The Evolution and Versatility of Microalgal Biotechnology: A Review

Article *in* Comprehensive Reviews in Food Science and Food Safety · September 2016

DOI: 10.1111/1541-4337.12227

CITATIONS
61

READS
4,948

1 author:

Imen Hamed
Cukurova University
18 PUBLICATIONS **655** CITATIONS

SEE PROFILE

Comprehensive REVIEWS in Food Science and Food Safety

The Evolution and Versatility of Microalgal Biotechnology: A Review

Imen Hamed

Abstract: Microalgal biotechnology has emerged due to the health-promoting properties of microalgae related to their bioactive compounds and the great diversity of products that can be developed from algal biomass. Microalgal biomasses have been produced industrially for applications in different fields such as food, pharmaceutical, nutraceutical, cosmetic, and animal feed industries. They can be cultivated either in open systems or in closed systems (photobioreactors). Another important area is the use of microalgal biomass for energy production. It has become obvious that petroleum-derived fuels are unsustainable, due to depleting world reserves and greenhouse gas emissions. Microalgae can provide several different types of renewable biofuels. These include methane produced by anaerobic digestion of the algal biomass, biodiesel derived from trans-esterification of microalgal lipids, bioethanol produced from carbohydrate fermentations, and photobiologically produced biohydrogen. The idea of using microalgae as a source of fuel is not new. However, it is now being taken seriously because of increases in petroleum prices and, more significantly, the increasing concern about global warming as associated with burning fossil fuels. This review offers an update on information about microalgae, specifically emphasizing their biotechnological importance.

Keywords: bioactive compounds, biomass production, genetic transformation, industrial applications, microalgal biotechnology, renewable energy

Introduction

The ocean is a rich source of biological and chemical diversity. It covers more than 70% of the Earth's surface. Therefore, it offers an enormous resource for novel compounds. Different marine creatures are subject to extreme conditions in their natural habitats; they are entirely different from the terrestrial organisms in many aspects as they adjust to their new environment (Lordan and others 2011). As a matter of fact, they produce a broad variety of unique potent substances. Among those organisms microalgae must be mentioned. They are photosynthetic prokaryotic (cyanobacteria) or eukaryotic microorganisms that grow rapidly and have the ability to live in different environments due to their unicellular or simple multicellular structure. They exist in various aquatic and terrestrial ecosystems (Rajvanshi and Sharma 2012). Despite the fact that more than 50000 species of them are known, only 30000 have been studied (Mata and others 2010).

Cyanobacteria and eukaryotic microalgae have great ecological and economical importance. Cyanobacteria, formerly named blue-green algae are the only known prokaryotes capable of oxygenic photosynthesis (Tamagnini and others 2002). They are considered among the oldest life forms on Earth and are the original producers of the Earth's oxygenic atmosphere (Chauvat and Cassier-Chauvat 2012; Saad and Atia 2014). Microalgae provide food and oxygen for many species in the aquatic environment. Many eukaryotic and prokaryotic species (*Euglena gracilis*, *Chlorococcum littorale*, *Cyanidium*, *Spirulina*, diatoms, and species of *Chlorella*) could be used for CO_2 bio-fixation (Ono and Cuello 2003; Hopkinson and others 2011). CO_2 is converted to microalgal biomass, which could be used to produce commercially valuable products. Mitigation of CO_2 by microalgae is considered more environmentally friendly and sustainable than chemical/physical CO_2 removal (Zhang 2015). These microorganisms are also a source of highly valuable molecules such as proteins, PUFAs (polyunsaturated fatty acids), photosynthetic pigments, and polysaccharides (Lordan and others 2011; Ibañez and others 2011). Those compounds find many uses in the food, pharmaceutical, and cosmetic industries, due to their numerous biological activities (antioxidant, anticancer, antihypertension, immunomodulatory, and prevention of cardiovascular diseases).

Microalgae have been used for thousands of years by indigenous populations (Blackburn and Volkman 2012). The first use dates back 2000 y to the Chinese, who used *Nostoc* to survive during famine (Spolaore and others 2006). *Spirulina* also seems to be one of the most used genera as food. *Spirulina* has been consumed by different cultures such as the Maya civilization and by African communities in Chad and Niger near the alkaline lakes (Ciferri and Tiboni 1985; Belay 2007). However, the biomass production of microalgae is still new (Gouveia and others 2008).

Microalgal biotechnology has emerged due to the great diversity of the products that can be developed from the biomass. Facing an uncertain future with food and energy shortages and increasing

MS 20160752 Submitted 16/5/2016, Accepted 12/8/2016. *Authors are with Biotechnology Research and Application Centre, Cukurova Univ., Adana, Turkey. Direct inquiries to author Hamed (E-mail: imen_hh@yahoo.fr).*

doi: 10.1111/1541-4337.12227

climate change, microalgal biotechnology has became one of the most serious tracks for such emerging problems that are encountered nowadays (Stephens and others 2013; Saifullah and others 2014).

Despite its importance, microalgal biotechnology began really to develop only in the middle of the last century (Pulz and Gross 2004; Hallmann 2007). Currently, there are different industrial uses of microalgae, such as for human nutrition, animal feed, fertilizer use, and ingredients to be incorporated in cosmetic and pharmaceutical products (Milledge 2011; Yaakob and others 2014). Moreover, many studies have been conducted for the use of microalgae as energy source (biodiesel, bioethanol, and biohydrogen) (Hannon and others 2010; Huang and others 2010; Zhu and others 2012; Slade and Bauen 2013).

Biotechnology that involves the use of living organisms in industrial processes has been around since the dawn of time (bread, cheese, and wine) (Clark and Pazdernik 2016). But with scientific progress, biotechnologies have evolved also. Actually, microalgal species can be altered through genetic engineering to diversify and improve competitiveness (Spolaore and others 2006).

This review focuses on the biotechnological applications of microalgae. Microalgal bioactive compounds and their methods of extraction are presented. Information about their numerous industrial uses and their promising exploitation as a source of renewable energy are also provided. Finally, their cultivation, harvesting, and dewatering are explained and the possibilities of their improvement by genetic engineering are discussed.

Microalgal Cells

Algae are among the oldest group of organisms (Lee 2008). Microalgae are prokaryotic and eukaryotic photosynthetic microorganisms. There are 2 groups of prokaryotic (Cyanophyta and Prochlorophyta) and different eukaryotic divisions (Chlorophyta, Rhodophyta, Phaeophyta, Bacillariophyta, and Chrysophyta) (Mutanda 2013). Microalgae are ubiquitous. Thus, they can be found almost anywhere on Earth, in fresh water (ponds, puddles, canals, and lakes) and in marine and hyper-saline environments (Williams and Laurens 2010).

Algae can have an autotrophic or heterotrophic metabolism. The first group requires only inorganic components (salts, CO_2, and a light energy) for growth. The second one represents non-photosynthetic organisms. Thereby, microalgae need an external source of organic components. Some algae are mixotrophic, which means that they have the capacity to both do photosynthesis and obtain exogenous organic nutrients (Brennan and Owende 2010; Dragone and others 2010; Behera and others 2014).

Anoxygenic photosynthetic bacteria are the earliest photoautotrophic life forms that developed 3.5 billion years ago (Schopf and Packer 1987; Nisbet and Sleep 2001). They use light energy to pull protons and electrons from donor molecules (H_2S) to reduce CO_2 to create organic compounds (Masojidek and others 2013). Prokaryotic algae (cyanobacteria) and eukaryotic algae appeared later and created our oxygenous atmosphere (Buick 2008). Photosynthesis (Figure 1) is a procedure of sunlight energy transformation. In this process, photoautotrophs convert inorganic compounds and light energy to organic compounds. All photosynthetic organisms possess pigments for harvesting light energy. There are 3 main groups of pigments: the lipophilic chlorophylls and carotenoids and the hydrophilic phycobilins (Bittencourt Sydney and others 2013).

In eukaryotic organisms, the photosynthetic unit is organized in special organelles, the chloroplasts, which contain alternating layers of lipoprotein membranes (thylakoids) and aqueous phases, the stroma. The photosynthetic reactions are located in the thylakoid membranes. Those membranes form closed, flat vesicles around the intrathylakoidal space, the lumen. The thylakoid membrane contains 5 major complexes: light-harvesting antennae, photosystem II (PS II) and photosystem I (PS I), cytochrome b6/f, and ATP synthase, which maintain photosynthetic electron transport and photophosphorylation (Masojidek and others 2004).

The photosynthetic apparatus of cyanobacteria is very similar to that of eukaryotic organisms (Drews 1999). The major differences are that the thylakoids occupy the peripheral part of the cytoplasm since there is no chloroplast in prokaryotic cells. The thylakoid membranes are arranged in parallel layers like onion skins and folded in complex patterns, but they are not stacked like grana membranes in chloroplasts. Cyanobacteria contain phycobiliproteins, which are organized in phycobilisomes attached to the surface of the thylakoid membrane (Tomaselli 2004). Phycobilisomes are the major antenna systems of cyanobacteria, while in eukaryotic groups chlorophyll *a* (Chl) is the light-harvesting antenna and it is accompanied by Chl *b* or Chl *c*. The so-called accessory (antennae) pigments Chl *b*, *c*, and *d* extend the range of light absorption (Drews 1999).

Bioactive Compounds in Microalgae

Many marine molecules have been determined as possessing a variety of biological effects. Microalgae are well-known for their original chemical composition and their incredible effects have been proven. Microalgal bioactive compounds such as lipids, starch, proteins or carotenoids, depending on the species, are accumulated under stress conditions including nutrient deprivation, pH, light intensity, temperature, and salinity (Duong and others 2015). The lipids content increases greatly under nutrient starvation (nitrogen, phosphorus, sulfur, and silicon), temperature change, salinity, pH, heavy metals stress (cadmium, iron, copper, and zinc), and light irradiation for some species such as *Chlamydomonas reinhardtii*, *Dunaliella salina*, *Nannochloropsis oculata*, *Spirulina platensis*, *Chlorella vulgaris*, and *Euglenia gracilis* (Sharma and others 2012). Increasing temperature and light intensity beyond the optimum reduces protein synthesis and results in decreased growth rates as reported regarding *Phaeodactylum tricornutum, Scenedesmus* sp., and *Dunaliella viridis*. Increased temperature leads to degradation of the starch produced as noticed for *Chlorella vulgaris*. Carotenoids that protect chlorophyll from photodamage are accumulated in algae with increased temperature and UV radiation. Effects of phosphorus deficiency on *Ankistrodesmus falcatus* include decrease in chlorophyll *a* and a higher lipid/protein ratio and an increase in carbohydrate content. Furthermore, with nutrient starvation a degradation of phycolbillisomes was noticed for cyanobacteria and red algae and an accumulation of astaxanthin under phosphate deficiency was demonstrated for *Haematococcus pluvialis*. Regarding the effect of heavy metals on pigments, a reduction in carotenoid composition was reported when iron concentration was decreased (Juneja and others 2013). Table 1 shows the health-enhancing properties of some of those components.

Amino acids, peptides, and proteins

Microalgae can be used as an alternative protein source because of the high protein content of various species and their amino acid pattern (Gouveia and others 2008). For example, *Spirulina* has a significant protein content (60% to 70%) (Lordan and others 2011). This genus possesses many activities, such as reducing potential animal brain damage; it also shows a reduction

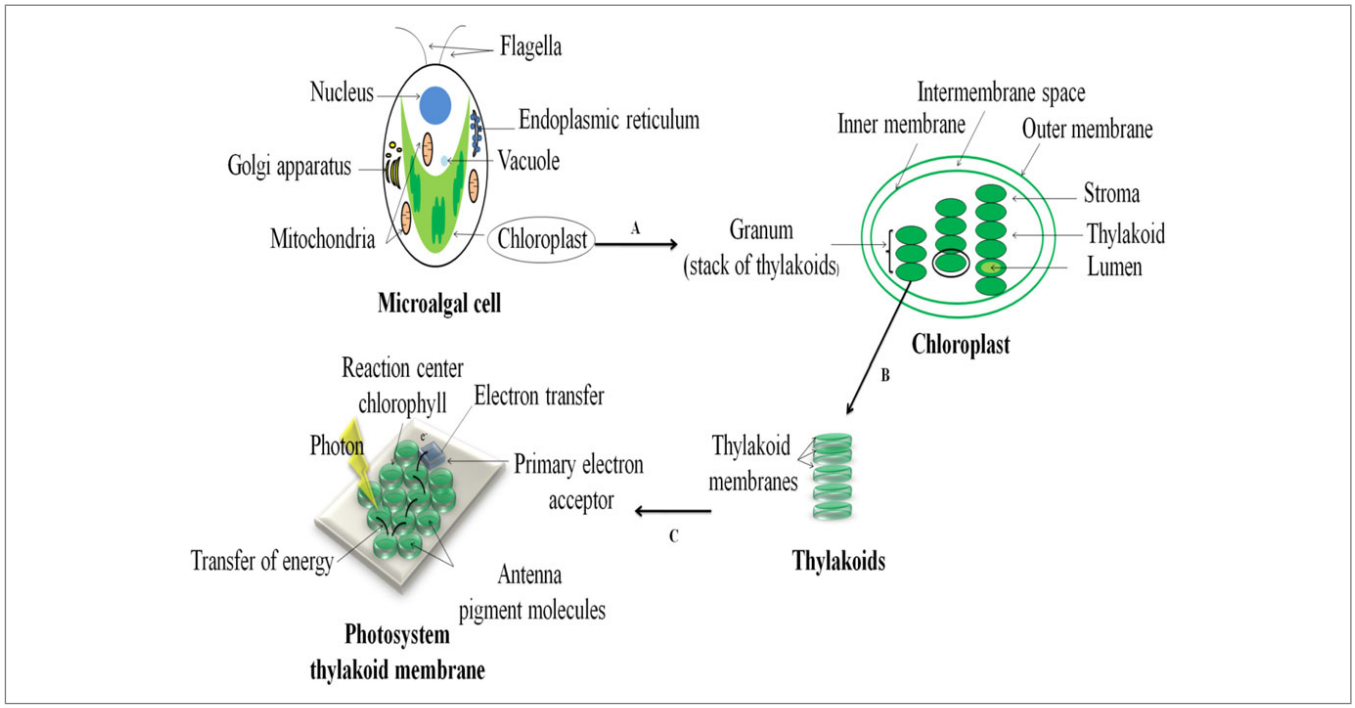


Figure 1–Photosynthesis in microalgal cells.

of inflammation, and a quotidian addition of *Spirulina* is able to decrease allergies (Rasmussen and Morrissey 2007). Furthermore *Dunaliella,* at an industrial-scale, can produce more protein compared to terrestrial harvests (about 100 X greater yield). Peptides extracted from *Chlorella vulgaris* have important preventive impacts on cellular damage (Lordan and others 2011).

