and allophycocyanin with maximum absorbance at 650 nm and identified as A-PC. From the red microalgae, phycocyanin is identified as R-PC [13]. The molecular structure of phycocyanin changes with the pH of the medium, exhibiting the $(\alpha\beta)_3$ trimeric structure at pH 7. However, at the pH range of 5–6, the much more available phycocyanin, C-PC, assumes the hexameric structural conformation $(\alpha\beta)_6$. Phycocyanin boosts the human and animal immune systems and protects against certain diseases. It exhibits hepatoprotection, cytoprotection, and neuroprotection. Persons undergoing chemotherapy and radiation for cancer are placed on Phycocyanin from spirulina as a dietary supplement to ease negative symptoms during treatment as well as rejuvenate post-treatment. Phycocyanin is used in the food industry as a food additive [12, 14].

2.5 Phycoerythrin (PE)

Phycoerythrin is an accessory pigment to the main chlorophyll pigment complex found in red algae and cryptophytes; it is part of a covalently bonded phycobilin chromophore in the family of phycobilins, typical of which is phycoerythrobilin, the phycoerythrin acceptor chromophore. Phycoerythrin is made up of (αβ) monomers aggregates. Except for phycoerythrin 545 (PE545), these monomer aggregates are assembled into $(\alpha\beta)_3$ trimers or $(\alpha\beta)_6$ hexamers with 3 or 32 symmetry and enclosing central channel [13, 14]. In red algae, they are attached to the stroma of thylakoid membranes of chloroplasts, whereas in cryptophytes, phycobilisomes are reduced and housed inside the lumen of thylakoids. Phycoerythrin captures light energy from the electromagnetic radiation and directs it to the reaction site through the phycobiliproteins, phycocyanin, and through A-PC. Each trimer and hexamer in the phycobilisome (PBS) has a minimum of one linker protein at the central channel. The α and β chains in B-phycoerythrin (B-PE) and R-phycoerythrin (R-PE) from the red algae also have γ sub-units conferring both link and light-capturing capabilities due to the presence of chromophores [14] (**Figure 4**).

The chloroplast of algal cell contains the water-soluble phycobilin pigments and while the same phycobilin pigments are found in the phycocyanin and phycoerythrin of Cyanobacteria and the red algae, the Rhodophyta. The algal chlorophyll has a structural difference from Bacteriochlorophylls (Bchl) of cyanobacteria, the latter having one of the porphyrin rings saturated, and absorbing longer wavelengths of light as opposed to chlorophylls. *Rhodopseudomonas viridis* has its bacteriochlorophyll b absorb 960 nm wavelength of light [15].

2.6 The Chromophore

The colors of pigments are the reflections of the electromagnetic spectrum from the pigments. A portion of the pigment molecule causes the formation of the color perceived, and this moiety is referred to as **chromophore**. A chromophore has two energy levels referred to as orbitals, and the difference in their energies lie within the visible spectrum of electromagnetic radiation. Thus, a photon of incident light can excite an electron from its ground-state orbital to the excited state. Chromophores are generally either **conjugated pi-systems** or transition **metal complexes** [16]. For **conjugated pi-systems,** electrons are excited between **pi**-orbitals distributed over alternating single and double bonds. Where conjugated systems are less than eight conjugated double bonds, absorbance occurs only in the ultraviolet region and is visible to the human eye. But blue or green compounds essentially do not rely on conjugated pi-bonds alone. Typical chromophores in this category are the azo compounds, lycopene, β-carotene, anthocyanin, and retinenes. **Metal complex chromophores** have transition metals whose d-orbitals are incomplete but are shared with the ligands. Chromophores in this category are the chlorophylls, hemoglobin, and hemocyanin [17].

In general, chromophores comprise four pyrrole rings; identified as (i) open-chain pyrroles with no transition metal involved – typically, carotenoids, phycobilins, and phytochromes, (ii) pyrroles arranged as a porphyrin ring with a central transition metal atom – typically, chlorophylls and bacteriochlorophylls ($C_{55}H_{74}MgN_4O_6$). Chlorophyll absorbs all other visible components of light except green, which is the color the human eye sees of plants in their leaves. Various chlorophylls and accessory pigments (as discussed in sections 2.1–2.5) have characteristic *absorption spectra*; and the *action spectrum* that drives photosynthetic

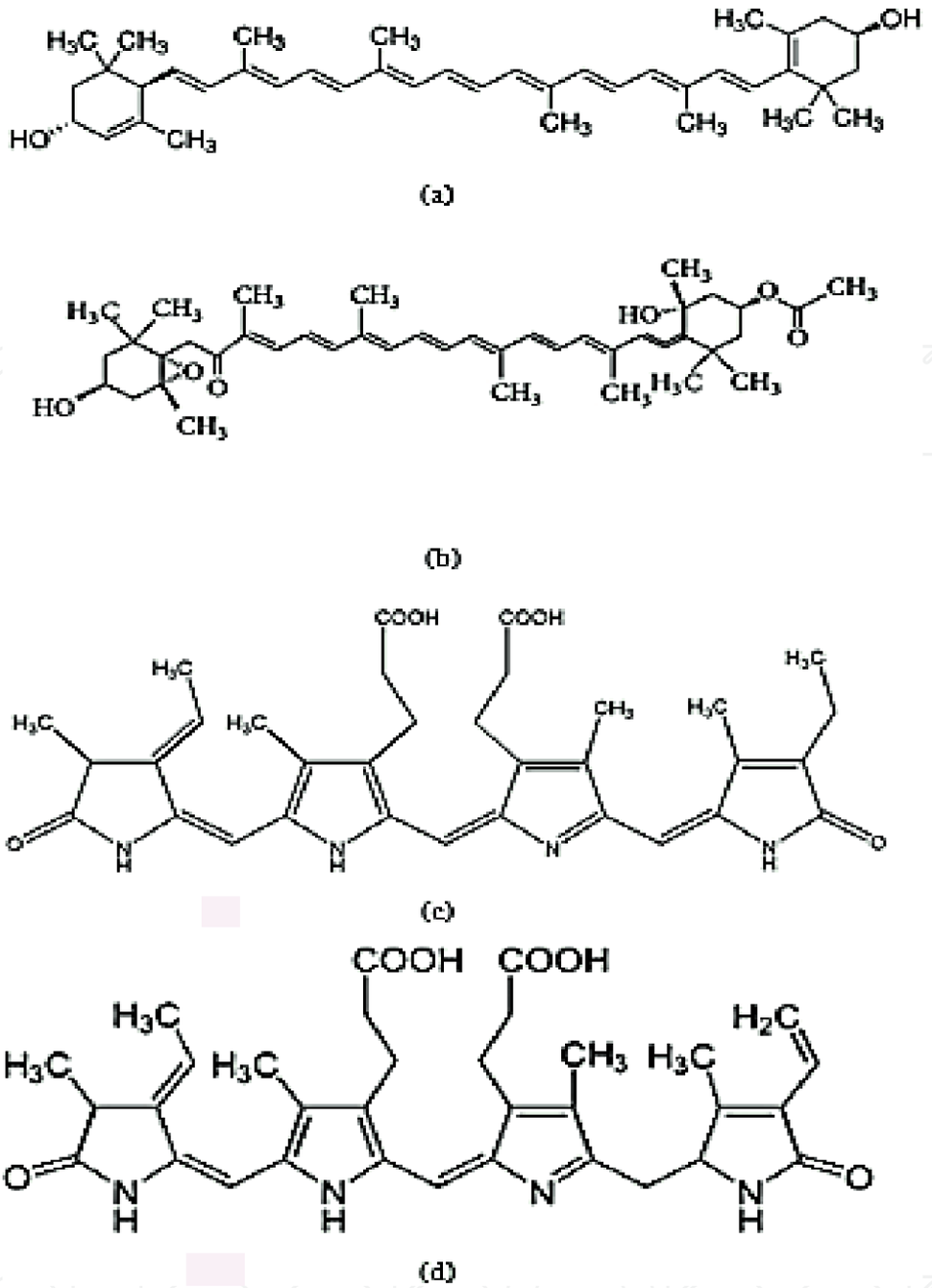

Figure 4.
The structure of the pigments: (a) xanthophyll (b) Fucoxanthin (c) Phycocyanin and (d) Phycoerythrin [12–14].