Lipids and fatty acids

The total oil or fat content of microalgae ranges from 1% to 70% of the dry weight and tends to be inversely proportional to the rate of growth with greater accumulations during the stationary phase (Spolaore and others 2006). Microalgal lipids can be divided in 2 groups; storage lipids (nonpolar lipids mainly triacylglycerides) and structural lipids (polar lipids such as phospholipids and sterols) (Sharma and others 2012). Algal species have different fatty acid profiles, which depend on various parameters: age, growth stage, and environmental conditions (Metting 1996).

Biodiesel can be produced from algal biomass, since microalgae accumulate lipids, especially triacylglycerides (TAGs) within their cells (100 times more fat per acre than terrestrial plants). TAGs could be used as starting material for high-energy-density fuels such as biodiesel (transesterification of TAGs to yield fatty acid methyl esters) and green gasoline (combination of hydroprocessing and catalytic cracking to yield alkanes) (Pienkos and Darzins 2009; Mubarak and others 2015).

Sterols are another group of interesting lipids from algal sources. Sterols and some of their derivatives were determined to have anti-inflammatory and anti-oxidative activities and to participate in lowering LDL cholesterol levels *in vivo* (Fernandes and Cabral 2007). Moreover, phytosterols (C_{28} and C_{29} sterols) are important precursors of compounds including vitamins. For example, ergosterol is a precursor of vitamin D_2 and cortisone (Ibañez and others 2011; Francavilla and others 2013).

Polyunsaturated fatty acids (PUFAs) with more than 18 carbons are not synthesized by humans. Therefore, they have to obtain them from an exogenous source (food). PUFAs are essential for the development and the physiology and they have been proven to have health benefits, such as blood pressure maintenance, blood coagulation, and effects on the functions of the brain and the nervous system. They also reduce the occurrence of various chronic diseases, including diabetes, arthritis, cardiovascular disease, and obesity (Wall and others 2010; Kris-Etherton and others 2003). Currently, fish and fish oil are the main sources of PUFAs, but their uses as food additives are limited due to the possible accumulation of toxins, fish odor, unpleasant taste, and poor oxidative stability (Brennan and Owende 2010). Therefore, microalgae have more advantages over fish oils. Besides, they are a primary source of PUFAs as they supply the whole food chains with these vital components (Brennan and Owende 2010; Monroig and others 2013). PUFAs in microalgae could be omega-3 (n-3) and omega-6 (n-6) fatty acids, specifically eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (AA), and γ-linolenic acid (GLA) (Sastre 2012).

Long-chain PUFAs are manufactured via microalgal cultivation and added to infant milk formulations. Besides PUFAs could be use as dietary supplements and food additives. Moreover, hens are fed with special microalgae like *Schizochytrium* and *Crypthecodinium* to produce "OMEGA" eggs. Such applications have proved to be profitable (Pulz and Gross 2004).

The major components of the lipidic parts of *Chlorella vulgaris* are oleic, palmitic, and linolenic acids (Mendes and others 1995). The green microalga *Haematococcus* contains short-chain fatty acids with antimicrobial activity (Rodríguez-Meizoso and others 2010). Concerning *Spirulina*, it represents a good source of γ-linolenic acid, which is a precursor of prostaglandins, leukotrienes, and thromboxans, all implicated in the regulation of cardiovascular, inflammatory, and immunological responses. This Cyanobacterium represents also a natural source of active fatty acids such as palmitic, lauric, and oleic acids, and the n-3 fatty acid docosahexaenoic acid (DHA). Clionasterol has been found in *Spirulina*. This

Table 1–Health benefits of some of the bioactive compounds produced by microalgae.

Bioactive compounds	Conditions of accumulation	Source	Health benefits	References
Proteins, peptides, and amino acids	Decreased temperature and light intensity Nutrient sufficiency	*Spirulina* *Chlorella vulagris*	• Reduce brain damage • Anti-inflammatory activity • Antioxidant activity • Reduce allergy symptoms • Protective effect against cellular damage	Rasmussen and Morrissey 2007; Lordan and others 2011; Juneja and others 2013
Lipids and fatty acids	Nutrient starvation (N, P, S, and Si) Temperature Salinity pH Heavy metals stress (Cd, Fe, Cu, and Zn) Light irradiation	*Haematococcus* *Spirulina*	• Antimicrobial activity • Implicated in the regulation of cardiovascular, inflammatory, and immunological responses • Dietary supplements	Rodríguez-Meizoso and others 2010; Lordan and others 2011; Sharma and others 2012
Polysaccharides	Nitrogen and phosphorus limitations	*Chlorella pyrenoidosa* *Chlorella ellipsoidea* Porphyridium *Nostac flegelliforme*	• Immunostimulating activity • Antimicrobiall effects against Listeria monocytogenes and Candida albicans • Free-radicals scavenger • Antiviral activity against Herpes simplex virus (HSV-1 and 2)	Pugh and others 2001; Bin and others 2013; Hasegawa and others 1995; Mata and others 2010; Spolaore and others 2006; Kanekiyo and others 2007; Vo and others 2011; Juneja and others 2013
Photosynthetic pigments β-carotene	Increased temperature and UV radiation Heavy metal sufficient medium	*Dunaliella salina*	• Natural food colorant • Provitamin A • Antioxidant additives for pharmaceutical, cosmetic and nutraceutical industries • Natural pigments for the aquaculture industry	Stahl and Sies 2003; Hejazi and Wijffels 2003; Ben-Amotz and Avron, 1990; Garcia-Gonzalez and others 2005; García-Chavarría and Lara-Flores 2013; Juneja and others 2013
Astaxanthin		*Haematococcus pluvialis*	• Natural red colorant for fish tissue (farmed salmon flesh) • Anticancer activity • Anti-inflammatory activity • Photoprotective effect • Improve immune response	Guerin and others 2003; Shields and Lupatsch 2012; Juneja and others 2013
Phycocyanobilin	Nutrient sufficiency	Spirulina	• Hepatoprotective and anti-inflammatory activities • Protective against neuro-degenerative diseases, gastric ulcers, and cancer • Antioxidant properties used as natural colorants in cosmetology and in the food industry	Ge and others 2006; Burtin 2003; Lordan and others 2011; Elias and others 2008; Juneja and others 2013
Phycoerythrobilin		*Porphyridium*		

sterol has been reported to reduce the generation of plasminogen-activating factor in vascular endothelial cells (Lordan and others 2011).

Diatoms are other examples that generally contain high levels of EPA (15% to 30% of total fatty acids) and no DHA. Besides, dinoflagellates have high potential for use in the commercial production of DHA, which ranges from 12% to 51% of total fatty acids (Rasmussen and Morrissey 2007).

Polysaccharides

Carbohydrates in microalgae can be composed by glucose, sugars, starch, and various polysaccharides (Saha and others 2015). Microalgae can be easily digested. Therefore there is no restriction in using them in the food and feed sectors (Spolaore and others 2006; Becker 2008).

Lately, research and development regarding production of polysaccharides from microalgae instead of macroalgae have increased. Microalgae can be used as a substitute to avoid problems such as raw-material shortages or pollution occurring during macroalgal production (Sastre 2012).

Cyanobacteria are considered by some to be a promising source of exocellular polysaccharides (EPSs) (De Philippis and Vincenzini 1998). EPSs are important in food industry as emulsion stabilizers, gelling agents, and inhibitors of crystal formation (in frozen foods). Moreover they are used in water clarification as flocculants, in beer and fire-fighting fluids as foam stabilizers, and in cosmetics and pharmaceuticals as hydrating agents (Jain and others 2005). Certain species of cyanobacteria are known to contain large amounts of released EPSs, which consist of a relatively large number of monosaccharides and display unique and rare properties. Cyanobacterial polysaccharides have demonstrated their potential to be used for the stabilization of emulsions or as thickening agents (De Philippis and others 2001).

Moreover, the biological activities of some microalgal species have been associated with polysaccharides (Raposo and others 2013). Polysaccharide complexes from *Chlorella pyrenoidosa*, and possibly *Chlorella ellipsoidea*, contain glucose and any combination of galactose, rhamnose, mannose, arabinose, *N*-acetylglucosamide, and *N*-acetylgalactosamine. These complexes are believed to have immunostimulating properties (Pugh and others 2001; Binand

others 2013) and can inhibit the proliferation of the pathogen *Listeria monocytogenes* and the fungus *Candida albicans* (Hasegawa and others 1995; Mata and others 2010).

β-1,3-Glucan is another polysaccharide from *Chlorella*; it is an active immunostimulator, a free-radical collector, and it is able to reduce blood lipids (Spolaore and others 2006). Diatoms such as *Skeletonema* can store high amounts of β-1,3-glucan during the stationary growth phase (Granum and others 2002). Furthermore, polysaccharides extracted from microalgae such as *Porphyridium* and *Nostac flegelliforme* showed potent effect against Herpes simplex virus (HSV-1 and 2) both *in vitro* and *in vivo* (Kanekiyo and others 2007; Vo and others 2011).

Algal polysaccharides have also other pharmacological properties. The results of screening programs to test *in vitro* immunologically relevant effects of polysaccharides from microalgae have shown that certain highly sulfated polysaccharides can trigger either the cellular or the humoral stimulation of the human immune system (Pulz and Gross 2004).

Photosynthetic pigments as antioxidants and natural colorants

Free radicals such as superoxide anions ($O_2.^-$) or hydroxyl radicals ($^{\bullet}HO$) are highly reactive, with high oxidizing potency, causing irreversible damage to human fat tissue, genetic material, and cell membranes. For stopping the nocif effects of these free radicals there is a need of antioxidants, which could be any substance that is able to inhibit oxidation when present at low concentrations in comparison to an oxidizable substrate (Jun and others 2004).

Microalgae, due to their phototrophic life, are exposed to high oxygen and radical stress. But the lack of oxidative damage in their structure suggests that their cells have protective antioxidative systems (Jiménez-Escrig and others 2001; Pulz and Gross 2004).

Photosynthetic pigments are classified as 3 groups: carotenoids, phycobilins, and chlorophylls. They are used by autotrophs (plants, algae, and cyanobacteria) to capture solar energy for photosynthesis (Rasmussen and Morrissey 2007).

Carotenoids are a family of natural pigments that are widely distributed in nature. There are more than 600 known carotenoid structures described in the literature. They are potent antioxidants (Krinsky 1989) and they have the ability to act as provitamin A (they can be converted into vitamin A by the human body). Carotenoids possess other bioactivities, for instance, they have protective activity against cancer, aging, ulcers, heart attack, and coronary artery disease (Ibañez and others 2011). They are also commonly used in food products as food colorants.

The most common carotenoid is β-carotene extracted from *Dunaliella salina*. It is mainly used in the food industry as a natural food colorant and as provitamin A. β-carotene can also be used in pharmaceutical industries to provide antioxidant activity for cancer prevention (Stahl and Sies 2003). Additionally, β-carotene can be used in the cosmetic and nutraceutical industries as an antioxidant additive and in the aquaculture industry as a natural pigment in fish tissues and as pro-vitamin A for animal feed (Ben-Amotz and Avron 1990; Hejazi and Wijffels 2003; Garcia-Gonzalez and others 2005; García-Chavarría and Lara-Flores 2013).

The second most important carotenoid from microalgae is astaxanthin, found in the green microalga *Haematococcus pluvialis*. It is used in aquaculture as a natural red colorant for farmed salmon flesh. Pharmaceutical products that contain astaxanthin are also found in the market (Sastre 2012). With an antioxidant activity up to 10 times stronger than other carotenoids (including β-carotene, zeaxanthin, canthaxanthin, and lutein) and 100 times greater than those of α-tocopherol (Miki 1991), astaxanthin provides protective activity against cancer, inflammation, UV light, and it also improves immune responses (Guerin and others 2003). Other carotenoids such as lutein, zeaxanthin, and canthaxanthin, produced in less important quantities, are used in animal feed and for pharmaceutical purposes (Rasmussen and Morrissey 2007; Sastre 2012).

The phycobiliproteins are protein-pigment complexes. Those pigments can be either phycocyanobilin (blue pigment) or phycoerythrobilin (red pigment). They are produced on a large scale from *Spirulina* (cyanobacterium) and *Porphyridium* (red microalga). They are partially responsible for many properties, mainly hepatoprotective and anti-inflammatory activities (Ge and others 2006), which could prevent many diseases (gastric ulcers, neurodegenerative diseases, and cancer) (Burtin 2003). Phycobiliproteins could be used for some immunological methods (fluorescent immunoassays and fluorescent immunohistochemistry) since they are spontaneously fluorescent compounds (Kronick 1986; Aneiros and Garateix 2004). They have also antioxidant properties and they can be used as natural colorants in cosmetology (eye liner and lipstick), and in the food industry (chewing gum, ice sorbets, popsicles, candy, and milk products) (Elias and others 2008; Lordan and others 2011).

Chlorophylls are green pigments that are found in photoautotrophic organisms (plants, algae, and cyanobacteria). This pigment is primarily used in the food industry as a natural colorant in foods and beverages. Additionally, chlorophylls and their derivatives exhibit anticancer activity (Hosikian and others 2010). Although the majority of industrial chlorophylls are extracted from vegetable sources, there is a growing interest in developing the biotechnological tools necessary for the production of chlorophylls from microalgae (Rasmussen and Morrissey 2007).

Vitamins

Vitamins are needed in the human body for different chemical and physiological processes. Vitamins are classified as water-soluble (group B vitamins and vitamin C) and fat-soluble vitamins (provitamin A and vitamins E, D, and K) (Skrovankova 2011).

Microalgae represent a valuable source of nearly all vitamins (A, B_1, B_2, B_6, B_{12}, C, E, nicotinate, biotin, folic acid, and pantothenic acid) (Lordan and others 2011). Those compounds enhance the nutritional value of microalgae, but their amount changes depending on many parameters (species, geographic area, season, environmental parameters, the harvesting procedure and the method of drying the cells) (Norziah and Ching 2000).

Extraction of Metabolites From Microalgae

Different methods exist for the extraction of specific components from microalgal biomass, including mechanical, chemical, and enzymatic methods. Each approach has its advantages and its drawbacks (Table 2).

Mechanical methods

Several methods have been used to recover bioactive compounds from microalgae. Expeller pressing or oil pressing is one of the simplest and oldest method used for extracting oil from seeds. It has also been applied for algal biomass. This technique consists of applying high mechanical pressure in order to break the cells and to squeeze out the oil from the biomass. However, this technique possesses several disadvantages including heat generation and choking problems which will decrease lipid recovery (Ramesh 2013). Another mechanical method called bead-beating has also been

Table 2–Advantages and limitations of extraction methods of microalgal bioactive compounds.

	Mechanical methods			Chemical methods		Enzymatic methods
	Expeller press	Bead-beating	Ultrasound	Use of solvents	Supercritical fluid extraction	Enzyme hydrolysis
Advantages	• Simple to use	• Biomass dehydration is not needed	• Not much thermal denaturation of compounds • No removing step of beads/chemicals in later process step	• Simple to use • Traditional method	• More efficient than liquid solvents • Not toxic	• Rapid • Effective • Specific
Drawbacks	• Heat generation • Choking problems	• Difficult to scale up • Use of a cooling jacket due to heat generation	• Generation of cell-damaging heat when prolonged	• Toxic • Not ecofriendly	• Requires high pressures • Costly process	• Enzymes are costlyIndustrial utilization is limited
References	Ramesh 2013	Ranjith Kumar and others 2015	Pernet and Tremblay 2003	Cuellar-Bermudez and others 2015	Ibañez and others 2011; González-Delgado and Kafarov 2011	Ranjith Kumar and others 2015

applied. This approach consists of direct damage to the cells caused by high-speed spinning of the biomass slurry with fine beads. Various beads are used for different types of cells. The optimal bead diameter for microalgae cells is 0.5 mm. Algal biomass dehydration is unnecessary, contrary to that of the expeller press method, which reduces the processing costs. However using bead-beating can be difficult to scale up and requires the use of a cooling jacket in order to prevent degradation of the final product due to heat generation by the rotating agitator inside the culture vessel (Ranjith Kumarand others 2015). The ultrasound is another technique, which consists of exposing the microalgae to sound waves of a specific frequency to destroy the cell wall (Pernet and Tremblay 2003). The advantage of sonication consists of generating only low temperatures. Thus it leads to less thermal denaturation of biomolecules. Besides, it is much more economical since there are no additions like beads or chemicals that would have to be removed later in the process. However, prolonged ultrasonication can generate heat that could be undesirable (Al Hattab and Ghaly 2015).

Chemical methods

Hexane, hexane-isopropanol, and chloroform-methanol are usually the most used solvents for lipid extraction. The adequate solvent, or mixture, for the extraction is chosen based on the polarity and/or solubility of the lipid content. A polar solvent could not extract lipids, while it is able to extract other microalgal components such as sugars, pigments, and amino acids. However, solvents are known not to be very environmentally friendly due to their toxicity (Cuellar-Bermudez and others 2015). Supercritical fluids are largely employed for the extraction since they are more effective than the traditional liquid solvents. Supercritical fluid extraction (SFE) is based on the use of solvents at temperatures and pressures above their critical points. One of the most valuable characteristics of SFE is the reduced employment of toxic organic solvents. Carbon dioxide (CO_2) is the most commonly used solvent to extract bioactive compounds. Supercritical CO_2 is nonflammable, nontoxic, and relatively inert. Other solvents have been proposed for SFE, including propane, butane, and dimethyl ether, but CO_2 remains the preferred solvent of choice (Ibañez and others 2011). SFE has been used with various species of microalgae to obtain different substances such as omega-3 fatty acids and pigments. SFE, compared to conventional liquid extraction, is expensive due to its requirement for high pressures which increases its cost (González-Delgado and Kafarov 2011).

Enzymatic methods

The cell wall of microalgae can be degraded with enzymes (sanilase, cellulase, protease, papain, lysozyme, and trypsin), which make the recovery of algal bioactive components much more rapid and effective. This method is advantageous since it is highly specific, which makes it desirable for specific by-products. However, enzymes can be costly which limits their use on an industrial scale (Ranjith Kumar and others 2015).

Microalgal Biomass Production

The biotechnological applications of microalgae have emerged due to the great diversity of products that can be recovered from the biomass. Microalgal cultivation on a large-scale has been studied for several decades (Lee 2001). The first unialgal cultivation was carried out with the microalga *Chlorella vulgaris* by Beijerinck (1890), who wanted to study the physiology of the organism.

Early attempts to increase algal cultivation using open ponds happened during World War II in Germany, where the green algae *Chlorella pyrenoidosa* and *Scenedesmus* and the diatom *Nitschea palea* were mass-cultured and their lipids were investigated. Back then, algae were cultivated mostly as food supplements. In the Carnegie Institute of Washington, as industrialization started, they realized the mass cultivation of algae for CO_2 remove from the environment (von Witsch and Harder 1953). In the 1970s, the manufacturing of algae for food started in East Europe and Japan in open ponds (Ugwu and others 2008; Vieira Costa and Greque de Morais 2013). With time, algal biomass became very important in many fields, including human nutrition (Mazo and others 2004), animal feed production, aquaculture (Lum and

Figure 2–Aztec harvesting *Spirulina* from lakes in Mexico. Drawing in Human Nature, March 1978, by Peter T. Furst.

Figure 3–Kanembu women harvesting *Spirulina* from Lake Boudou Andja, in Chad. Photo by Marzio Marzot from the FAO Report: The Future is an Ancient Lake, 2004.

others 2013), and for the pharmaceutical, nutraceutical, and cosmetic industries (Yaakob and others 2014).

There are 3 types of metabolism in microalgae: photoautotrophic, heterotrophic, and mixotrophic (Kunjapur and Eldridge 2010). Photoautotrophic production is autotrophic photosynthesis in which microalgae use light as the only energy source. Heterotrophic metabolism requires organic substances such as glucose to stimulate growth, and some algal species can combine autotrophic photosynthesis and heterotrophic assimilation of organic compounds in a mixotrophic process (Chojnacka and Noworyta 2004; Chojnacka and Zielińska 2012).

Large-scale microalgal culture can be classified into open systems, where the culture is directly exposed to the environment, and closed systems, where the culture is entirely enclosed within the culture container (Dormido and others 2014).

Open systems (ponds)

Algal cultivation in open pond production systems has been used since the 1950s (Brennan and Owende 2010). These systems can be divided into natural ponds and artificial ponds.

Natural ponds. Some natural environments have been used for the production of algal biomass. When microalgae find suitable climatic conditions and sufficient nutrients they grow profusely. The oldest records are from the Aztecs who used to harvest *Arthrospira* (*Spirulina*) from Lake Texcoco in present-day Mexico (Figure 2) (Borowitzka and Moheimani 2012).

There are many examples of eutrophic lakes or small natural basins that have been exploited for microalgal production. For instance, along the northeast border of Lake Chad, numerous temporary or permanent lakes can be found where the chemical compositions of the aquifer (high salinity, high pH, and high nutrient concentrations) create appropriate conditions for *Arthrospira* (*Spirulina*) growth. These lakes are highly productive natural systems and some of them are exploited by the Kanembu people (Figure 3) such as Lake Kossorom in which the bloomed biomass is harvested and used as food (Abdulqader and others 2000). In Myanmar (Burma), *Arthrospira* grows throughout the year in 4

Figure 4–*Spirulina* cultivated in *El Caracol* in Lake Texcoco near Mexico City.

old volcanic craters filled with alkaline waters, and it is harvested by simple filtration (sieving) during the growing season (Thein 1993). Moreover, in the 1970s and 1980s Sosa Texcoco Co. cultivated *Arthrospira maxima* in the outer parts of a spirally shaped solar evaporator, the *caracol* (Spanish word for snail) located near Mexico City (Figure 4) (Godinez and others 2001). *Dunaliella* is also produced in 2 lagoons in Australia: Hutt Lagoon (Western Australia) and Whyalla (South Australia) (Figure 5) (Tredici 2008).

Artificial ponds.

Circular ponds. Circular ponds are the oldest large-scale algal culture systems. They are not favored in microalgal production since they require expensive concrete construction and high energy input for mixing by a rotating arm mounted at the center of the pond. Those devices are, nevertheless, widely used in Japan

Figure 5–Hutt lagoon in Australia and its pink color due to *Dunaliella salina* pigments (β-carotene). Photo by Samuel Orchard.

Figure 6–Yaeyama on Okinawa Island, Southern Japan, where *Chlorella* algae are grown in circular ponds.

(Figure 6), Taiwan, and Indonesia for *Chlorella* biomass production (Lee 1997; Tredici 2008).

Raceway ponds. Raceway ponds (Figure 7) are the most widely used systems for the commercial production of microalgae. This is so because, generally, they are the cheapest to construct and their function is quiet simple (Borowitzka and Moheimani 2012; Enzing and others 2014). They are typically constructed of a closed loop and have oval-shaped recirculation channels, in which flow is guided around bends by baffles placed in the flow channel, and they are stirred with a paddlewheel to ensure some sort of homogenization of the culture. The paddlewheel is in continuous operation to prevent sedimentation. Raceways may be constructed of concrete, glass fiber, or a membrane (Borowitzka and Moheimani 2012).

Closed systems (Photobioreactors)

Photobioreactors (PBRs) are reactors in which phototrophs (microbial, algal, or plant cells) are grown or used to carry out photobiological reactions (Tredici 2008). PBRs represent a good alternative because most microalgae cannot be kept long enough in outdoor open systems due to the hazard of contamination by molds, bacteria, and protozoa, and competition from other microalgae (Mendes and Vermelho 2013). PBRs offer a closed-culture environment, which is protected and relatively safe from invasion by competing microorganisms and where conditions are better controlled (Patil and others 2005). They may be located indoors or outdoors, but since using free sunlight is better, outdoor locations are more common. PBRs can be classified into tubular, vertical column, and flat-plate PBRs (Dragone and others 2010).

Tubular photobioreactors (TPBRs). A tubular PBR is probably the most popular configuration of PBRs (Figure 8). It consists of an array of straight transparent glass or plastic (polypropylene, acrylic, or polyvinylchloride) tubes. The tubular display catches sunlight (solar collector) and can be aligned horizontally, inclined, vertically, or as a helix. In TPBRs continuous culture operation is used. Indeed, microalgal broth is circulated from a reservoir to the solar collector and back to the reservoir. The ground beneath the solar collector is often painted white, or covered with white sheets of plastic to increase reflectance. Biomass sedimentation in tubes is prevented by maintaining highly turbulent flow that is produced by using either a mechanical pump or an airlift pump (Chisti 2007;

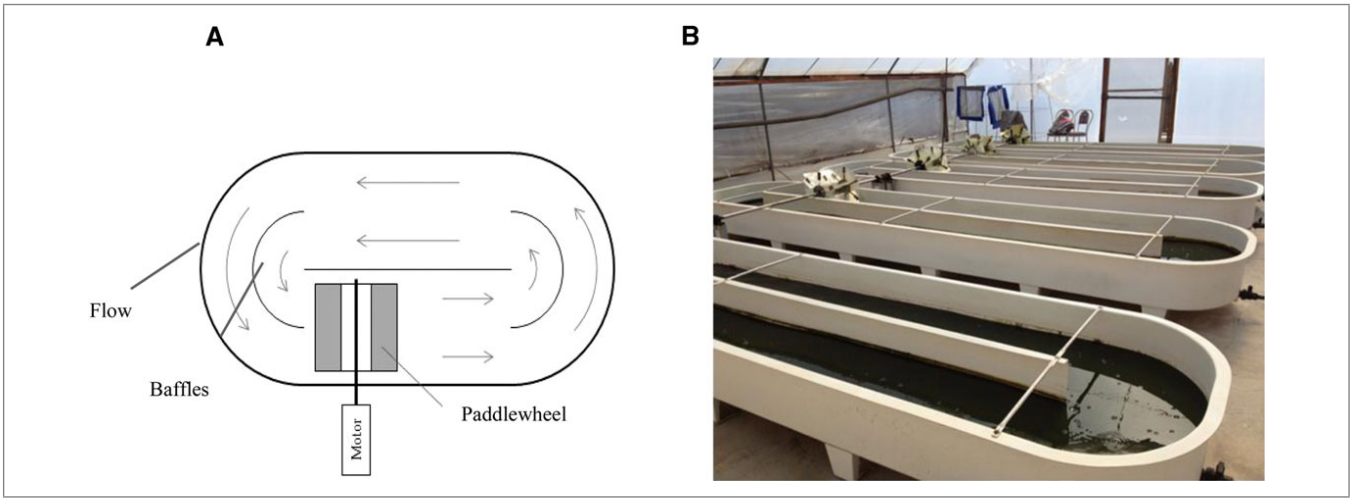


Figure 7–Raceway pond. (A) Schematic representation. (B) Photo taken at the Microalgal Biotechnology Lab of the Faculty of Fisheries of Çukurova University in Adana, Turkey.

Figure 8–Tubular Photobioreactor. Photo taken at the Microalgal Biotechnology Lab of the Faculty of Fisheries of Çukurova University in Adana, Turkey.

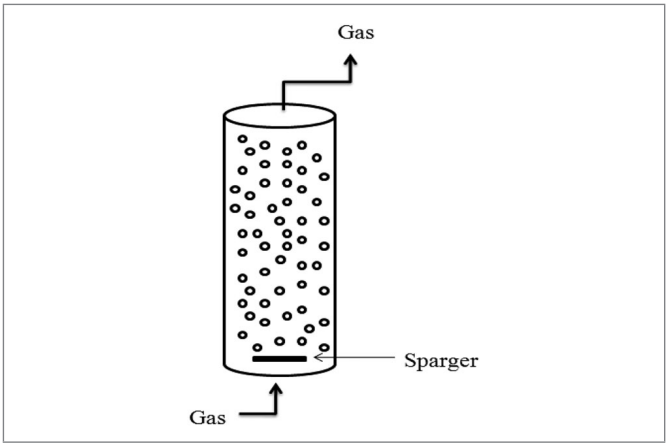


Figure 9–Vertical column photobioreactor.

Ugwu and others 2008; Brennan and Owende 2010; Wang and others 2012).

Vertical column photobioreactors. A vertical column photobioreactor (Figure 9) is composed by a vertical tube (glass or acrylic) that is transparent to permit the penetration of light. A gas sparger is placed at the bottom of the reactor. There is no physical agitation system implemented in the design of a vertical column photobioreactor. Consequently very little cell damage is associated with this PBR. This type of reactor can be categorized as a bubble column or an airlift reactor based on the liquid flow patterns inside the photobioreactor (Carvalho and others 2006).