reactions relates proportionately to the different wavelengths of light (see **Figure 5**). On absorbing light energy, one or more of the following effects happen in a pigment: (i) light energy is transformed to heat energy, or (ii) there is fluorescence, signifying that light energy is re-emitted at longer wavelengths, or (iii) the quantum of **energy is passed** from an excited **molecule of chlorophyll to another molecule** in a process called **exciton** transfer, or (iv) the reaction centre (RC) chlorophyll absorbs the energy and gives up an excited electron to an electron acceptor and (v) the RC chlorophyll is unstable and wants to replace its missing electron, which creates concentration gradients, leading to the production of ATP and NADPH, which are fed into the Calvin cycle (see **Figure 6**) to produce carbohydrates [18].

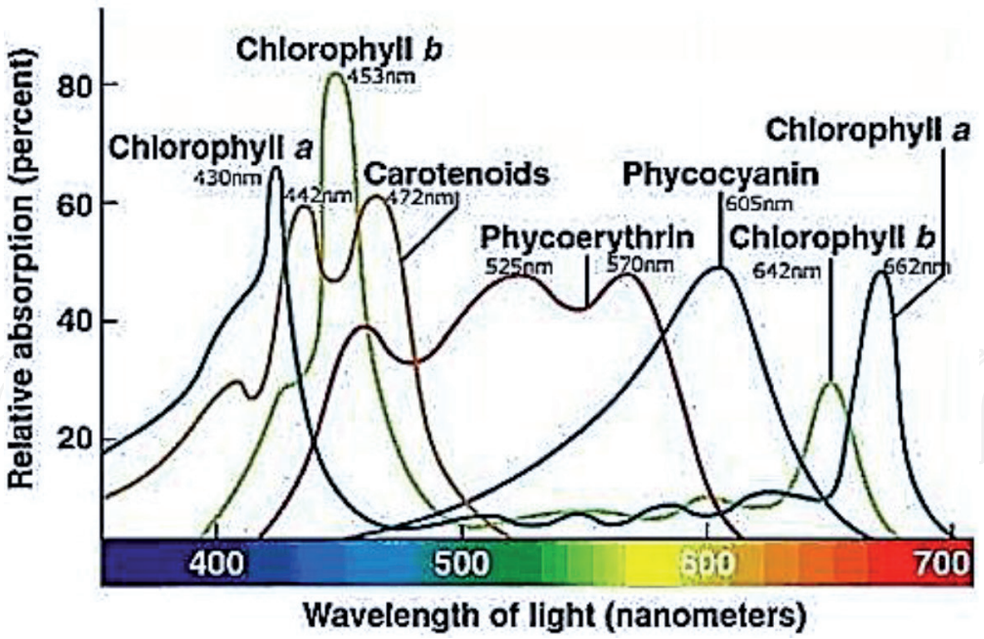

Figure 5.
Relative absorbance of photosynthetic pigments as a function of the wavelength of light [19].

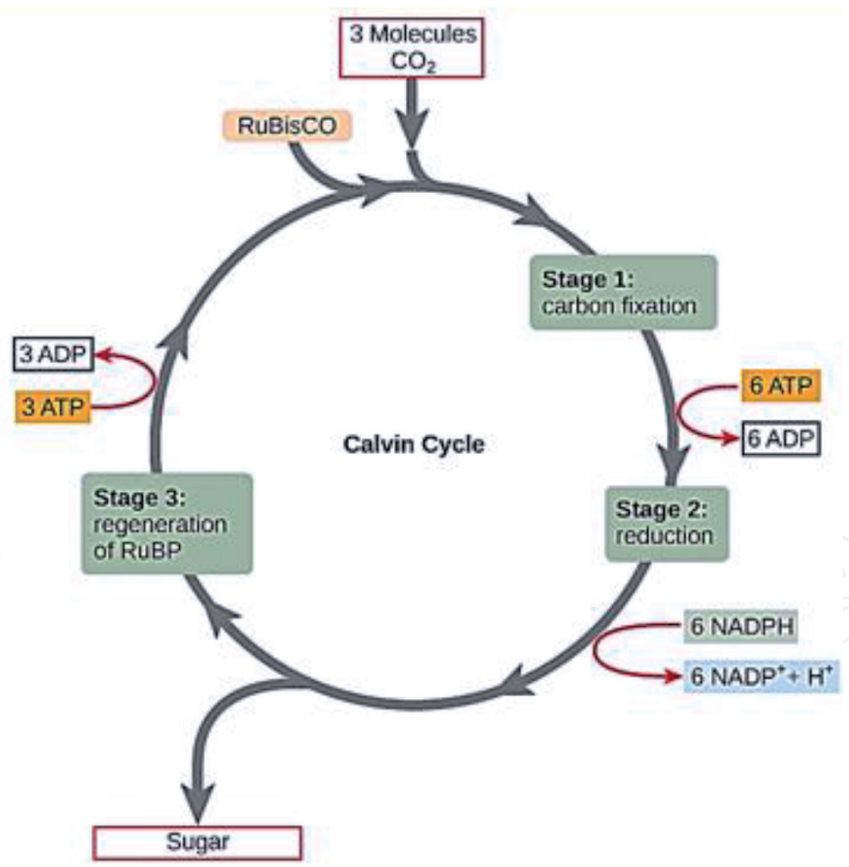

Figure 6.
The Calvin cycle [20].

2.7 The Calvin cycle

The dark reactions of photosynthesis occur in the stroma of the chloroplast and are referred to as the Calvin cycle. Although the Calvin cycle does not utilize light and can happen during the daytime or at night, they employ products of the

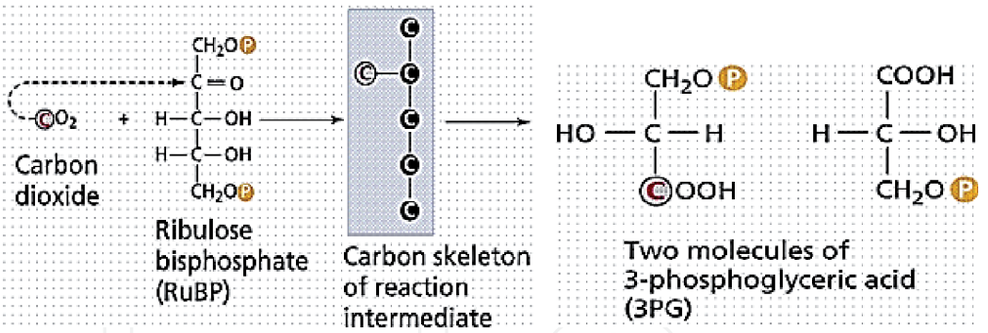

Figure 7.
The first reaction in the Calvin cycle: Carbon fixation.

light-dependent reactions to propagate. Products of the light-dependent reaction are ATP and reduced NADP; the energized electrons from the light-dependent reactions provide the energy to produce carbohydrates from carbon dioxide molecules.