Bubble column reactors are cylindrical vessels. The gas bubbling upward from the sparger allows the gas transfer and the mixing of the culture. In airlift reactors there is a container with 2 interconnecting zones. The first zone is constituted by a gas riser, in which the gas mixture flows upward to the surface from the sparger. The second region is formed by the downcomer, in which the medium flows down toward the bottom and circulates within the riser and the downcomer (Kumar and others 2011; Wang and others 2012; Yen and others 2013).

Flat-plate photobioreactors (FP-PBRs). FP-PBRs are some of the earliest forms of closed systems. Their construction dates back to the early 1950s. They have received much attention due to their panels' large surface area exposed to illumination (Brennan and Owende 2010). The simplest FP-PBR consists of translucent thin rectangular boxes, vertically disposed, opened at one end, illuminated on both sides, and stirred by aeration (Figure 10). The plate surface is made of a transparent material, usually glass or optical light film, for maximum utilization of solar light (Yen and others 2013). FP-PBRs have many advantages, such as suitability for outdoor cultivation and easiness to clean. However, they have also their limitations, including difficulties to scale up and to control the temperature (Hafez and others 2014; Sharma and others 2014). Recently, a new design of a vertical flat panel photobioreactor, consisting of a transparent plastic bag located on a rigid frame, has been proposed; this kind of reactor reduces the equipment cost (Sierra and others 2008).

Figure 10–Flat-plate photobioreactor. Photo taken at the Microalgal Biotechnology Lab of the Faculty of Fisheries of Çukurova University in Adana, Turkey.

Hybrid systems

Cultivation in a hybrid system is a method that combines 2 growth stages in 2 different systems (open and closed). This hybrid photobioreactor is designed to utilize the advantages of both systems (Adesanya and others 2014). The first stage of cultivation occurs in a photobioreactor where conditions are controlled to minimize contamination by microorganisms. The second step consists of growing the culture in an open pond. In this step the cells are exposed to a certain nutritional stress in order to increase the synthesis of a specific metabolite (lipid, protein, or carbohydrate) (Brennan and Owende 2010; Vieira Costa and Greque de Morais 2013).

New technologies

Inspired by the aforementioned PBRs, different technologies have been developed in order to improve some parameters such as light-capturing and light distribution by spectral shifting and internal illumination, mass transfer by membrane PBRs, and construction costs by use of plastic bag PBRs (Wang and others 2012).

Internally illuminated PBRs. Some photobioreactors can be internally illuminated with fluorescent lamps (Figure 11). Such a PBR is equipped with impellers for agitation of the algal culture. Air and CO_2 are supplied to the culture through spargers. This type of PBR can be modified in such a way that it can utilize both solar and artificial light systems. The artificial light is turned on whenever the solar light intensity is reduced to below a certain value during cloudy weather or at night. Therefore, supply of light can be sustained continuously (both day and night) (Ugwu and others 2008; Wang and others 2012).

Spectral shifting. PAR (photosynthetically active radiation) with the spectral range of 400 and 700 nm can be used by

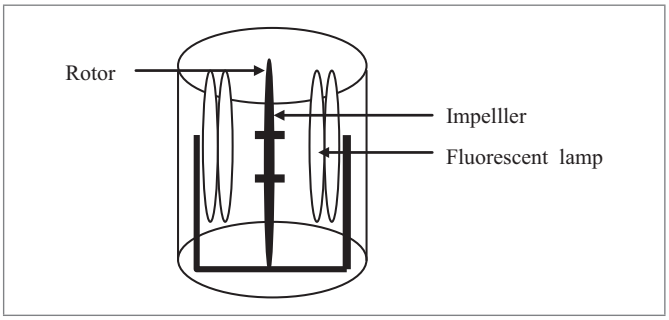


Figure 11–Schematic diagram of an internally illuminated photobioreactor.

photosynthetic organisms in the process of photosynthesis and it represents 50% of the total sunlight (Hall 1982).

This sets a natural barrier for the efficiency of photosynthesis. Furthermore, the radiance that is outside of the PAR range is the primary reason for temperature increase during cultivation, and non-PAR light of some frequencies such as UV is damaging to cells (Frohnmeyer and Staiger 2003). Studies have been conducted to increase the amount of PAR using spectral shifting, for example by using dyes that are capable of absorbing non-PAR and emitting fluorescent light that is PAR, which is better suited for algal growth (Prokop and others 1984). This technology was demonstrated to be capable of elevating the overall photosynthetic efficiency (Wang and others 2012).

Membrane PBRs. Membrane PBRs use the large surface areas provided by membranes to ease gas/liquid mass transfer or to allow long stable production periods by separating extracellular metabolites continuously. For example, the reactor could be equipped uniformly by hollow fiber membranes to function as a gas sparger by producing small bubbles. Another PBR coupled with an ultrafiltration system (immersed membranes) was tested for the continuous cultivation of the microalga *Haslea ostrearia* in order to enhance pigment (marennine) production and recovery. This system is interesting commercially because the energy costs were minimized and no shear stress due to pumping or circulation was applied on the cells (Lehr and Posten 2009; Wang and others 2012).

Offshore membrane enclosures for growing algae (OMEGA). OMEGA is a system of floating PBRs anchored offshore in protected bays. Effluents discharged from existing wastewater outlets enter the PBRs to provide water and nutrients required for microalgal growth. This approach possesses many advantages including the nonuse of terrestrial resources because of the offshore placement and the proximity to wastewater treatment installations; there is no need to pump the discharged water long distances. Therefore, the OMEGA system avoids competing with agriculture for water, fertilizer, and land. Besides, the OMEGA system significantly reduces the process cost compared to conventional PBRs, since cooling and structural supports are provided by the ocean. This system on a large scale could improve coastal water quality by removing nutrients from the incoming wastewater, and it can form artificial reefs which increase local species diversity by forming a habitat for an extensive community of marine organisms. However, the technical feasibility and performance of the OMEGA concept still have to be evaluated at various scales (Wiley and others 2013).

Each of the systems mentioned above has advantages and limitations, as shown in Table 3. For instance, compared to closed photobioreactors, an open pond is the cheapest method of large-scale algal biomass production. But, since they are in direct contact with the environment, the algal cultures are easily contaminated by other microorganisms (other algal species and protozoa). Even if PBRs have the advantage of possessing better controlled conditions, the equipment and processes are expensive, which make the mass cultivation of microalgae difficult. Despite the fact that much progress has been made in developing those systems, some further improvements are still required. In order to have efficient mass cultivation of algae, some further steps have to be taken such as maximization of outdoor solar radiation. In addition the improvements should have transparent surfaces, high illumination surfaces, high mass transfer rates, and should also be able to yield high biomass (Ugwu and others 2008). Moreover, design and construction of any system should depend on the type of species, the desired final product, the location, and the total cost of production (Hafez and others 2014).

Harvesting Microalgal Biomass

After microalgal production, the biomass must be harvested, which means separating the solid (biomass) from the liquid (culture medium). There are different methods for biomass harvesting; choosing the best of these technologies is crucial for economic reasons. However, the selection is not that easy, because of the

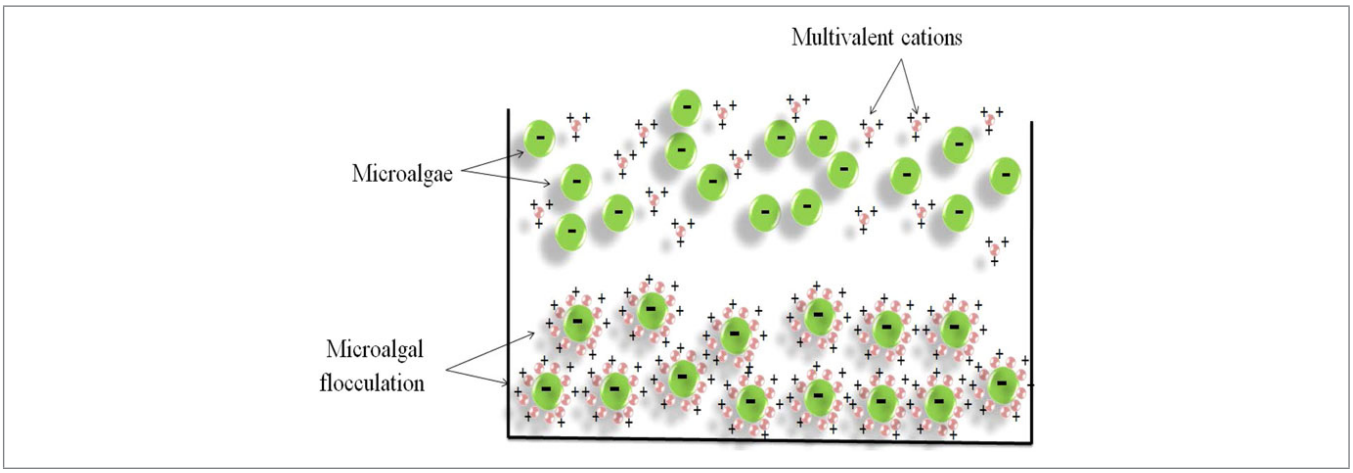
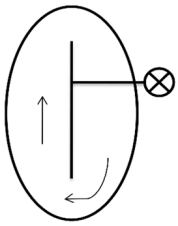
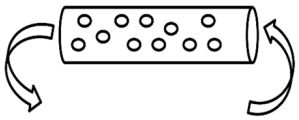
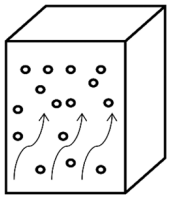
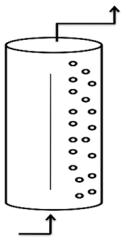


Figure 12–Flocculation method.

Table 3–Some advantages and limitations of open systems and photobioreactors.

Production systems	Shematic diagrams	Advantages	Limitations	References
Raceway pond		Relatively cheap Easy to clean Easy maintenance	Poor productivity Limited to a few strains of algae Cultures are easily contaminated Occupies large land area	Ugwu and others 2008; Mata and others 2010
Tubular photobioreactor		Suitable for outdoor cultures Relatively cheap Good biomass productivities	Fouling Requires large land space	Brennan and Owende 2010; Yen and others 2013
Flat plate photobioreactor		High biomass productivities Easy to sterilize Large illumination surface area	Difficulty to scale-up Difficulty to control the temperature	Hafez and others. 2014; Sharma and others 2014
Column photobioreactor		High mass transfer Easy to sterilize	Small illumination area Expensive compared to open pond	Dragone and others 2010; Lam and Lee 2013

small size of some algal cells (2 to 40 μm) which make the recovery of biomass difficult (Lee and others 2014).

Biomass aggregation

Flocculation. Flocculation is usually used as a preparatory step before other harvesting methods, such as filtration, flotation, centrifugal recovery, or sedimentation (Brennan and Owende 2010). In this method (Figure 12) there is aggregation of the microalgal cells in order to increase the size and to facilitate the other techniques (Mata and others 2010).

Microalgal cells carry a negative charge that prevents aggregation of cells in suspension (negative cells repel other negatively charged cells). The surface charge can be neutralized or reduced by adding flocculants, such as multivalent cations and cationic polymers, to the broth (Muradov and others 2015). The low cost effectiveness in low concentration and nontoxicity are some of the criteria that should be associated with the flocculants. As a nontoxic flocculant chitosan (polymer of acetylglucosamine), an edible flocculant, has shown efficacy in harvesting microalgae (Chen and others 2014). Bioflocculants, which result from the synthesis of extracellular polymers by living cells, are another example of flocculants used for the cells harvesting (Gao and others 2006). It has been known that the bacterium *Paenibacillus* sp. AM49 is able to produce a bioflocculant with good efficiency for harvesting *Chlorella vulgaris* (Grima and others 2003). Sometimes, just by changing the pH of the algal broth or medium, effective flocculation is reached (Guo and others 2013).

Ultrasound. Ultrasound has been used to induce aggregation followed by enhanced sedimentation (Bosma and others 2003). The biggest advantage of ultrasonic harvesting is that it does not produce shear stress on the biomass even if it is used continuously. Shear stress could destroy potentially valuable metabolites (Brennan and Owende 2010).

Flotation

Flotation is different from flocculation because it consists of dispersing micro-air bubbles without adding chemicals. The purpose is to trap algal cells on the surface of the water (Velan and

Saravanane 2013). Some microalgal species naturally float to the surface when their lipid contents increase. Even if flotation is mentioned as a harvesting technique, it is limited technically and economically, since in most cases flocculants are applied to enhance the flotation which results in high operational costs (Gultom and Hu 2013).

Centrifugal recovery

A centrifuge is a sedimentation reservoir with improved gravitational force to enhance the biomass recovery (Chisti and Moo-Young 1999). Centrifugal recovery can be rapid, but the disadvantages of the process are that it requires the use of energy input and maintenance which both have a considerable impact on the cost (Grima and others 2003).

Filtration

Conventional filtration, which can operate under pressure or vacuum, is the most appropriate method for harvesting large (>70 μm) microalgae (*Coelastrum* and *Spirulina*). But the small dimensions of some species of microalgae (<30 μm), close to the size of bacteria, make the use of this method impossible. For that reason, microfiltration (biomass harvesting) and ultrafiltration (isolation of metabolites) are used as an alternative to conventional filtration for recovering those smaller cells (Gultom and Hu 2013). Both methods rely on porous membrane filter media. The basic difference between the 2 operations is the particle size range (Grima and others 2013).

The choice between all the mentioned harvesting methods depends on many characteristics of microalgae, such as size, density, and the desired final products. Centrifugation of microalgal suspensions is often quite efficient but expensive, and the centrifugal effect is responsible for cell damage. Flocculation can also induce toxic effects such as chemical contamination of the biomass. Filtration may be unsatisfactory because it can be relatively slow. For processing of low broth volumes, membrane filtration can be more inexpensive than centrifugation. But in large-scales production, centrifugation may be a more economical method of harvesting due to the cost of membrane replacement and pumping.

For all those reasons, the selection of a harvesting technology is crucial for the economic production of microalgal biomass.

Biomass Dehydration

After separation from the culture medium, algal biomass (5% to 15% dry weight) must be quickly processed or it can spoil in only a few hours in a hot climate (Grima and others 2013). The specific postharvest processes depend strongly on the desired product. Drying of the wet algal biomass is used to extend its shelf-life, especially if the biomass is the final product. Some of the methods that have been used include spray-drying, freeze-drying, and sun-drying (Grima and others 2003; Munir and others 2013).

Spray-drying

Spray-drying is rapid and ideal for the drying of microalgal cultures. This method consists of generating a fine spray of suspension droplets, which are brought into continuous contact with hot air in a large chamber (Figure 13). The result of this operation is a dry powder that settles to the bottom from where it is removed. Spray-drying is the most extended method for dewatering microalgal biomass because of its many advantages (continuous operation, powdered product requiring no further size reduction, and rapid drying, which leads to good product quality) (Mujumdar 2000; Grima and others 2013).

Lyophilization

Lyophilization, known also as freeze-drying, it is the most gentle of all drying methods. The algal biomass to be dehydrated is frozen and the ice crystals are sublimed by slight warming without defrosting. Lyophilization is represented by 3 phases: freezing to solidify the material, drying by sublimation to decrease the moisture to below 20% w/w, followed by a secondary drying to lower the bound moisture to the required final value (often below 1% w/w) (Grima and others 2013).

Sun-drying

This technique is based on the use of solar energy (Figure 14). Thus, it is considered as the cheapest method compared to the other techniques. However, this approach is both weather- and volume- dependent and requires large land areas (Guldhe and others 2014; Milledge and others 2014).

Comparison of the dehydration techniques

Spray drying is the method of choice for high-value operations, but it can cause significant deterioration of some algal components such as pigments. Lyophilization has been widely used for drying microalgae at the laboratory-scale; however, freeze-drying is too expensive for large amounts (Grima and others 2003). Sun drying is the cheapest dehydration method, but the main disadvantages include long drying times and the requirement of large surfaces (Brennan and Owende 2010), also the risk of contamination by animals or wind-blown debris exists.

Safety and Hazard Aspects of Microalgae for Food and Feed Applications

Microalgae can accumulate pesticides, heavy metals, and toxins. Toxins produced by microalgae are potent and represent a serious hazard for human health. Algal toxins accumulate especially in filter-feeding shellfish, such as clams, mussels, oysters, or scallops. This could cause numerous illnesses including neurotoxic shellfish poisoning (NSP), paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), diarrhetic shelfish poisoning (DSP), and ciguatera fish poisoning (CFP). Besides, there is a risk of microbial contamination by pathogens due to the cultivation in open ponds, which are exposed to various animals (birds, insects, and rodents) and the cultivation could also be in an unsuitable location (near industrial or agricultural areas) (van der Spiegel and other 2013; Rzymski and others 2015) . In order to use microalgae for food or feed, it is very important to know their safety. Some algae used in the food and feed sectors have already been given the GRAS status (generally recognized as safe) by FDA (Food and Drug Administration). For instance *Spirulina*, species of *Chlorella*, *Haematococcus pluvialis,* and *Porphyridium cruentum* are widely commercialized and sold as food supplements. Besides, food ingredients found on the market such as, β-carotene from *Dunaliella*, docosahexaenoic acid (DHA) from *Crypthecodinium cohnii*, *Haematococcus pluvialis* extract containing astaxanthin, and *Laminaria japonica* broth and extract powder have already been approved by FDA and EFSA (European Food Safety Authority) (Enzing and others 2014). *Nannochloropsis oculata* could also be used as a source of omega-3 fatty acids, specifically eicosapentaenoic acid (EPA), after an investigation of its safety *in vivo* no toxicity and no mortalities were reported during the study (Kagan and Matulka 2015).

Commercial Applications of Microalgal Bioactive Compounds

The various aforementioned bioactive compounds could be largely used in different industrial sectors (pharmaceutical,

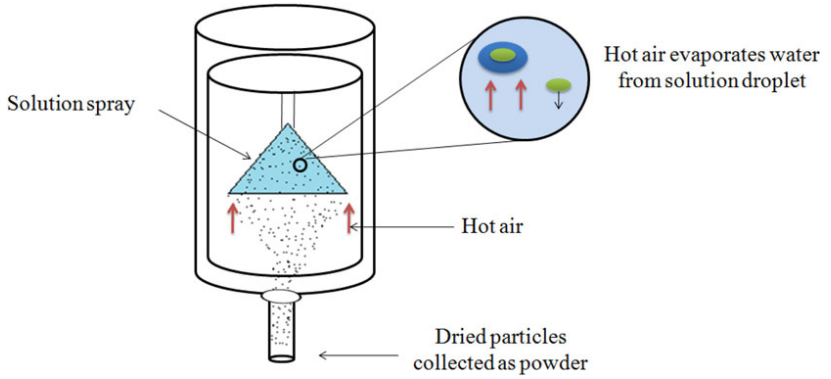


Figure 13–Spray dryer

Figure 14–Women preparing to sun-dry *Spirulina* in a sand filter. Photos by Marzio Marzot from the FAO Report: The Future is an Ancient Lake, 2004.

cosmetic, nutraceutical, feed, and food). After drying the biomass, it will be used as it is or specific components may be extracted. The many uses of microalgae are summarized in Table 4.

Microalgae a source of human nutrition

Around 2400 y ago, Hippocrates set out guidelines for his medical students. One of his principles was "let food be your medicine and medicine be your food." The principle stressed the obvious relationship between food and good health (Chadwick 2003). Modern consumers are increasingly concerned about their health and are trying to control their diet. Numerous illnesses (cholesterol, heart disease, and osteoporosis) have been linked to bad eating habits. Microalgae are now present on the market, dominated by these species: *Arthrospira* (*Spirulina*), *Chlorella,* and *Dunaliella salina* (Brennan and Owende 2010).

Arthrospira (*Spirulina*) could be consumed because of its significant protein content (55% to 70% of total dry weight) and its excellent nutritive value that is also based on the high content of iron and essential unsaturated fatty acids. It is also one of the richest natural plant sources of vitamin B_{12} (Doshi and others 2010). In addition, this microalga has various beneficial effects on health: antihypertensive, prevention of renal failure, improvement of the growth of beneficial intestinal *Lactobacillus* bacteria (Beheshtipour and others 2013).

Spirulina platensis and *Spirulina maxima* are the most popular, in terms of human consumption. For this reason, *Spirulina* has been used as a source of food by many civilizations, among the first the Aztecs who used to harvest this microalga in Lake Texcoco (now Mexico). This genus is still part of the diet of certain population where this microalga grows naturally, for example in the lakes of Chad (Metting 1996; Spolaore and others 2006; Mata and others 2010).

The polysaccharide β-1,3-glucan is the main component in *Chlorella* that could be an active immunostimulator, an antioxidant, and a reducer of the lipid levels in the blood. Other health-promoting effects have been identified (efficacy on gastric ulcers, wounds, and constipation) *Chlorella* can also be administered as a food supplement (Spolaore and others 2006; Mata and others 2010; Sastre 2012).

The β-carotene content, for which *Dunaliella salina* is exploited, can reach up to 14% of dry weight (Tafreshi and Shariati 2009). Under stress conditions of growth, such as high salinity or high temperature, the cells accumulate high amounts of carotenoids and change their color from green to orange (Sastre 2012; Tran and

Table 4–Some industrial applications that use microalgae.

Application fields	Examples of species used	Purpose of utilization	References
Food and nutrition	Spirulina platensis, Spirulina maxima	High protein content High content at iron and essential unsaturated fatty acids Richest sources of vitamin B_{12} Anti-hypertension property Protection against renal failure Better growth of intestinal bacteria (*Lactobacillus*)	Doshi and others 2010; Beheshtipour and others 2013
	Chlorella	Active immunostimulator Free radical scavenger Reduce blood lipids Prevention of gastric ulcers, wounds, and constipation Food additive	Sastre, 2012; Spolaore and others 2006; Mata and others 2010
	Muriellopsis sp.	Accumulate high levels of carotenoids (lutein) Pigment used for the treatment of degenerative diseases	Guedes and others 2011
Animal feeds	Chlorella, Tetraselmis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Spirulina, Dunaliella, Skeletonema, and Thalassiosira	High nutritional value (protein, vitamin, and highly unsaturated fatty acid contents) Zooplankton culture (rotifers, cladocerans, brine shrimp, copepods) Coloring the flesh of salmon, trout, and chickens Coloring the yolk of hen eggs and the exoskeleton and skin of shrimps and prawns Increase the value of aquaculture Improve the physiology and external aspect of pets and farm animals	Hemaiswarya and others 2011; Sirakov and others 2015; Priyadarshani and others 2012; Das and others 2012; Sommer and others 1991; Mata and others 2010; Gouveia and others 2008; Spolaore and others 2006
Fertilizer	Anabaena Nostoc	Improving quality and fertility of soils and retain water Release phosphate, nitrogen, and trace elements Decrease chemical nitrogen demands Production of plant-protecting substances with antiviral and antibacterial activities	Painter 1993; Mandal and others 1999; Hannon and others 2010; Abd El Baky and El-Baroty 2013
Cosmetic	Spirulina Chlorella	Source of bioactive proteins, vitamins, minerals, and pigments Development of skin care and hair-care products Delay wrinkles Skin tightening Stretch-mark repairing effects Collagen synthesis and stimulation Improve tissue regeneration	Kim and Wijesekara 2011; Spolaore and others 2006; Adarme-Vega and others 2012; Yaakob and others 2014
	Nannochloropsis oculata	Inhibition of tyrosinase activity by the pigment zeaxanthin used in whitening creams	Babitha and Kim 2011
Pharmaceutical	*Tolypothrix byssoidea*	Tubercidin production showed activity against P-388 lymphocytic leukemia	Biabani and others 2002
	Chlamydomonas	L-asparaginase inhibits growth of lymphosarcoma in mice	Ahmad and others 2012; Paul 1982

Figure 15–Some commercially available products containing *Spirulina* and *Chlorella vulgaris*.

others 2014). Other microalgal species have been investigated as a nutritional food source. For example, *Muriellopsis* sp., among other microalgae, is able to accumulate high levels of carotenoids, such as lutein, that can be used for the prevention and treatment of certain degenerative diseases (Guedes and others 2011). *Haematococcus pluvialis* produces astaxanthin, a high-value carotenoid that is well known for its antioxidant activity and other properties (anticancer, photoprotection, and anti-inflammatory) (Yuan and others 2011; Dhankhar and others 2012). The U.S. FDA has cleared *Haematococcus pluvialis* for marketing as a dietary supplement; and this specie has also been approved in several European countries for human consumption (Mata and others 2010). *Aphanizomenon flos-aquae*, are another commercial species claimed to be able to promote good overall health (Spolaore and others 2006). Many countries (Germany, France, Japan, U.S., China, and Thailand) have started to market functional foods containing microalgae and cyanobacteria. Different forms are found on the market (Figure 15), for instance capsules and tablets. Microalgae can also be incorporated into pasta, bread, yogurt, soft drinks, snack foods, candy bars, or chewing-gum (Pulz and Gross 2004; Mohamed and others 2013).

Microalgae a source of animal feed

Microalgae can be found in animal feed ranging from aquaculture species (fish, molluscs, and shrimps) to pets and farm animals. Actually, 30% of the worldwide algal manufacturing is sold to the feed industries and more 50% of the world production of *Spirulina* is employed as a feed additive (Spolaore and others 2006; Moradikor and Mohamadi 2015).

In aquaculture the most used species are from the genera *Chlorella*, *Tetraselmis*, *Dunaliella, Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Spirulina*, *Skeletonema*, *Isochrysis*, and *Thalassiosira* (Hemaiswarya and others 2011; Sirakov and others 2015). A microalgal species has to meet different standards before utilization: It has to be readily produced and must be nontoxic. It also needs to possess the appropriate size and shape to be absorbed by organisms. Besides, it has to have high nutritional properties and digestible cell wall material to make the cell's nutrients available. Protein, vitamin, and highly unsaturated fatty acid contents are important factors that determine the nutritional value of microalgae (Priyadarshani and others 2012). Indeed, some fatty acids are essential for many marine animals, including the growth and metamorphosis of many larvae (Patil and others 2005). To get better results by providing better balanced nutrition and improving animal growth, a diet that combines several species from the ones listed above has demonstrated better efficacy than a diet composed of only 1 algal species (Spolaore and others 2006). Microalgae can also be used for culturing several types of zooplankton (rotifers, cladocerans, brine shrimp, or copepods) used as live food in crustacean and finfish farming (Das and others 2012).

Currently, the largest market for astaxanthin is probably salmon and trout for coloring the muscle tissue (Sommer and others 1991). Astaxanthin has been approved in Japan and Canada as a pigment in salmonid feeds. Other animals, such as shrimp, prawns, chickens, and laying hens also benefit from astaxanthin supplementation in their diets (for exoskeleton and skin coloration, muscle tissue, and yolk) (Mata and others 2010).

In order to increase the value of aquaculture species, different methods have been used. For instance, feeds including 5% to 20% *Arthrospira* enhance the red and yellow patterns in carp, while leaving a brilliant white color (Gouveia and others 2008). In addition, the traditional French technique called the greening of oysters, which consists in creating a blue-green color on the gills and labial palps of oysters using the diatom *Haslea ostrearia,* can increase by 40% the product's market value (Hemaiswarya and others 2011).

Microalgae are also added to pet and farm animal diets. In fact, *Arthrospira* is largely used with different types of animal: horses, cats, dogs, aquarium fish, birds, and cows. Algae enhance the physiology (supplying vitamins, minerals, and essential fatty acids, thus boosting the immune system and improving fertility and weight control) and the external aspect (healthy skin and lustrous coat) of animals (Spolaore and others 2006).

Microalgae a source of fertilizer

Microalgae play an important role in the soil ecosystem by improving its quality and fertility. In fact, they have the ability to retain water (important in arid regions), to release phosphate and trace elements from insoluble minerals, and to store nitrogen and release it slowly under field conditions (Painter 1993). Cyanobacteria such as *Anabaena and Nostoc* have already been used as bioferilizer for rice production in tropical and subtropical agriculture due to their ability to perform air-nitrogen fixation (Mandal and others 1999). These biofertilizers improve the biologically fixed nitrogen by reducing chemical nitrogen demands (Sastre 2012). Microalgae can also be a source of bioactive compounds that could be used as plant-protecting substances against diseases caused by viruses or bacteria (Hannon and others 2010; Abd El Baky and El-Baroty 2013).

Microalgae a source of ingredients for cosmetic products

Extracts from microalgae are rich sources of bioactive proteins, vitamins, minerals, and carotenoid pigments such as astaxanthin. Some microalgal species are already well established in the skin care market, the main ones being *Spirulina* and *Chlorella* (Kim and Wijesekara 2011). Applications include facial and body skin care (anti-aging, moisturizing, and regenerant products), shower gels, body lotions, sun screen cream, and hair-care products (Spolaore and others 2006).