Stage 1: Carbon fixation - Carbon-Fixing Reactions are also known as the Dark Reactions during which CO_2 gas diffuses through and dissolves in the water around the walls of mesophyll cells, diffuses through the cytoplasm and chloroplast membrane into the stomata. In the stroma, CO_2 undergoes a ribulose bisphosphate carboxylase (Rubisco) enzymatic catalyzed reaction with ribulose bisphosphate (RuBP) [19] (**Figure 7**).

The Calvin Cycle first produces phosphoglyceric acid (PGA), which is phosphorylated, using the energy carriers ATP and NADPH generated by the photosystems I and II, to produce 12 molecules of phosphoglyceraldehyde (PGAL). Two molecules of PGAL are ejected from the cycle in the form of a glucose molecule. The other ten molecules of PGAL are converted to 6 RuBP molecules, using the inherent energy in ATP and the cycle continues [19, 20].

Stage 2: Reduction and sugar production – The cell utilizes the high energy molecules ATP and NADPH and reduces 3-PGA to form triose phosphate, G3P.2G3P, which leaves the cycle to produce glucose, starch, cellulose, lipids, amino acids, and nucleotides [20].

Stage 3 Regeneration – The remaining then G3P (3-GPA) in the cycle are regenerated to RuBP, which is a 6-carbon molecule with 2 phosphates, and it requires energy to generate. This process utilizes the high energy ATP made during the light-dependent reactions. The RuBP molecule formed then interacts with more CO_2 from the atmosphere and generates more PGA to keep the cycle going [20].

The summary of the reactions in the Calvin cycle (see Eq. (2))

$$3\,CO_2 + 6\,NADPH + 6\,H^+ + 9\,ATP \rightarrow 3-PGAL$$
$$+6\,NADP^+ + 9\,ADP + 3\,H_2O + 8\,P_i \left(P_i = \text{inorganic phosphate}\right) \qquad (2)$$

2.8 The inherent energy of a photon

Light has properties of both waves and particles, from the quantum mechanics point of view [20]. The particulate behavior of light presents light as a stream of particles of energy, known as photons, which interact with electrons to cause the energy contained in the light to disappear and then reappear as the kinetic energy of the ejected electrons plus a work function.

$$E_{photon} = h\nu = KE_{electron} + \varphi \qquad (3)$$

where E_{photon} is the energy of a photon, h is Planck's constant (6.626×10^{-34} J·s), and v is the frequency of the light wave, $KE_{electron}$ is the kinetic energy of electron and φ is the work function, which defines the minimum amount of energy that is necessary to induce photoemission of electrons from the surface of a metal, and the value of φ depends on the metal. We are dealing with biotic materials in the context of this chapter, so we may assume that $\varphi = 0$.

By definition, $v = \frac{c}{\lambda}$, where c is the velocity of light (3×10^8 m/s in a vacuum), and λ is the wavelength of light. It is important to note that the energy content of light of shorter wavelength is higher than that of longer wavelengths; and for one mole of photons, the energy is the total of the energies of all the particles in one mole, which is given in Eq. (4).

$$E = NE_{electron} \tag{4}$$

where N is the Avogadro's number (6.02×10^{23} molecules or photons/mol). Thus

$$E = NE_{electron} = hv = Nh\frac{c}{\lambda} \tag{5}$$

Thus for sunlight with a wavelength of 650 nm (650×10^{-9} m), the energy is computed in Eq. (6).

$$\begin{aligned} E &= \left(6.02\text{x}10^{23}\ \text{photons}/mol\right)\left(6.626\text{x}10^{-34}\ \text{J.s}\right)\left(\frac{3\text{x}10^{8}\ \text{m/s}}{650\text{x}10^{-9}\ \text{m}}\right) \\ &= 184100.86\ \text{J/mol} \\ &\cong 184.1\ \text{kJ/mol} \end{aligned} \tag{6}$$

If all this were to be used for synthesizing ATP from ADP and **P**i it would be enough to synthesize several moles [20].

Chlorophylls b, c, d, and e are accessory pigments with xanthophylls, and carotenoids in algae and protistans, Pigments that are not accessory to chlorophyll absorb light energy at wavelengths that do not stimulate chlorophyll. Light energy absorbed by accessory pigments is channeled to the reaction site and is converted into chemical energy. The ability to absorb some energy from the longer, more penetrating wavelengths probably conferred an advantage to the benthic photosynthetic algae. Depending upon turbidity of the water, the shorter, high energy wavelengths penetrate very little in the euphotic zone (below 5 meters) in seawater [7, 8]. Chlorophyll molecules being the main producers of pigments are bound to proteins of the photosynthetic membranes and capture the sunlight in oxygenic plants, and convert light energy into chemical energy. This is facilitated by pigment-protein complexes known as Photosystem I (PSI) and Photosystem II (PSII) reaction sites [9]. In PS II water is *split* and the electrons are used to replenish excited electrons that are lost from the photosystem. The loss of electrons during the oxidation of water results in the formation of O_2 gas. In PS I the electron acceptor is first in an electron transport system in the thylakoid membrane. Electrons pass through the chain via a series of redox reactions until it hit the final electron acceptor. The final electron acceptor is $NADP^+$ which is reduced to NADPH. ATP is produced throughout the whole process via chemical osmosis, meaning using an H^+ gradient during electron transport (photophosphorylation). It has been shown that [6] the protein is composed of seven transmembrane helices with a retinal

chromophore covalently bonded in the central region via a protonated Schiff base to a lysine residue (see **Figure 8**).

The most common chlorophylls are chlorophyll a, chlorophyll b, and chlorophyll c1, and chlorophyll c2. Each pigment registers a maximum signal at a particular wavelength of maximum absorption ($\lambda_{\max}$), this coupled with the selective scattering of light, microalgae are seen in their defined color. Consequently, in the blue region of the spectrum, $\lambda_{\max} = 440\,nm$ and in the red region of the spectrum, $\lambda_{\max} = 675\,nm$ (see **Figure 5**) [22]. As the microalgae cells are irradiated with light from a source, the photosynthetic process is initiated and propagated. The photosynthetic rate increases as the intensity of the irradiance are increased; and the level of irradiance is reached where the rate of photosynthesis attains a maximum and begins to retard (see **Figure 9**). At this stage, the cells are said to be in photoinhibition. Photoinhibition is thus a phenomenon that describes the inability of the photosynthetic organism to support photosynthesis in the presence of excess illumination from a light source [19].

The saturation irradiance ($I_k = \frac{P_m^B}{\alpha^B}$) and photo-inhibition (β) parameters indicate the half-saturation constant when the photosynthetic rate is half the maximum value of the photosynthetic rate of the system $\left(P = \frac{P_m^B}{2} \right)$. α^B is the measure of photosynthetic efficiency of solar energy conversion into chemical energy, and it takes into account that the light absorbed by the algal cell is

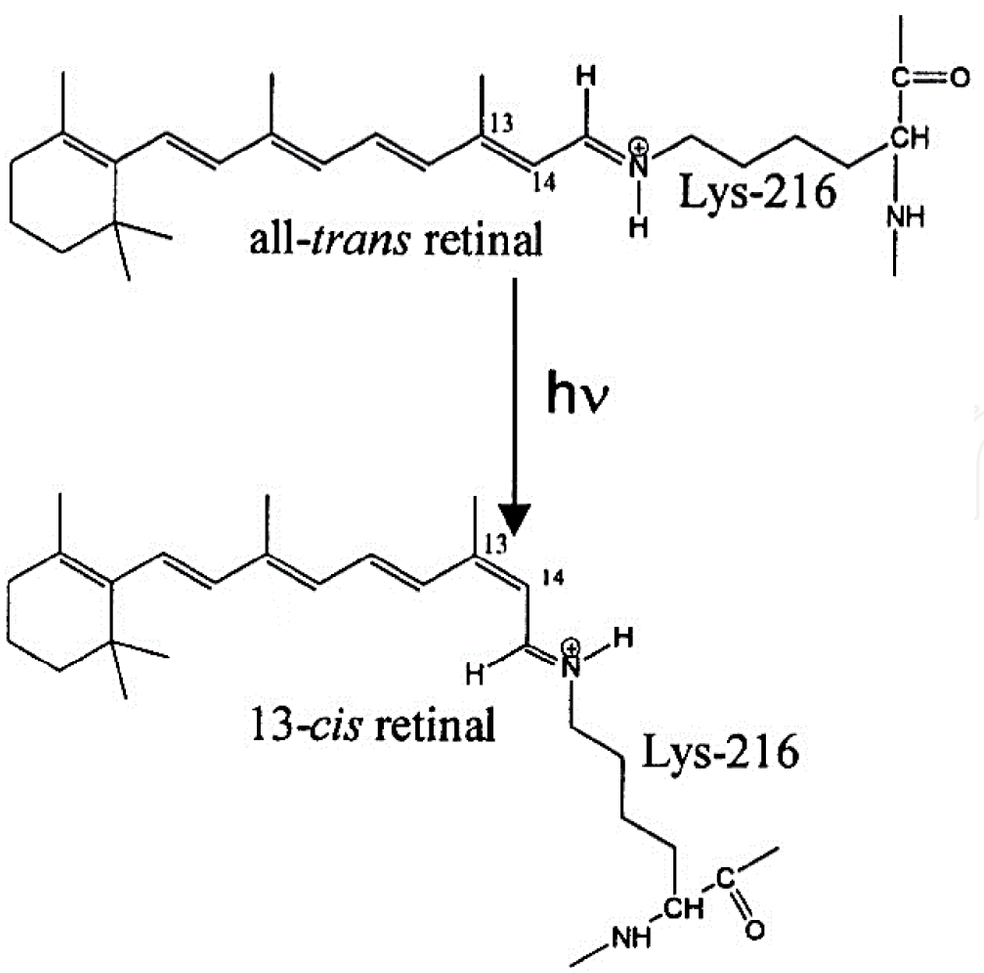

Figure 8.
Photo-isomerization of all-trans to 13-cis retinal in bR [21].

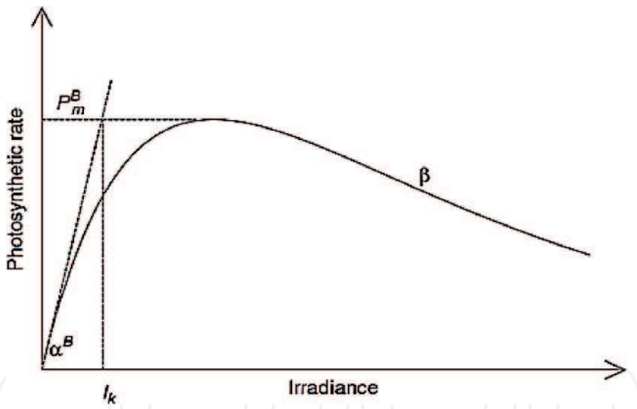

Figure 9.
Photosynthesis – Irradiance curve.

proportional to the functional absorption cross-section of the effective area that PS II presents to an incoming photon. P_m^B is the assimilation number which is the maximum photosynthetic rate [22].

3. The microalgae biomass

Microalgae is a promising renewable resource for biofuels, and optimization and control of the biomass growth production have gained economic and commercial interests. Algae do not compete with traditional food crops for space and resources [5]. Microalgae are highly diverse and differences within and between both species and populations lead to significant differences in biogeography and the environment. The macromolecular composition of the microalgae is of interest for understanding nutrient competition within microalgal communities, food web interactions, and developing algal systems for the development of biofuels, nutraceuticals, and mariculture [3]. Production of microalgae-derived metabolites requires processes for culturing the algae, recovery of the biomass, and further downstream processing to purify the metabolite. The cost of producing microalgal bioactive agents has to be weighed as the downstream recovery of the microalgal products can be substantially more expensive than the culturing of the microalgae [5]. Depending on their origin, algae are referred to as terrestrial algae, snow algae, seaweeds, and phytoplankton. Ubiquitous in marine, freshwater, and terrestrial habitats and possessing broad biochemical diversity, which is the basis for many biotechnological and industrial applications [3].

3.1 Algaculture (culture of microalgae in hatcheries)

Hatcheries are used to produce a range of microalgae biomass, which are used in a variety of ways for commercial purposes. Studies have adduced the success of a microalgae hatchery system to the following factors: (i) the dimensions of the container/bioreactor where microalgae are cultured, (ii) exposure to illumination, and (iii) concentration of microalgal cells within the reactor [23, 24]. Photosynthesis is one of the basic biochemical transformations of photosynthetic micro-organisms that convert solar energy into chemical energy. Many microalgae are autotrophs, which use photosynthesis to produce food. Some heterotrophic microalgae can grow in the dark by utilizing organic carbon. Some microalgae grow by combining both autotrophy and heterotrophy into a hybrid cultivation mode called mixotrophy [4, 6]. Diatoms and dinoflagellates are the two types of microalgae. Diatoms can be spheres, triangles,

elliptical or stars. Many dinoflagellates have two flagella for their movement through the water. Both diatoms and dinoflagellates contain oils in their cells, helping them to swim. Both diatoms and dinoflagellates can grow very quickly and cause algal blooms [3].

3.1.1 Biomass by open pond cultivation

There are two main advantages of culturing microalgae using the open pond system. Firstly, an open pond system is easier to build and operate. Secondly, open ponds are cheaper than closed bioreactors because closed bioreactors require parts that are expensive to acquire. However, where the temperature is the growth or lipid accumulation limiting factor, using open pond systems may decrease the productivity of certain commercially important strains such as *Arthrospira sp.* Waste heat and CO_2 from industrial sources can be used to compensate for this [24]. Some organizations use the open raceway pond approach, employing foam fractionation to concentrate microalgal cells before they are lysed by the cavitation bubble collapse. Some commercial outdoor raceway ponds are located near power plants where 4–15% CO_2 from the flue gas is fed to the raceway ponds. 1.8 units of CO_2 are required to produce one unit of algal biomass, and the practical operation of open ponds has shown that dissolved CO_2 in water is not enough; therefore, the bubbling of air into water improves CO_2 dissolution [25]. Maintaining algae monocultures in open ponds poses serious challenges due to contamination with local algae species and invasion of algae predators. Some strategic operation models adopting higher salinity, pH, or temperature operating conditions have been proposed to provide a selective microenvironment to cultivate some commercial strains. In this regard, therefore, successes have been recorded in open ponds *Spiriluna* monoculture commercial cultivation at high pH values ranging from 9.0 to 11.0. In another operation, β-carotene is produced from *Dulaliella* sp. in open ponds wi high salinity values [25, 26] (**Figure 10**).