It was found that tyrosinase activities were inhibited by the extract of several microalgae. Antityrosinase zeaxanthin from *Nannochloropsis oculata* has been discovered and the extract from this microalga has been used in whitening creams (Babitha and Kim 2011). There are many microalgae-based products available now on the market, such as Protulines® which is an extract rich in proteins from *Spirulina*, helping against first wrinkles and having skin tightening and stretch-mark repairing effects (Adarme-Vega and others 2012). Dermochlorella® is another product containing an extract from *Chlorella vulgaris* that stimulates collagen synthesis in skin cells improving tissue regeneration and combating wrinkles (Yaakob and others 2014).

Microalgae a source of pharmaceutical products

Macroalgae have attracted more attention than microalgae because of their large size and accessibility. But for several decades now, microalgae have received increasing interest because of their bioactive compounds which are important for the pharmaceutical industry (Krishnakumar and others 2013; Pradhan and others 2014). Screening of marine organisms for bioactive components began in the 1970s (Ellinger and others 2014). Early discoveries included tubercidin, a heterocyclic nitrogen compound from the cyanobacterium *Tolypothrix byssoidea,* which showed to have *in vitro* activity against P-388 lymphocytic leukemia (Biabani and others 2002). Another compound, an L-asparaginase from the green

microalga *Chlamydomonas* inhibits growth of lymphosarcoma in mice (Paul 1982; Ahmad and others 2012). Many cyanobacteria have been shown to produce antineoplastic compounds. A large number of microalgal extracts have also been found to have antimicrobial and antiviral activities against Herpes simplex virus types II and respiratory syncytial virus (Borowitzka 1995; Metting 1996).

Biodiesel from Microalgae

Energy production has always been one of our major concerns as it plays a vital role in our lives. Global energy sources are classified into 2 groups, fossil (nonrenewable) and renewable. The world's excessive demand for energy, the oil crisis, the gulf war in 1991, in addition to the reduced availability of petroleum and more severe government regulation on exhaust emission, have made researchers look for alternative solutions of fuel development. Hence, energy sources including solar, wind, geothermal, hydro, nuclear, and hydrogen have been investigated (Demirbas and Demirbas 2010). Biomass is among those renewable energies (McKendry 2002a, 2002b). Biofuels are a renewable energy source produced from biomass, which can be used as a substitute for petroleum fuels. The benefits over traditional fuels include greater energy security and reduced environmental impact (Demirbas 2010).

There are 3 types of generation of biofuels that have been developed. The first generation of biofuels comes from terrestrial crops (maize, sugarcane, rapeseed, and sugar beet) and has many limitations including contribution to water shortages and precipitation of the destruction of forests. The second generation is obtained from forest residues, lignocellulosic agriculture, and from nonfood crop feedstocks. However, its main disadvantage is the land use. Therefore, the third generation of biofuels derived from microalgae, is seen as an alternative for sustainable bioenergy production that can overcome the limitations of first and second generation biofuels (Antizar-Ladislao and Turrion-Gomez 2008; Naik and others 2010; Li-Beisson and Peltier 2013). Therefore microalgae could be a very attractive source of renewable biofuels, especially biodiesel. Many species of microalgae contain high amounts of lipids, including triacylglycerides, which are adequate for the production of biodiesel. Algae also contain carbohydrates which can be fermented to produce ethanol. Algal biomass, like other biomasses, can be converted to biofuels by different processes such as thermochemical conversion (gasification, thermochemical liquefaction, pyrolysis, and direct combustion) and biochemical conversion (anaerobic digestion, alcoholic fermentation, and photobiological hydrogen production) (Borowitzka and Moheimani 2013).

Using microalgal-derived biofuels has many advantages. For example microalgae can be produced throughout the year. Therefore, oil productivity of microalgal cultures surpasses the yield of oilseed crops. In addition, even if they grow in aqueous media, they need less water than terrestrial crops; thus, there is a reduction in the use of freshwater (Dismukes and others 2008). Microalgae are able to accumulate lipids in the range of 20% to 50% dry weight of biomass. Microalgal biomass production can also fixate CO_2 waste and algal cultivation does not require herbicides or pesticides application (Brennan and Owende 2010). Plus, microalgae can be cultivated in wastewater which offers nutrients (phosphorus and nitrogen) that can be used by algae as growth medium; hence, besides from algal cultivation there is a treatment of effluents by removal of contaminants (Chinnasamy and others 2010; Ruiz-Martinez and others 2012).

Genetic Engineering of Microalgae

Microalgae represent a much simpler system for genetic manipulations compared to higher plants, due to the absence of cell differentiation. The aim of microalgal transformation is to improve the production of valuable bioactive compounds (Pulz and others 2001).

Until now, about 30 microalgal species have been successfully transformed. These include green algae (*Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *Volvox carteri*, and *Dunaliella salina*), diatoms (*Phaeodactylum tricornutum* and *Thalassiosira pseudonana*), red algae (*Cyanidioschyzon merolae* and *Galdieria sulphuraria*), brown algae, euglenids, and dinoflagellates (Radakovits and others 2010; Medipally and others 2015). New genes are introduced into the cells by different methods such as glass bead transformation, which consists in agitating cells in the presence of exogenous DNA, glass beads, and polyethylene glycol. This method was successfully applied to the green algae *Chlamydomonas* by Kindle (1990). Electroporation is another transformation technique that requires the delivery of an electrical pulse to cells that opens pores in the membrane through which DNA can pass down a concentration gradient into the cell. Microalgal cells can also be transformed by Biolistic (gene gun), which consists in the propulsion of metal particles (microprojectiles) coated with DNA at high velocities into cells under partial vacuum (He 2004).

Despite those great advances there are certain cautions that have to be taken for various reasons. For instance, the accumulation of valuable substances in algae via genetic transformation can reach a point where cellular metabolism begins to be negatively affected (Nazari and Raheb 2015). In addition transgenic algae could represent a significant threat to the ecosystem and have to be banned from outdoor cultivation systems and be under strict regulation (Pulz and Gross 2004).

Conclusion

Microalgae have an enormous biodiversity and they can be a source of bioactive compounds (proteins, lipids, pigments, and vitamins) with numerous biological activities (antioxidant, antibacterial, antiviral, and anti-inflammatory). The interest in microalgae has increased as a result of the need for additional food supplies, energy resources and various raw materials. Production of biofuel from microalgae at industrial scales at low cost can facilitate their use, since it can compete with fossil fuel and even replace it. The residual biomass could be used for other applications (in food, pharmaceutical, and cosmetic industries). Moreover, the genetic improvement of algal species could be an important step. Actually, the use of transgenic microalgae for commercial application has not yet been reported, but it holds significant promise. Modified species could overproduce desirable algal bioactive compounds. Despite of their many advantages, the use of microalgae is still limited due to their high costs processing. Algal cultivation, harvesting, and dewatering should be improved and new scale-up methods should be introduced. More funds have to be made available for the study of microalgae. Of the 50000 existent species, only a few thousand are now kept in collections and are investigated for their chemical content, and even fewer are cultivated in industrial quantities. Therefore, microalgae are still not a well-studied life form from a biotechnological point of view.

References

Abd El Baky HH, El-Baroty GS. 2013. Healthy benefit of microalgal bioactive substances. J Fish Aquat Sci 1:11–23.

Abdulqader G, Barsanti L, Tredici MR. 2000. Harvest of *Arthrospira platensis* from Lake Kossorom (Chad) and its household usage among the Kanembu. J Appl Phycol 12:493–8.

Adarme-Vega TC, Lim DKY, Timmins M, Vernen F, Li Y, Schenk PM. 2012. Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. Microb Cell Fact 11:96–105.

Adesanya VO, Cadena E, Scott SA, Smith AG. 2014. Life cycle assessment on microalgal biodiesel production using a hybrid cultivation system. Bioresour Technol 163:343–55.

Ahmad N, Pandit NP, Maheshwari SK. 2012. L-asparaginase gene–a therapeutic approach towards drugs for cancer cell. Intl J Biosci 2:1–11.

Al Hattab M, Ghaly A. 2015. Microalgae oil extraction pre-treatment methods: critical review and comparative analysis. J Fundam Renewable Energy Appl 5:172.

Aneiros A, Garateix A. 2004. Bioactive peptides from marine sources: pharmacological properties and isolation procedures. J Chromatogr B: Anal Technol Biomed Life Sci 803:41–53.

Antizar-Ladislao B, Turrion-Gomez JL. 2008. Second-generation biofuels and local bioenergy systems. Biofuels Bioprod Biorefin 2:455–69.

Babitha S, Kim E-K. 2011. Effect of marine cosmeceuticals on the pigmentation of skin. In: Kim S-K, editor. Marine cosmeceuticals: trends and prospects. Boca Raton, Fla.: CRC Press. p 63–5.

Becker W. 2008. Microalgae in human and animal nutrition. In: Richmond A, editor. Handbook of microalgal culture: biotechnology and applied phycology. Cornwall, UK: Blackwell Publishing. p 312–52.

Behera S, Singh R, Arora R, Kumar Sharma N, Shukla M, Kumar S. 2014. Scope of algae as third generation biofuels. Front Bioeng Biotechnol 2:1–13.

Beheshtipour H, Mortazavian AM, Mohammadi R, Sohrabvandi S, Khosravi-Darani K. 2013. Supplementation of *Spirulina platensis* and *Chlorella vulgaris* algae into probiotic fermented milks. Compr Rev Food Sci Food Saf 12:144–54.

Beijerinck MW. 1890. Culturversuche mit Zoochlorellen, Lichenengonidien und anderen niederen Algen. Bot Z 48:726–40.

Belay A. 2007. *Spirulina* (*Arthrospira*): production and quality assurance. In: Gershwin ME, Amha Belay A, editors. Spirulina in human nutrition and health. Boca Raton, Fla.: CRC Press. p 2–23.

Ben-Amotz A, Avron M. 1990. The biotechnology of cultivating the halotolerant alga *Dunaliella*. Trends Biotechnol 8:121–6.

Biabani MF, Gunasekera SP, Longley RE, Wright AE, Pomponi SA. 2002. Tubercidin, a cytotoxic agent from the marine sponge *Caulospongia biflabellata*. Pharm Biol 40:302–3.

Bin S, Zhi-ping W, Xin-ying L, Jin-xin Y, Jin-jin L, Jing-mei W, Li-fang M, Zi-yuan C. 2013. Breeding of a *Chlorella* strain with high yield of polysaccharide and its effect on growth and immunoregulation of *Litopenaeus vannamei*. J Nucl Agric Sci 27:168–72.

Bittencourt Sydney E, Novak AC, Cesar de Carvalho J, Soccol CR. 2013. Respirometric balance and carbon fixation of industrially important algae. In: Pandey A, Lee D-J, Chisti Y, Soccol CR, editors. Biofuels from algae. San Diego, U.S.A.: Newnes. p 67–84.

Blackburn SI, Volkman JK. 2012. Microalgae: a renewable source of bioproducts. In: Turgut Dunford N, editor. Food and industrial bioproducts and bioprocessing. Oxford, UK: John Wiley & Sons. p 221–37.

Borowitzka MA. 1995. Microalgae as sources of pharmaceuticals and other biologically active compounds. J Appl Phycol 7:3–15.

Borowitzka MA, Moheimani NR. 2012. Open pond culture systems. In: Borowitzka MA, Moheimani NR, editors. Algae for biofuels and energy. Dordrecht, Netherlands: Springer. p 133–52.

Borowitzka MA, Moheimani NR. 2013. Sustainable biofuels from algae. Mitig Adapt Strateg Glob Change 18:13–25.

Bosma R, van Spronsen WA, Tramper J, Wijffels RH. 2003. Ultrasound, a new separation technique to harvest microalgae. J Appl Phycol 15:143–53.

Brennan L, Owende P. 2010. Biofuels from microalgae-a review of technologies for production, processing, and extractions of biofuels and co-products. Renewable Sustainable Energy Rev 14:557–77.

Buick R. 2008. When did oxygenic photosynthesis evolve? Philos Trans R Soc B 363:2731–43.

Burtin P. 2003. Nutritional value of seaweeds. Electron J Environ Agric Food Chem 2:498–503.

Carvalho AP, Meireles LA, Malcata FX. 2006. Microalgal reactors: a review of enclosed system designs and performances. Biotechnol Prog 22:1490–506.

Chadwick R. 2003. Nutrition and health. In: Chadwick R, Henson S, Moseley B, Koenen G, Liakopoulos M, Midden C, Palou A, Rechkemmer G, Schröder D, von Wright A, editors. Functional food. Berlin, Germany: Springer. p 39–60.

Chauvat F, Cassier-Chauvat C. 2012. Preface. In: Chauvat F, Cassier-Chauvat C, editors. Genomics of cyanobacteria. San Diego, Calif.: Academic Press. p 9–16.

Chen G, Zhao L, Qi Y, Cui YL. 2014. Chitosan and its derivatives applied in harvesting microalgae for biodiesel production: an outlook. J Nanomater 2014:1–9.

Chinnasamy S, Bhatnagar A, Hunt RW, Das KC. 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresour Technol 101:3097–105.

Chisti Y, Moo-Young M. 1999. Fermentation technology, bioprocessing, scale-up and manufacture. In: Moses V, Cape RE, editors. Biotechnology—the science and the business. Amsterdam, Netherlands: CRC Press p 177–222.

Chisti Y. 2007. Biodiesel from microalgae. Biotechnol Adv 25:294–306.

Chojnacka K, Noworyta A. 2004. Evaluation of *Spirulina* sp. growth in photoautotrophic, heterotrophic and mixotrophic cultures. Enzyme Microb Technol 34:461–5.

Chojnacka K, Zielińska A. 2012. Evaluation of growth yield of *Spirulina* (*Arthrospira*) sp. in photoautotrophic, heterotrophic and mixotrophic cultures. World J Microbiol Biotechnol 28:437–45.

Ciferri O, Tiboni O. 1985. The biochemistry and industrial potential of *Spirulina*. Annu Rev Microbiol 39:503–26.

Clark DP, Pazdernik NJ. 2016. Basics of biotechnology. In: Clark DP, Pazdernik NJ, editors. Biotechnology. London, UK: Academic Cell. p 1–32.

Cuellar-Bermudez SP, Aguilar-Hernandez I, Cardenas-Chavez DL, Ornelas-Soto N, Romero-Ogawa MA, Parra-Saldivar R. 2015. Extraction and purification of high-value metabolites from microalgae: essential lipids, astaxanthin and phycobiliproteins. Microb Biotechnol 8:190–209.