3.1.2 Biomass by vertical reactor systems

Many photobioreactors have been suggested for commercial production of algal biomass. However, only a few of them are suitable for practical application because of poor gas mass transfer. The vertical tubular photobioreactor provides a greater

Figure 10.
Algae raceway pond: The microalgae culture broth is constantly kept in motion with a powered paddle wheel [23].

surface area for the interaction of light and the algal cells, increasing the time of gas mass transfer in the culture broth, and the efficient uptake of nutrients. Most times, commercial cultivation of microalgae in vertical reactor systems and reactors of other configurations is not economically viable in batch mode, due to the time taken to load, unload, and clean the reactor systems. The vertical tubular reactor can be made of alveolar panels, polyethylene sleeves, or glass tubes and supported on steel frames (see **Figure 11**). The low productivity characterizing this reactor system is overcome when the surface area to volume ratio is increased. The O_2 gas mass transfer is aided by bubbling air through the culture broth [23].

3.1.3 Biomass by horizontal photobioreactors

This is an outdoor microalgal cultivation system, which has tubes laid on the ground to form a network of loops (see **Figure 12 (b)**). A pump is used to mix the microalgal suspended culture, which raises the culture vertically periodically into a photobioreactor. Pulsed mixing at intervals produces better results than continuous mixing. *Arthrospira sp.* used as a dietary supplement was reported to have higher productivity because of a better-suited temperature range and an extended cultivation period during warm weather periods [23]. The horizontal tubular photobioreactor was assembled with three loops of 80 m each (see **Figure 12**) connected via a manifold to a bubble column used for oxygen removal, temperature control, nutrient, and antifoam addition. The horizontal photobioreactor was operated at a

Figure 11.
Vertical tubular photobioreactors for culturing microalgae [23].

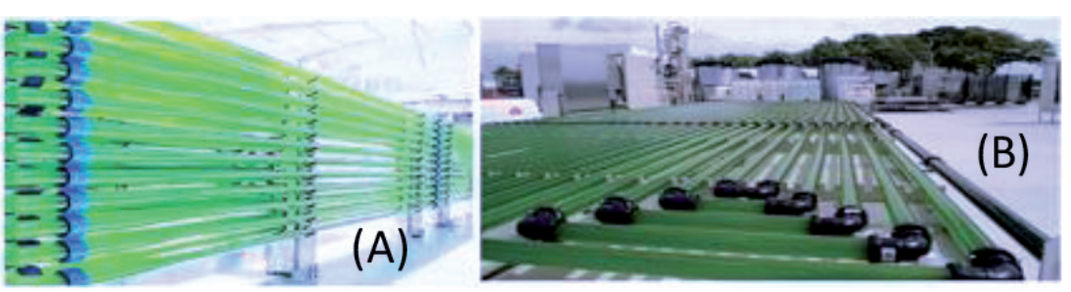

Figure 12.
Horizontal tubular photobioreactor of different orientations [27]: (A) standing tubular bioreactor. The cultivation area occupied is less compared to the floor-type. (B) floor tubular reactor. This occupies a large cultivation area.

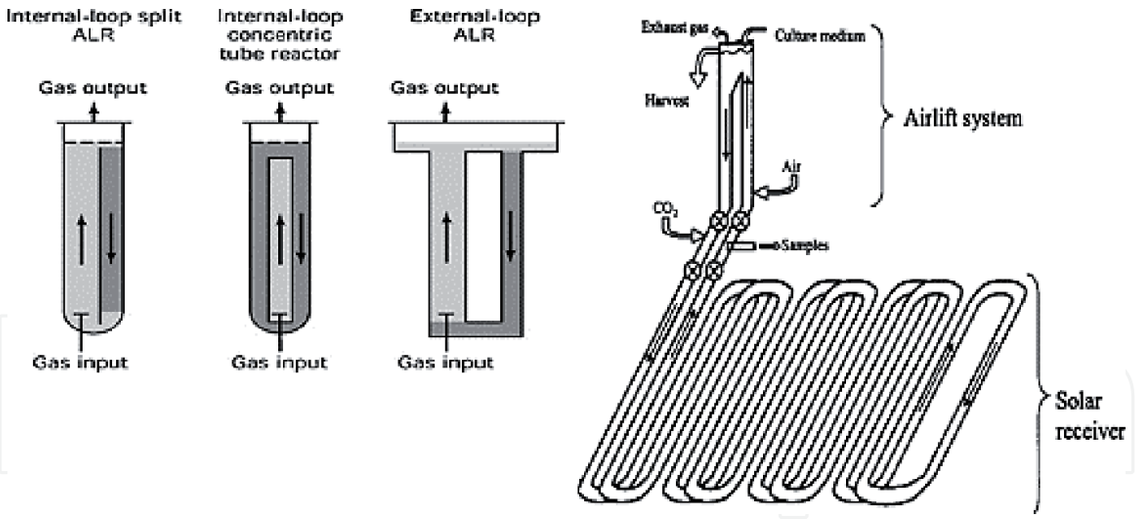

Figure 13.
Different types of airlift photobioreactor [26].

liquid velocity of 0.45 m s^{-1}. To prevent high concentrations of dissolved oxygen, a superficial gas velocity (vgs) of 0.04 m s^{-1} was used in the bubble column [23]. The productivity of *Nannochloropsis sp*. suffered a setback in 250% air-saturated dissolved oxygen solution; and as the dissolved oxygen concentration was raised above 300%, the growth of *Nannochloropsis sp.* was stalled, and that of *Neochloris oleoabundans* was inhibited. However, increasing the airflow rate in the reactor removed the growth inhibitory effect of dissolved oxygen [24].

3.1.4 Biomass by the air-lift method

This is an outdoor microalgal cultivation technique for the production of biomass and metabolites under a highly controlled environment. By this technique, the air is moved within the system to circulate the medium in which microalgae is growing. The culture is grown in transparent tubes that lie horizontally on the ground and are connected by a network of pipes (see **Figure 13**). Air is passed through the tube such that air escapes from the end that rests inside the reactor that contains the culture and creates an effect like stirring [28]. Other configurations of the airlift reactor are an improvement over this design. The external-loop ALR is a promising configuration for breakthrough scale-up *Scenedesmus sp.* biomass production [26].

4. Metabolic platforms for microalgal growth

Different microalgae strains acclimate in different environments, evolving their metabolic pathways to stimulate and propagate growth. However, the extent of growth depends on the composition of the culture media which can be enhanced by either inorganic or organic carbon metabolism or both. Other co-factors such as nutrient availability, pH, chemical oxygen demand (COD), and temperature also influence growth, and the accumulation of metabolites in microalgae (see **Table 3**) [29].

4.1 Autotrophic metabolism

The photosynthetic CO_2-fixation in microalgae suffices to possess a greater ability to fix CO_2. Photo trophy refers to an autotrophic mode of metabolism in which

Metabolic mode	Energy source	Carbon source	Light availability	Metabolism availability
Photo-autotrophic	Light	Inorganic	Obligatory	Fixed
Heterotrophic	Organic	Organic	Not required	Switch between sources
Photoheterotrophic	Light	Organic	Obligatory	Switch between sources
Mixotrophic	Light & organic	Inorganic & organic	Not obligatory	Simultaneous utilization

Table 3.
Microalgal metabolic requirements.

organisms can harness light energy with the help of photosynthetic pigments and convert it to chemical bond energy in the form of ATP (photophosphorylation).

Autotrophy is the ability of PMOs to use inorganic carbon in the form of CO_2 as the sole source of carbon to synthesize organic compounds necessary to build cell components. This is also referred to as carbon-autotrophy to distinguish the ability of some organisms to use molecular nitrogen as the sole source of nitrogen. Such organisms are referred to as nitrogen autotrophs. However, autotrophy as used in this chapter is carbon autotrophy. This is a property that is present primarily, in plants, algae, and phototrophic bacteria including cyanobacteria [30].

Aside from these organisms, all of which are photosynthetic, several groups of non-photosynthetic bacteria can grow using CO_2 as the sole source of carbon by their ability to oxidize inorganic compounds. Such organisms are chemoautotrophic or chemolithotrophic [31].

CO_2 is the end-product of aerobic respiration, a process that releases the energy of respiratory substrates. Carbon dioxide is, therefore, poor in energy content. In autotrophic metabolism, this energy-poor compound is used to build organic molecules which are much richer in energy content. Therefore, It is noted that the conversion of CO_2 to organic compounds requires the input of energy from an external source. The ultimate source in the case of photosynthesis is radiant energy and in the case of chemolithotrophy is the oxidation energy of inorganic chemical compounds. In either case, the immediate source of energy for driving the endergonic reaction involved in the conversion of CO_2 to organic compounds is ATP [32].

In photosynthesis, ATP is generated with the help of photosynthetic pigments through a process known as photophosphorylation. In chemoautotrophy, the energy of oxidation of inorganic compounds is channelized into the respiratory chain for ATP synthesis by oxidative phosphorylation.

Thus, autotrophic metabolism consists of two sets of reactions viz. (1) the ATP and the reducing force are generated and, (2) they are used for the reduction of CO_2 to organic compounds.

The reactions in (1) are different in phototrophic and non-phototrophic autotrophs. But the reactions in (2) are common between the two groups. In the majority of autotrophs, the reactions involved in the reduction of CO_2 proceed via a cyclic pathway, known as the reductive pentose phosphate pathway or, more commonly, as the Calvin-Benson cycle, or simply the Calvin cycle, although other pathways are also known to operate in some organisms, both in the phototrophic green plants and bacteria. The reduction of CO_2 to yield organic compounds is commonly known as CO_2-fixation [32, 33].

4.2 Heterotrophic metabolism

The supply of sufficient light for massive growth is the main goal and a limiting factor for microalgal cultivation. To ignore the requirement for illumination and present the possibility of high cell concentration, points at heterotrophic cultivation as a promising, efficient, and sustainable strategy for certain microalgae to produce metabolites of value by using carbon substances as the sole carbon and energy source. The optimized preliminary cell culturing of microalgae species is an important stage in culturing microalgae biomass at the commercial scale. The growth environment during the culturing process can be [32] either autotrophic (inorganic carbon) or heterotrophic (organic carbon) depending upon the nature of cells and their growth tendencies. Heterotrophic and mixotrophic microalgae are more capable of growing much faster with higher cellular oil accumulation as compared to autotrophic microalgae species. However, heterotrophic microalgae require organic carbon sources like glycerol, glucose, or acetate as a sole source of carbon for growth, which is responsible for about 80% of the costs of culture media [33]. The metabolism of respiration is applied to produce energy. The respiration rates, intimately geared to the growth and division, are determined by the oxidization of organic substrates of the given microalgae [32]. Glucose provides the organic carbon needed and it is preferred because of its high energy density compared to other sources. The oxidative assimilation of glucose employs either the Embden–Meyerhof–Parnas (EMP) pathway or the pentose phosphate (PP) pathway depending on the cycle position. During the dark cycle, PMOs assimilate and metabolize glucose via the PP pathway. However, during the daytime cycle, glycolysis in the cytosol is via the EMP pathway [34]. The growth rate, lipid content, and the ATP of microalgae under the heterotrophic metabolic strategy are higher compared to those under the photoautotrophic metabolic strategy but depend mainly on the PMO's species and strain used. The PMO's growth is steady and rapid in a nutrient-rich culture media using a high level of system control, to achieve biomass production of 50–100 g L^{-1} in heterotrophy which is higher than that achieved in photoautotrophy [35].

Heterotrophic metabolism eliminates the two main problems associated with autotrophic metabolism viz. (i) it allows the use of practically any vessel as a bioreactor, and (ii) low energy and high yield, as major outcomes, giving a significant reduction in costs for the process. Cost-effectiveness and relative simplicity of operations and daily maintenance are the main attractions of the heterotrophic growth approach. A significant benefit is that it is possible to obtain, heterotrophically, high densities of microalgae cells that provides an economically feasible method for large scale, mass production cultivation [34].

Heterotrophy has its drawbacks viz. (1) The microalgae species and strains that can grow by the heterotrophic strategy are limited; (2) Increasing energy expenses and costs by adding organic carbon substrate; (3) Contamination and competition with local microorganisms; (4) Inhibition of growth by excess organic substrate; and (5) Inability to produce light-induced metabolites [35]. Nonetheless, heterotrophic cultures are gaining increasing application for producing a wide variety of microalgal metabolites from bench experiments to commercial scale.

4.3 Mixotrophic metabolism

Mixotrophic cultivation of microalgae strategies provides both carbon dioxide and organic carbon simultaneously and both chemoheterotrophic and photoautotrophic metabolisms operate concurrently. Microalgae biomass produced by this approach has high density and contains high-value lipids, proteins, carbohydrates, and

pigments; and the product range is very versatile [7–10]. These products range from high-value nutraceuticals, food supplements, and cosmetics to the lower value commodities biofuels, food, fertilizer, and application in wastewater treatment [10–12].

4.4 Microalgal metabolites

Microalgal biomass contains considerable amounts of bioactive molecules such as carotenoids (astaxanthins, β-carotenes, and xanthophylls), omega-3 fatty acids, polysaccharides, and proteins, which can be used in several applications as colorants, pharmaceuticals, food, food additives, and feed and as bioplastics.

4.4.1 Carotenoids

Microalgae produce carotenoids and all known xanthophylls found in terrestrial plants (e.g., zeaxanthin, lutein, antheraxanthin). Astaxanthin is a carotenoid pigment that occurs in microalgae, trout, yeast, and shrimp, among other sea creatures. It is found in abundance in Pacific salmon and the fish appears pinkish due to the presence of astaxanthin. Astaxanthin is an antioxidant; it is said to have many health benefits. Carotenoids as accessory pigments, capture light energy during photosynthesis and promote photoprotection. Stains of *Nannochloropsis sp., Rhodotorula glutinis,* and *Neochloris oleoabundans* have high contents of carotenoids. The red ketocarotenoid, astaxanthin (3, 30-dihydroxy-b,β-carotene 4,40-dione) is an antioxidant and the green microalga *Haematococcus pluvialis* is said to be a good natural source of astaxanthin [36–40].

4.4.2 Lutein

Lutein, a xanthophyll, is one of the many known naturally occurring carotenoids. Lutein is synthesized only by plants and is found in large quantities in green leafy vegetables like kale, spinach, yellow carrots, and in dietary supplements. The lutein-rich microalgae *Scenedesmus almeriensis* and *Desmodesmus sp.* could be considered as promising sources of lutein for their tolerance to harsh environmental growth conditions. It is a food colorant with the potential for preventing cancer. It is used for maintaining eye health and to reduce the risk of retinal macular degeneration. The performance of three *Chlorella* species on the production of biomass, lipid, and lutein showed high productivities, presenting the microalgae as a promising resource for these products [41].