Das P, Mandal SC, Bhagabati SK, Akhtar MS, Singh SK. 2012. Important live food organisms and their role in aquaculture. In: Sundaray JK, Sukham M, Mohanty RK, Otta SK, editors. Frontiers in Aquaculture. New Delhi, India: Narendra Publishing House. p 69–86.

De Philippis R, Sili C, Paperi R, Vincenzini M. 2001. Exopolysaccharide-producing Cyanobacteria and their possible exploitation: a review. J Appl Phycol 13:293–9.

De Philippis R, Vincenzini M. 1998. Exocellular polysaccharides from Cyanobacteria and their possible applications. FEMS Microbiol Rev 22:151–75.

Demirbas A, Demirbas MF. 2010. Energy demand and availability. In: Demirbas A, Demirbas MF, editors. Algae energy: algae as a new source of biodiesel. London, UK: Springer. p 1–26.

Demirbas A. 2010. Use of algae as biofuel sources. Energy Convers Manage 51:2738–49.

Dhankhar J, Kadian SS, Asha Sharma A. 2012. Astaxanthin: a potential Carotenoid. Intl J Pharm Sci Res 3:1246–59.

Dismukes GC, Carrieri D, Bennette N, Ananyev GM, Posewitz MC. 2008. Aquatic phototrophs: efficient alternatives to land-based crops for biofuels. Curr Opin Biotechnol 19:235–40.

Dormido R, Sánchez J, Duro N, Dormido-Canto S, Guinaldo M, Dormido S. 2014. An interactive tool for outdoor computer controlled cultivation of microalgae in a tubular photobioreactor system. Sensors 14:4466–83.

Doshi H, Ray A, Kothari IL. 2010. *Spirulina* Biotechnology. In: Jain SK, Khan AA, Rai MK, editors. Geomicrobiology. Boca Raton, Fla.: CRC Press. p 209–36.

Dragone G, Fernandes BD, Vicente AA, Teixeira JA. 2010. Third generation biofuels from microalgae. In: Vilas AM, editor. Current research, technology and education topics in applied microbiology and microbial biotechnology. Badajoz, Spain: Formatex Research Center. p 1355–66.

Drews G. 1999. Utilization of light by prokaryotes. In: Lengeler J, Drews G, Schlegel H, editors. Biology of the prokaryotes. Stuttgart: Germany: Georg thieme verlag. p 328–40.

Duong VT, Thomas-Hall SR, Schenk PM. 2015. Growth and lipid accumulation of microalgae from fluctuating brackish and sea water locations in South East Queensland-Australia. Front Plant Sci 6:359

Elias RJ, Kellerby SS, Decker EA. 2008. Antioxidant activity of proteins and peptides. Crit Rev Food Sci Nutr 48:430–41.

Ellinger B, Silber J, Prashar A, Landskron J, Weber J, Rehermann S, Müller F-J, Smith S, Wrigley S, Taskén K, Gribbon P, Labes A, Imhoff JF. 2014. A phenotypic screening approach to identify anticancer compounds derived from marine fungi. Assay Drug Dev Technol 12:162–75.

Enzing C, Ploeg M, Barbosa M, Sijtsma L. 2014. Microalgae-based products for the food and feed sector: an outlook for Europe. In: Vigani M, Parisi C, Cerezo ER, editors. EUR - Scientific and Technical Research Reports. Lxembourg: Publications Office of the European Union. p 9–18.

Fernandes P, Cabral JMS. 2007. Phytosterols: Applications and recovery methods. Bioresour Technol 98:2335–50.

Francavilla M, Franchi M, Monteleone M, Caroppo C. 2013. The red seaweed *Gracilaria gracilis* as a multi products source. Mar Drugs 11:3754–76

Frohnmeyer H, Staiger D. 2003. Ultraviolet-B radiation-mediated responses in plants. Balancing damage and protection. Plant Physiol 133:1420–28.

Gao J, Bao HY, Xin MX, Liu YX, Li Q, Zhang YF. 2006. Characterization of a bioflocculant from a newly isolated *Vagococcus* sp. W31. J Zhejiang Univ Sci B 7:186–92.

García-Chavarría M, Lara-Flores M. 2013. The use of carotenoid in aquaculture. Res J Fish Hydrobiol 8:38–49.

Garcia-Gonzalez M, Moreno J, Manzano JC, Florencio FJ, Guerrero MG. 2005. Production of *Dunaliella salina* biomass rich in 9-cis-beta-carotene and lutein in a closed tubular photobioreactor. J Biotechnol 115:81–90.

Ge B, Qin S, Han L, Lin F, Ren Y. 2006. Antioxidant properties of recombinant allophycocyanin expressed in *Escherichia coli*. J Photochem Photobiol B 84:175–80.

Godinez, JS, Ortega MM, Garduno G, Oliva MG, Vilaclara G. 2001. Traditional knowledge of Mexican continental algae. Jf Ethnobiol 21: 57–88.

González-Delgado ÁD, Kafarov V. 2011. Microalgae based biorefinery: issues to consider. CT&F-Ciencia, Tecnología y Futuro 4:5–22.

Gouveia L, Batista AP, Sousa I, Raymundo A, Bandarra NM. 2008. Microalgae in novel food products. In: Papadopoulos KN, editor. Food chemistry research developments. New York, U.S.A.: Nova Publishers. p 75–111.

Granum E, Kirkvold S, Myklestad SM. 2002. Cellular and extracellular production of carbohydrates and amino acids by the marine diatom *Skeletonema costatum*: diel variations and effects of N depletion. Mar Ecol Prog Ser 242:83–94.

Grima EM, Acien Fernandez FG, Medina AR. 2013. Downstream processing of cell mass and products. In: Richmond A, Hu Q, editors. Handbook of microalgal culture: applied phycology and biotechnology. West Sussex, UK: John Wiley & Sons. p 267–309.

Grima EM, Belarbi EH, Acién Fernandez FG, Medina AR, Chisti Y. 2003. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol Adv 20:491–515.

Guedes AC, Amaro HM, Malcata FX. 2011. Microalgae as sources of carotenoids. Mar Drugs 9:625–44.

Guerin M, Huntley ME, Olaizola M. 2003. *Haematococcus* astaxanthin: applications for human health and nutrition. Trends Biotechnol 21:210–6.

Guldhe A, Singh B, Rawat I, Ramluckan K, Bux F. 2014. Efficacy of drying and cell disruption techniques on lipid recovery from microalgae for biodiesel production. Fuel 128:46–52.

Gultom SO, Hu B. 2013. Review of microalgae harvesting via co-pelletization with filamentous fungus. Energies 6:5921–39.

Guo S-L, Zhao X-Q, Wan C, Huang Z-Y, Yang Y-L, Alam MA, Ho S-H, Bai F-W, Chang J-S. 2013. Characterization of flocculating agent from the self-flocculating microalga *Scenedesmus obliquus* AS-6-1 for efficient biomass harvest. Bioresour Technol 145: 285–9.

Hafez H, Nakhla G, Naggar HEl, Ibrahim G, Elnashaie SSEH. 2014. Biological hydrogen production: light-driven process. In: Sherif SA, Yogi Goswami D, Stefanakos EK, Steinfeld A, editors. Handbook of hydrogen energy. Boca Raton, Fla.: CRC Press. p 321–68.

Hall DO. 1982. Solar energy through biology: fuels from biomass. In: Mislin H, Bachofen R, editors. New trends in research and utilization of solar energy through biological systems. Basel, Switzerland: Birkhäuser. p 9–15.

Hallmann A. 2007. Algal transgenics and biotechnology. Transgc Plant J 1:81–98.

Hannon M, Gimpel J, Tran M, Rasala B, Mayfield S. 2010. Biofuels from algae: challenges and potential. Biofuels 1:763–84.

Hasegawa T, Okuda M, Makino M, Hiromatsu K, Nomoto K, Yoshikai Y. 1995. Hot water extracts of *Chlorella vulgaris* reduce opportunistic infection with *Listeria monocytogenes* in C57BL/6 mice infected with LP-BM5 murine leukemia viruses. Intl J Immunopharmacol 17:505–12.

He Q. 2004. Microalgae as platforms for recombinant proteins. In: Richmond A, editor. Handbook of microalgal culture: biotechnology and applied phycology. Oxford, UK: Blackwell Science. p 471–84.

Hejazi M, Wijffels RH. 2003. Effect of light intensity on β-carotene production and extraction by *Dunaliella salina* in two-phase bioreactors. Biomol Eng 20:171–5.

Hemaiswarya S, Raja R, Kumar RR, Ganesan V, Anbazhagan C. 2011. Microalgae: a sustainable feed source for aquaculture. World J Microbiol Biotechnol 27:1737–46.

Hopkinson BM, Dupont CL, Allen AE, Morel FMM. 2011. Efficiency of the CO_2-concentrating mechanism of diatoms. Proc Natl Acad Sci 108:3830–37.

Hosikian A, Lim S, Halim R, Danquah MK. 2010. Chlorophyll extraction from microalgae: a review on the process engineering aspects. Intl J Chem Eng 2010:1–11.

Huang GH, Chen F, Wei D, Zhang XW, Chen G. 2010. Biodiesel production by microalgal biotechnology. Appl Energy 87:38–46.

Ibañez E, Herrero M, Mendiola JA, Castro-Puyana M. 2011. Extraction and characterization of bioactive compounds with health benefits from marine resources: macro and micro algae, cyanobacteria, and invertebrates. In: Hayes M, editor. Marine bioactive compounds: sources, characterization and applications. New York, U.S.A.: Springer. p 55–98.

Jain R, Raghukumar S, Tharanathan R, Bhosle NB. 2005. Extracellular polysaccharide production by thraustochytrid protists. Mar Biotechnol 7:184–92.

Jiménez-Escrig A, Jiménez-Jiménez I, Pulido R, Saura-Calixto F. 2001. Antioxidant activity of fresh and processed edible seaweeds. J Sci Food Agric 81:530–4.

Jun SY, Park PJ, Jung WK, Kim SK. 2004. Purification and characterization of an antioxidative peptide from enzymatic hydrolysate of yellowfin sole (*Limanda aspera*) frame protein. Eur Food Res Technol 219:20–6.

Juneja A, Ceballos RM, Murthy GS. 2013. Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: a review. Energies 6:4607–38.

Kagan ML, Matulka RA. 2015. Safety assessment of the microalgae *Nannochloropsis oculata*. Toxicol Rep 2:617–623.

Kanekiyo K, Hayashi K, Takenaka H, Lee JB, Hayashi T. 2007. Anti-Herpes simplex virus target of an acidic polysaccharide, nostoflan, from the edible blue-green alga *Nostoc flagelliforme*. Biol Pharm Bull 30:1573–5.

Kim S-K, Wijesekara I. 2011. Cosmeceuticals from marine resources: Prospects and Commercial Trends. In: Kim S.K, editor. Marine cosmeceuticals: trends and prospects. Boca Raton, Fla.: CRC Press. p 1–10.

Kindle KL. 1990. High-frequency nuclear transformation of *Chlamydomonas reinhardtii*. Proc Natl Acad Sci USA 87:1228–1232.

Krinsky NI. 1989. Antioxidant functions of carotenoids. Free Radl Biol Med 7:617–35.

Kris-Etherton PM, Harris WS, Appel LJ. 2003. Fish consumption, Fish oil, omega-3 fatty acids, and cardiovascular disease. Arterioscler Thromb Vasc Biol 23:20–30.

Krishnakumar S, Dooslin Mercy Bai V, Alexis Rajan R. 2013. Evaluation of bioactive metabolites from halophilic microalgae *Dunaliella salina* by GC-MS analysis. Intl J Pharm Pharm Sci 5:296–303.

Kronick MN. 1986. The use of phycobiliproteins as fluorescent labels in immunoassay. J Immunol Methods 92:1–13.

Kumar K, Dasgupta CN, Nayak B, Lindblad P, Das D. 2011. Development of suitable photobioreactors for CO_2 sequestration addressing global warming using green algae and Cyanobacteria. Bioresour Technol 102:4945–53.

Kunjapur AM, Eldridge RB. 2010. Photobioreactor design for commercial biofuel production from microalgae. Ind Eng Chem Res 49: 3516–26.

Lam MK, Lee KT. 2013. Scale-up and commercialization of algal cultivation and biofuel production. In: Pandey A, Lee D-J, Chisti Y, Soccol CR, editors. Biofuels from algae. San Diego, U.S.A.: Elsevier. p 261–82.

Lee RE. 2008. Basic characteristics of the algae. In: Lee RE, editor. Phycology. New York, U.S.A.: Cambridge University Press. p 1–27.

Lee S-H, Oh H-M, Jo B-H, Lee S-A, Shin S-Y, Kim H-S, Lee S-H, Ahn C-Y. 2014. Higher biomass productivity of microalgae in an attached growth system, using wastewater. J Microbiol Biotechnol 24:1566–73.

Lee YK. 1997. Commercial production of microalgae in the Asia-Pacific rim. J Appl Phycol 9:403–11.

Lee YK. 2001. Microalgal mass culture systems and methods: their limitation and potential. J Appl Phycol 13:307–15.

Lehr F, Posten C. 2009. Closed photo-bioreactors as tools for biofuel production. Curr Opin Biotechnol 20:280–95.

Li-Beisson Y, Peltier G. 2013. Third-generation biofuels: current and future research on microalgal lipid biotechnology. OCL 20:1–8.

Lordan S, Ross RP, Stanton C. 2011. Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. Mar Drugs 9:1056–100.

Lum KK, Kim J, Lei XG. 2013. Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. J Anim Sci Biotechnol 4:53.

Mandal B, Vlek PLG, Mandal LN. 1999. Beneficial effects of blue-green algae and Azolla, excluding supplying nitrogen, on wetland rice fields: a review. Biol Fertil Soils 28:329–42.

Masojidek J, Koblizek M, Torzillo G. 2004. Photosynthesis in Microalgae. In: Richmond A, editor. Handbook of microalgal culture: biotechnology and applied phycology. Oxford, UK: Blackwell Science. p 20–39.

Masojidek J, Torzillo G, Koblizek M. 2013. Photosynthesis in Microalgae. In: Richmond A, Hu Q, editors. Handbook of microalgal culture: applied phycology and biotechnology, 2nd ed. West Sussex, UK: John Wiley & Sons. p 21–36.

Mata TM, Martins AA, Caetano NS. 2010. Microalgae for biodiesel production and other applications: a review. Renewe Sustain Ener Rev 14:217–32.

Mazo VK, Gmoshinskiĭ IV, Zilova IS. 2004. Microalgae *Spirulina* in human nutrition. Voprosy Pitaniia 73:45–53.