4.4.3 Poly-unsaturated fatty acids

Microalgae are the dominant sources of polyunsaturated fatty acids in the marine food chain. *Schizochytrium sp.* is a type of marine microalgae with the natural capacity to produce oil extremely rich in docosahexaenoic acid (DHA) omega-3 fatty acids [42]. DHA-rich extracts from *Schizochytrium sp*. are presented as a feed supplement to swine for their muscle tissue development and as a raw material for the production of aquafeed. *N. oculata* and *P. tricornotum* have a favorable omega-3: omega-6 ratio that is adequate to enrich food [43]. The growth conditions are deliberately manipulated to achieve the desired fatty acid composition of the biomass. At low nutrient concentrations, the microalgal lipids accumulated are rich in triglycerides and are more suitable for biodiesel production; and high nutrients supply to the growth medium leads to the accumulation of long-chain unsaturated fatty acids [44]. DHA applications include healthcare, pharmaceutical, and food & beverage sectors. Within this segment, the pharmaceutical application holds a larger share of it [45].

The acetyl-CoA condensation to fatty acyls is one of the methods by which biohydrocarbons are produced in-situ biotic organisms. The second biohydrocarbon production pathway is the isopentenyl pyrophosphate (IPP) condensation to higher isoprenoids, which is responsible for the diverse isoprene derivatives, many of which are suitable for fuels or fuel additives due to their desirable cetane and pour point and other fuel properties [5]. The low-to-zero-oxygen content of isoprenoids results in energy densities similar to the alkanes in current diesel fuels and diversity of ring structures affords lower cloud points [46, 47]. Additionally, it has been found that slight modifications to enzymes involved in the final steps of higher isoprenoid synthesis can result in subtle product variants with distinct thermochemical and thermophysical properties [47]. The precursors for the majority of these compounds are metabolic intermediates in photosynthetic microorganisms (PMOs). Genetic engineering of microalgae and cyanobacteria would be required to enhance the productivity of PMOs [5].

4.4.4 Microalgal triglycerides

Triglycerides are lipids or waxes, formed by biochemically combining glycerol and fatty acids in the ratio of 1: 3 respectively. This combination may be a simple type or a mixed type. Triglycerides in which the glycerol backbone is attached to three molecules of the same fatty acid are referred to as simple triglycerides. Typical in this category is tripalmitin, $C_3H_5(OCOC_{15}H_{31})_3$. Only a few of the glycerides occurring in nature are of the simple type; most are mixed triglycerides (see **Figure 14**) [48]. Based on saturation and unsaturation of the attached fatty acids, triglycerides can be classified as saturated, monounsaturated, and polyunsaturated. In saturated triglycerides, all the fatty acids are saturated. Saturated fats abound in

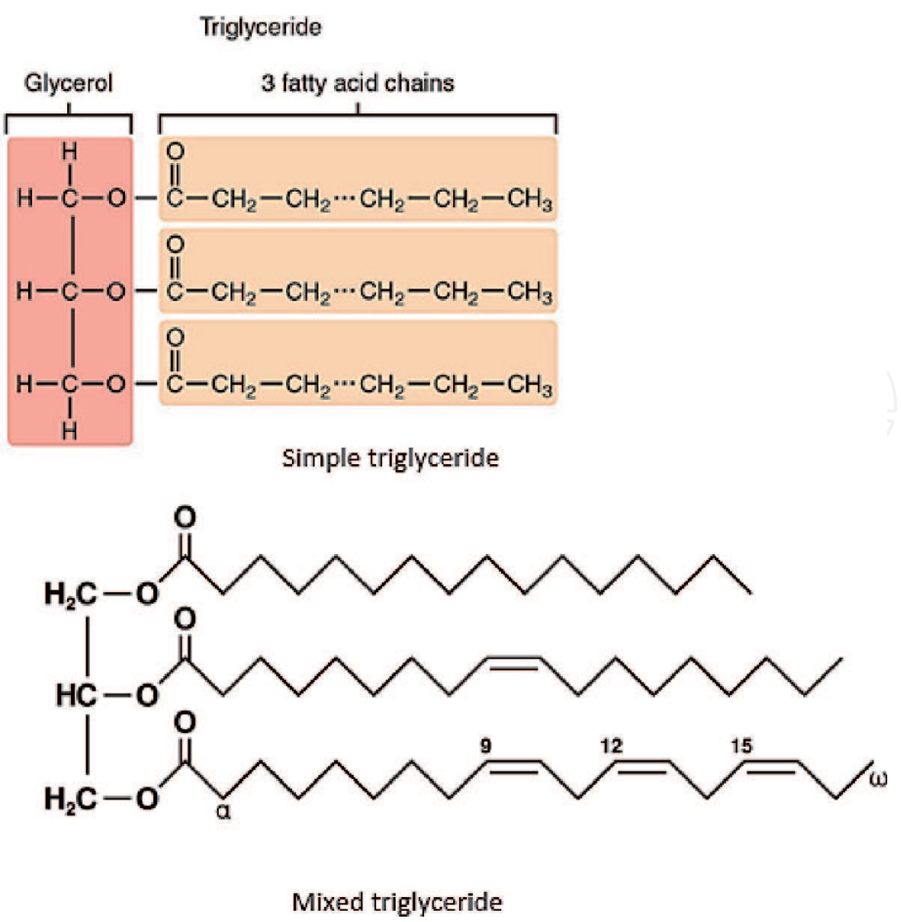

Figure 14.
The structure of triglyceride showing the simple and mixed types.

many animal products such as butter, cheese, cream, and fatty meats, ice cream, and whole milk. In monounsaturated triglycerides most of the fatty acids are monounsaturated. Vegetable oils such as canola oil, olive oil, peanut oil, and sesame oil have high levels of monounsaturated fats and polyunsaturated triglycerides. Omega-3 and omega-6 fatty acids are polyunsaturated.

Microalgae are a promising renewable resource for green production of triacylglycerols (TAGs), which can be used as a biofuel feedstock. Nitrogen starvation is the most effective strategy to induce TAG biosynthesis in microalgae [48]. One of the best microalgae for lipid production is *Botryococcus braunii* Kutzing, above 70% of lipid in its cell content. Whereas other microalgae like *Scenedesmus sp., Chlorella sp*., and *Nanochloropsis sp*. also produce lipid up to 40% [49–51].

4.4.5 Microalgal phospholipids

Phospholipids are made up of four components viz. fatty acids, a platform to which the fatty acids are attached, phosphate, and an alcohol attached to the phosphate. Phospholipids may be built on either glycerol or sphingosine framework. Phospholipids built on glycerol framework are called phosphoglycerides (or glycerophospholipids). A phosphoglyceride consists of a glycerol molecule, two fatty acids, a phosphate, and choline, which is an alcohol. Phosphoglycerides are the most abundant phospholipid molecules found in cell membranes. The phospholipids built on sphingosine framework are referred to as sphingolipids or glycolipids, depending on the number of glucose or galactose molecules they contain; and lipoproteins, which are complexes of cholesterol, triglycerides, and proteins that transport lipids in the aqueous environment of the bloodstream. These are complex lipids. The algae contain three major phospholipids, phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and phosphatidylcholine (PC). Phospholipids are synthesized by both prokaryotic and eukaryotic organisms. They are the major component of most eukaryotic cell membranes, which play a fundamental role in compartmentalizing the biochemistry of life [52]. The hydroxyl groups at positions C-1 and C-2 in phosphoglycerides are esterified to the carboxyl groups of the two fatty acid chains. The hydroxyl group at position C-3 hydroxyl group of the glycerol backbone is esterified to phosphoric acid. At this extent of conversion, the product is phosphatidic acid, which is the simplest phosphoglyceride. Phosphatidic acid now serves as the backbone on which most phosphoglycerides are derived having moieties such as serine, ethanolamine, choline, glycerol, and the inositol. Consequently, we have phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, and phosphatidylinositol respectively (see **Figure 15**) [52].

4.4.6 Vitamins and fine chemicals from microalgal biomass

Metabolites from both microalgae and cyanobacteria have attended to both human and animal health and food needs and these microorganisms have become attractive resources for bioactive natural products that have wide applications in pharmaceutical, food, and chemical industries. Algae-derived bioactive substrates are employed for drug screening, given their tremendous structural diversity and biological availability. Microalgae biomass has a wide range of physiological and biochemical characteristics and contains 50–70% protein compared to 50% in meat, and 15–17% in wheat, with 30% lipids, more than 40% glycerol, 8–14% carotene, and a reasonably high levels of vitamins B1, B2, B3, B6, B12, E, K, D, and others [54–56].

Microalgae that have been cultivated on commercial scales and are available include *Chlorella, Dunaliella, Nannochloris, Nitzschia, Crypthecodinium, Schizochytrium, Tetraselmis, Skeletonema,* etc. and the cyanobacterium, *Spirulina,* and

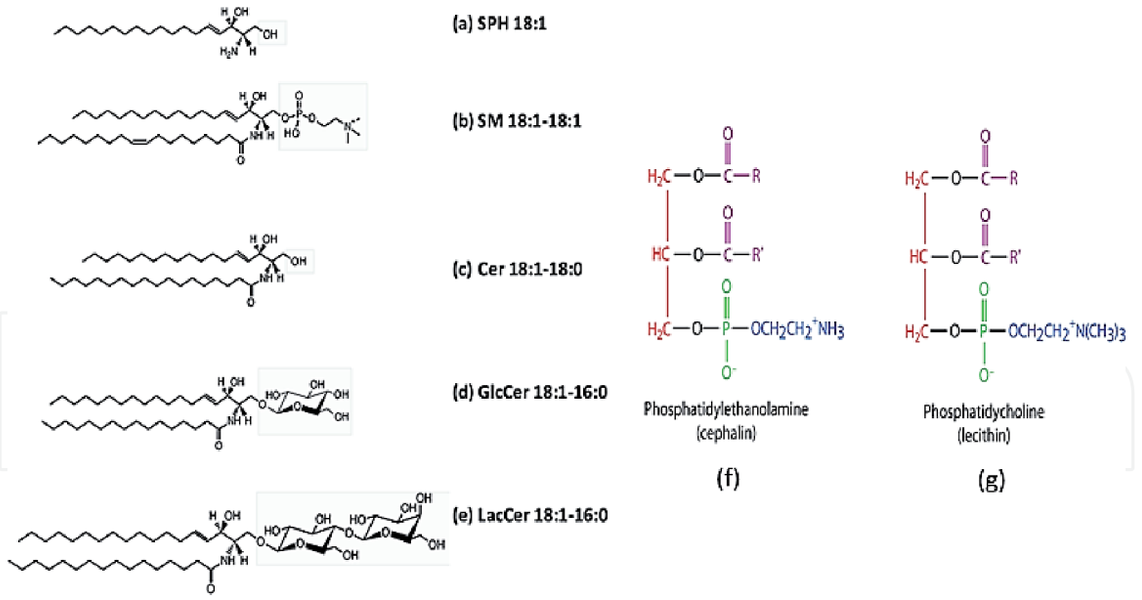

Figure 15.
Sphingolipids and phospholipids: The classification of sphingolipids is based on the group attached to the sphingosine (LCB) backbone (a). Sphingomyelin (b) and ceramides (c-e) differ in fatty acid length, unsaturation, and in the type of attached head group and hydroxylation. Phospholipids with glycerol framework: (f) phosphatidylethanolamine, (g) phosphatidylcholine [53].

a host of others. Most of the commercially produced microalgal biomass is presented to the market as a food supplement, and they are presented as tablets and capsules. Breakfast cereals, noodles, beverages, wines, and cosmetics now contain microalgae and their extracts. More than 75% of pharmaceutical product development is carried out by the microalgal food supplement production outfits. In the recent several years, microalgal and cyanobacterial research has explored diverse cultivation protocols aimed at improving growth rates, biomass yields, and accumulating metabolites for high nutritional value, and high-value chemicals (pigments and vitamins) [55]. Many more bioactive metabolites have been reported in microalgae. Dried microalgae biomass could be used as high-protein feeds for animals such as shrimp and fish, and microalgal biomass is a significant resource for cytotoxic agents with applications in cancer chemotherapy. The blooms of *Phaeocystis sp*., a marine microalga have antibiotic substances listed therein. *Phaeocystis pouchetii* produces acrylic acid, which makes up to 7.0% of its dry weight. The antibiotic metabolites so produced migrate in the food chain through the digestive system of some Antarctic marine animal species. Also, the alga *Dunaliella sp.* produces β-carotene and certain vitamins, which have boosted the Mariculture activities. Some cyanobacteria and microalgae such as *Ochromonas* sp. and *Prymnesium parvum* produce toxins, which may have the potential for pharmaceutical applications. These marine cyanobacteria produce bioactive metabolites such as acetogenins, bromophenols, fatty acids, terpenes, sterols, alkaloids, etc. with antibiotics, and antifungal activities. Diverse strains of cyanobacteria produce intracellular and extracellular metabolites with bioactive functions such as antitumor, anti-inflammatory, antialgal, antibacterial, antifungal, and antiviral activity [55, 56].

4.5 Microalgal biomass production limiting factors

Abiotic, Biotic, and process-related factors influence the growth of algae. Some of the abiotic factors are illumination and luminous intensity, daytime to night-time ratio, the temperature of the culture medium, nutrient availability, O_2, and CO_2 mass transfer, pH value, the hydraulic retention time (HRT), salinity, and presence

of growth-inhibiting chemical agents [30]. Some of the biotic factors are the presence of pathogens (bacteria, fungi, viruses) and the presence of more than one algae strains. Each algae strain has a different capacity to assimilate nutrients, and in mixed cultures, there is competition for the available nutrients in the media, which may afferent the growth of some strains [36]. Process related factors that may influence algal growth are hydrodynamics of the culture broth, which is influenced by the choice of the bioreactor, the initial algal cell concentration in the reactor, and the related frequency of harvesting algal biomass [57, 58].

5. Conclusions

There is a major difference between microalgae and cyanobacteria in terms of their cell structure and this work has presented unmistakable evidence that microalgae have a nucleus and chloroplast, and their makeup includes their full identity in a two-stranded DNA. On the other hand, cyanobacteria are identified by one-stranded DNA and do not have a nucleus and neither a chloroplast. However, Microalgae and cyanobacteria do photosynthesize to produce their food.

It is seen from research as discussed in this chapter that value products aimed to meet pharmaceutical and food needs are obtainable by continuous availability of nutrients to the microalgae in the culture media. It is also seen that to accumulate lipid in the order of triglycerides for biodiesel production, microalgae must experience nutrients deficiency in the culture media at the stationary stage of growth.

The hydrodynamics of the microalgal culture broth depends on the choice of bioreactor for a particular cultivation activity and contributes to the algal growth factor.

The versatility of the microalgal biomass is expressed in the diversity of metabolites produced by manipulation of the growth factors in favor of the desired product. Also, the choice of the strain will drive towards the targeted product.

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Conflict of interest

The authors have declared that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this chapter.

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Author details

Donald Tyoker Kukwa* and Maggie Chetty
Department of Chemical Engineering, Durban University of Technology, Durban, South Africa

*Address all correspondence to: tyokerdoo@gmail.com

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