McKendry P. 2002a. Energy production from biomass (part 1): overview of biomass. Bioresour Technol 83:37–46.

McKendry P. 2002b. Energy production from biomass (part 2): conversion technologies. Bioresour Technol 83:47–54.

Medipally SR, Yusoff FM, Banerjee S, Shariff M. 2015. Microalgae as sustainable renewable energy feedstock for biofuel production. BioMed Res Intl 2015: 1–13.

Mendes LBB, Vermelho AB. 2013. Allelopathy as a potential strategy to improve microalgae cultivation. Biotechnol Biofuels 6:152.

Mendes RL, Fernandes HL, Coelho JP, Reis EC, Cabral JM, Novais JM, Palavra AF. 1995. Supercritical CO_2 extraction of carotenoids and other lipids from *Chlorella vulgaris*. Food Chem 53:99–103.

Metting FB. 1996. Biodiversity and application of microalgae. J Ind Microbiol 17:477–89.

Miki W. 1991. Biological functions and activities of animal carotenoids. Pure Appl Chem 63:141–6.

Milledge JJ. 2011. Commercial application of microalgae other than as biofuels: a brief review. Rev Environ Sci Biotechnol 10:31–41.

Milledge JJ, Smith B, Dyer PW, Harvey P. 2014. Macroalgae-derived biofuel: a review of methods of energy extraction from seaweed biomass. Energies 7:7194–222.

Mohamed AG, Abo-El-Khair BE, Shalaby SM. 2013. Quality of novel healthy processed cheese analogue enhanced with marine microalgae *Chlorella vulgaris* biomass. World Appl Sci J 23:914–25.

Monroig Ó, Tocher DR, Navarro JC. 2013. Biosynthesis of polyunsaturated fatty acids in marine invertebrates: recent advances in molecular mechanisms. Mar Drugs 11:3998–4018.

Moradikor N, Mohamadi N. 2015. The Effects of different levels chlorell microalgae on performance and immune response of laying hens under heat stress condition. Intl J Life Sci 9:71–4.

Mujumdar AS. 2000. Classification and selection of industrial dryers. In: Devahastin S, editor. Practical guide to industrial drying. Montreal, Canada: Exergex Corporation. p 23–77.

Munir N, Sharif N, Shagufta N, Saleem F, Manzoor F. 2013. Harvesting and processing of microalgae biomass fractions for biodiesel production (a review). Sci Technol Dev 32:235–43.

Mubarak M, Shaija A, Suchithra TV. 2015. A review on the extraction of lipid from microalgae for biodiesel production. Algal Res 7:117–23.

Muradov N, Taha M, Miranda AF, Wrede D, Kadali K, Gujar A, Stevenson T, Ball AS, Mouradov A. 2015. Fungal-assisted algal flocculation: application in wastewater treatment and biofuel production. Biotechnol Biofuels 8:24.

Mutanda T. 2013. Introduction. In: Bux F, editor. Biotechnological applications of microalgae: biodiesel and value-added products. Boca Raton, Fla.: CRC Press. p 1–4.

Naik SN, Goud VV, Rout PK, Dalai AK. 2010. Production of first and second generation biofuels: a comprehensive review. Renew Sustain Ener Rev 14:578–597.

Nazari F, Raheb J. 2015. Genetic engineering of microalgae for enhanced biodiesel production suitable fuel replacement of fossil fuel as a novel energy source. Am J Life Sci 3:32–41.

Nisbet EG, Sleep NH. 2001. The habitat and nature of early life. Nature 409:1083–91.

Norziah MH, Ching CY. 2000. Nutritional composition of edible seaweed *Gracilaria changgi*. Food Chem 68:69–76.

Ono E, Cuello JL. 2003. Selection of optimal microalgae species for CO_2 sequestration. Proceedings of the 2nd Annual Conference on Carbon Sequestration, Alexandria. p 1–7.

Painter TJ. 1993. Carbohydrate polymers in desert reclamation: the potential of microalgal biofertilizers. Carbohydr Polym 20:77–86.

Patil V, Reitan KI, Knustsen G, Mortensen LM, Kallqvist T, Olsen E, Vogt G, Gislerod HR. 2005. Microlage as source of polyunsaturated fatty acids for aquaculture. Curr Top Plant Biol 6:57–65.

Paul JH. 1982. Isolation and characterization of a *Chlamydomonas* L-asparaginase. Biochem J 203:109–15.

Pernet F, Tremblay R. 2003. Effect of ultrasonication and grinding on the determination of lipid class content of microalgae harvested on filters. Lipids 38:1191–95.

Pienkos PT, Darzins A. 2009. The promise and challenges of microalgal-derived biofuels. Biofuels Bioprod Biorefin 3:431–40.

Pradhan J, Das S, Kumar Das B. Antibacterial activity of fresh water microalgae: A review. 2014. Afr J Pharm Pharmacol 8:809–18.

Priyadarshani I, Sahu D, Rath B. 2012. Algae in aquaculture. Intl J Health Sci Res 2:108–14.

Prokop A, Quinn MF, Fekri M, Murad M, Ahmed SA. 1984. Spectral shifting by dyes to enhance algae growth. Biotechnol Bioeng 26:1313–22.

Pugh N, Ross SA, ElSohly HN, ElSohly MA, Pasco DS. 2001. Isolation of three high- molecular-weight polysaccharide preparations with potent immunostimulatory activity from *Spirulina platensis, Aphanizomenon flos-aquae* and *Chlorella pyrenoidosa*. Planta Med 67:737–42.

Pulz O, Gross W. 2004. Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol 65:635–48.

Pulz O, Scheibenbogen K, Gross W. 2001. Biotechnology with Cyanobacteria and microalgae. In: Rehm HJ, Reed G, Puhler A, Stadler P, editors. Biotechnology. 2nd, completely revised edition. Weinheim, Germany: Wiley-VCH. p 107–34.

Radakovits R, Jinkerson RE, Darzins A, Posewitz MC. 2010. Genetic engineering of algae for enhanced biofuel production. Eukaryotic Cell 9:486–501

Rajvanshi S, Sharma MP. 2012. Microalgae: a potential source of biodiesel. J Sustain Bioener Syst 2:49–59.

Ramesh D. 2013. Lipid identification and extraction techniques. In: Bux F, editor. Biotechnological applications of microalgae: biodiesel and value-added products. Boca Raton, Fla.: CRC Press. p 89–97.

Ranjith Kumar R, Hanumantha Rao P, Arumugam M. 2015. Lipid extraction methods from microalgae: a comprehensive review. Front Energy Res 2:61.

Raposo MFDJ, de Morais RMSC, Bernardo de Morais AMM. 2013. Bioactivity and applications of sulphated polysaccharides from marine microalgae. Mar Drugs 11:233–52.

Rasmussen RS, Morrissey MT. 2007. Marine biotechnology for production of food ingredients. Adv Food Nutr Res 52:237–92.

Rodríguez-Meizoso I, Jaime L, Santoyo S, Señoráns F, Cifuentes A, Ibáñez E. 2010. Subcritical water extraction and characterization of bioactive compounds from *Haematococcus pluvialis* microalga. J Pharm Biomed Anal 51:456–63.

Ruiz-Martinez A, Garcia NM, Romero I, Seco A, Ferrer J. 2012. Microalgae cultivation in wastewater: nutrient removal from anaerobic membrane bioreactor effluent. Bioresour Technol 126:247–53

Rzymski P, Niedzielski P, Kaczmarek N, Jurczak T, Klimaszyk P. 2015. The multidisciplinary approach to safety and toxicity assessment of microalgae-based food supplements following clinical cases of poisoning. Harmful Algae 46:34–42.

Saad A, Atia A. 2014. Review on freshwater blue-green algae (Cyanobacteria): occurrence, classification and toxicology. Biosci Biotechnol Res Asia 11:1319–25.

Saha SK, McHugh E, Murray P, Walsh DJ. 2015. Microalgae as a source of nutraceuticals. In: Botana LM, Alfonso A, editors. Phycotoxins: chemistry and biochemistry, 2nd ed. West Sussex: UK: John Wiley & Sons. p 255–92.

Saifullah AZA, Abdul Karim Md, Ahmad-Yazid A. 2014. Microalgae: an alternative source of renewable energy. Am J Eng Res (AJER) 3:330–8.

Sastre RR. 2012. Products from microalgae: An overview. In: Posten C, Walter C, editors. Microalgal biotechnology: integration and economy. Berlin, Germany: Walter de Gruyter. p 13–44.

Schopf JW, Packer BM. 1987. Early Archean (3.3-billion to 3.5 billion-year-old) microfossils from Warrawoona Group, Australia. Science 237:70–3

Sharma KK, Schuhmann H, Schenk PM. 2012. High lipid induction in microalgae for biodiesel production. Energies 5:1532–53.

Sharma P, Khetmalas MB, Tandon GD. 2014. Biofuels from green microalgae. In: Salar RK, Gahlawat SK, Siwach P, Duhan JS, editors. Biotechnology: prospects and applications. New Delhi, India: Springer. p 95–110.

Shields R, Lupatsch I. 2012. Alage for aquaculture and animal feeds. In: Posten C, Walter C, editors. Microalgal biotechnology: integration and economy. Berlin, Germany: Walter de Gruyter. p 79–96.

Sierra E, Acién FG, Fernandez JM, Garcia JL, Gonzalez C, Molina E. Characterization of a flat plate photobioreactor for the production of microalgae. Chem Eng J 2008;138:136–47.

Sirakov I, Velichkova K, Stoyanova S, Staykov Y. 2015. The importance of microalgae for aquaculture industry. Review. Intl J Fisher Aquat Stud 2:31–7.

Skrovankova S. 2011. Seaweed vitamins as nutraceuticals. In: Kim S-K, editor. Marine medicinal foods: Implications and applications, macro and microalgae. Waltham, U.S.A.: Academic Press. p 358–66.

Slade R, Bauen A. 2013. Micro-algae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects. Biomass Bioener 53:29–38.

Sommer TR, Potts WT, Morrissy NM. 1991. Utilization of microalgal astaxanthin by rainbow trout (*Oncorhynchus mykiss*). Aquaculture 94:79–88.

Spolaore P, Joannis-Cassan C, Duran E, Isambert A. 2006. Commercial applications of microalgae. J Biosci Bioeng 101:87–96.

Stahl W, Sies H. 2003. Antioxidant activity of carotenoids. Mol Aspects Med 24:345–51.

Stephens E, Ross IL, Hankamer B. 2013. Expanding the microalgal industry-continuing controversy or compelling case? Curr Opin Chem Biol 17:1–9.

Tafreshi AH, Shariati M. 2009. *Dunaliella* biotechnology: methods and applications. J Appl Microbiol 107:14–35.

Tamagnini P, Axelsson R, Lindberg P, Oxelfelt F, Wünschiers R, Lindblad P. 2002. Hydrogenases and hydrogen metabolism of cyanobacteria. Microbiol Mol Biol Rev 66:1–20.

Thein, M. 1993. Production of *Spirulina* in Myanmar (Burma). Bull Inst Oceanogr 12:175–78.

Tomaselli L. 2004. The microalgal cell. In: Richmond A, editor. Handbook of microalgal culture: biotechnology and applied phycology. Oxford, UK: Blackwell Science. p 3–19.

Tran D, Doan N, Louime C, Giordano M, Portilla S. 2014. Growth, antioxidant capacity and total carotene of *Dunaliella salina* DCCBC15 in a low cost enriched natural seawater medium. World J Microbiol Biotechnol 30:317–22.

Tredici MR. 2008. Mass production of microalgae: photobioreactors. In: Richmond A, editor. Handbook of microalgal culture: biotechnology and applied phycology. Cornwall, UK: Blackwell Publishing. p. 178–98.

Ugwu CU, Aoyagi H, Uchiyama H. 2008. Photobioreactors for mass cultivation of algae. Bioresour Technol 99:4021–28.

van der Spiegel M, Noordam MY, van der Fels-Klerx HJ. 2013. Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. Compr Rev Food Sci Food Saf 12:662–678.

Velan M, Saravanane R. 2013. Pollution abatement and utilization of flue gas for bioenergy production—a review. Intl J Emerg Technol Advanced Eng 3:94–9.

Vieira Costa JA, Greque de Morais M. 2013. An open pond system for microalgal cultivation. In: Pandey A, Lee D-J, Chisti Y, Soccol CR, editors. Biofuels from Algae. San Diego, USA: Newnes. p 1–20.

Vo T-S, Ngo D-H, Ta QV, Kim S-K. 2011. Marine organisms as a therapeutic source against Herpes simplex virus infection. Eur J Pharm Sci 44:11–20.

von Witsch H, Harder R. 1953. Production of organic material by green algae and diatoms. In: Burlew JS, editor. Algal culture from laboratory to pilot plant. Washington, DC: Carnegie Institution of Washington. p 154–65.

Wall R, Ross RP, Fitzgerald GF, Stanton C. 2010. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. Nutr Rev 68:280–9.

Wang B, Lan CQ, Horsman M. 2012. Closed photobioreactors for production of microalgal biomasses. Biotechnol Adv 30:904–12.

Wiley P, Harris L, Reinsch S, Tozzi S, Embaye T, Clark K, Mckuin B, Kolber Z, Adams R, Kagawa H, Richardson TJ, Malinowski J, Beal C, Claxton MA, Geiger E, Rask J, Campbell JE, Trent J. 2013. Microalgae cultivation using offshore membrane enclosures for growing algae (OMEGA). J Sustain Bioenerg Syst 3:18–32.

Williams PJB, Laurens LML. 2010. Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetics & economics. Energ Environ Sci 3:554–90.

Yaakob Z, Ali E, Zainal A, Mohamad M, Takriff MS. 2014. An overview: biomolecules from microalgae for animal feed and aquaculture. J Biol Res 21:6–15.

Yen HW, Hu IC, Chen CY, Chang JS. 2013. Design of photobioracators for algal cultivation. In: Pandey A, Lee DJ, Chisti Y, Soccol CR, editors. Biofuels from algae. San Diego, U.S.A.: Elsevier. p 23–47.

Yuan JP, Peng J, Yin K, Wang JH. 2011. Potential health-promoting effects of astaxanthin: a high-value carotenoid mostly from microalgae. Mol Nutr Food Res 55:150–65.

Zhang X. 2015. Microalgae removal of CO_2 from flue gas. London: UK: IEA Clean Coal Centre. p 1–95.

Zhu L, Naaranoja M, Hiltunen E. 2012. Environmental sustainability of microalgae production as a biofuel source. Adv Mater Res 378-379:433–8.