Microalgae-based Pharmaceuticals and Nutraceuticals: An Emerging Field with Immense Market Potential

Durga Jha[1],‡, Vishakha Jain[1],‡, Brinda Sharma[1], Anil Kant[1], Vijay Kumar Garlapati[1],*

Abstract

Microalgae are one of the renewable sources for pharmaceutical compounds as well as biofuels. The microalgae sector is growing rapidly due to the scarcity of substrate sources, more yields, and the GRAS (generally recognized as safe) status of compounds associated with microalgae. Due to this GRAS status, the algal products are beneficial not only for the pharmaceutical but also for the food industry. In this review, insights into the different process aspects and obstacles of pharmaceutical and nutraceutical compounds derived from microalgae on large-scale are discussed. Various culture production methods like photoautotrophic, heterotrophic, and mixotrophic processes have been included and recent advances in metabolic strategies for upgrading the microalgal technology for pharmaceutical and nutraceutical compounds are highlighted as well as the prospects in the field presented. Overall, this review discusses the question, how microalgae can be a great advantage for the pharmaceutical and nutraceutical industry.

Keywords: Nutraceuticals, Metabolic engineering, Microalgae, Pharmaceuticals, Production technologies

Received: October 14, 2016; *revised:* March 05, 2017; *accepted:* March 24, 2017

DOI: 10.1002/cben.201600023

1 Introduction

Blue green algae are microscopic photosynthetic organisms which grow in different types of aquatic systems. They are distinguished from plants by their cellular structure and functions similar to bacteria and have been placed into the kingdom *Monera* of prokaryotic organisms. On the other hand, algae are eukaryotic organisms with more resemblance to plants in terms of photosynthetic pigments and metabolic pathways, but without specialized tissue organization. They belong to the kingdom *Protista*. The term microalgae is used to represent unicellular photosynthetic organisms that include both prokaryotic and eukaryotic organisms. The photosynthetic reaction leads to the ability to produce many bioactive compounds that have been investigated for human use. Microalgae also stabilize the marine ecosystem as they are the food source for other marine organisms. Due to the increasing need for food, pharmaceuticals, bioenergy and other bioactive compounds, microalgal technology seems to be a viable resource to meet the market potential [1]. Algae are a diverse group of microorganisms with various types of physiological and biochemical characteristics.

Different kinds of microalgae exist like green algae, blue algae, and red algae. Green algae have been exploited commercially for their carbohydrates, lipids, proteins, and enzymes [2]. Besides their nutritional value, microalgae are also a source for different pigments, proteins, and fatty acids for human consumption. They have been reported for the production of different secondary metabolites under various stress conditions like nutrient deprivation, light, temperature, and pH which will be discussed further below. So far, little is known about the possible effects of stress conditions on microalgae for the production of hydrogen and different other metabolites. All these properties of microalgae make them an excellent choice for the production of nutraceutical and pharmaceutical components. Also, the economic viability of processes in case of minimizing operational and maintenance cost along with maximum production is a factor that leads to commercial success [1].

The various products from microalgae include chemicals, nutraceuticals, β-carotene, omega-3 fatty acids, algal oil, etc. The growing demand for these compounds paves the way for sustainable microalgal technology. The algae can also produce different types of secondary metabolites under stress conditions [3–5]. Until to date, the most commercially available biochemical product is β-carotene by the *Dunaliala salina*, a halophilic green algae [6]. Significant progress in strain development and sustainable cultivation technologies are required to reduce the

[1] Durga Jha, Vishakha Jain, Brinda Sharma, Dr. Anil Kant, Dr. Vijay Kumar Garlapati (corresponding author)
Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, Himachal Pradesh 173 234, India.
E-Mail: shanepati@gmail.com

‡These authors contributed equally as first authors

current high production costs [7]. In the following sections, the different pharmaceuticals and nutraceuticals from microalgae are summarized along with the discussion of different cultivation practices, commercial aspects, and safety considerations.

2 Pharmaceuticals

Initially, the secondary metabolites extracted from plants have profoundly been used in the health sector. The lesser yields coupled with seasonal variations of plant sources made researchers switch to microalgae as production platform. The bioactive compounds from microalgae are considered as natural similar to aquatic natural communities. Microalgae systems have the potential of producing new chemical entities which are considered to be difficult to obtain through chemical synthesis. There is a vast range of pharmaceutical products produced from microalgae.

2.1 Antimicrobial, Antiviral, and Antifungal Compounds

The extracts of microalgae show antimicrobial, antiviral, and antifungal properties while the products of Chlorella sp. and Spirulina sp. are also used as ingredients of different skin care, sun protection, and hair care formulations [8]. Microalgae such as *Ochromonas* sp. produce toxins with pharmaceutical potential [9]. Cyanobacteria are also used to produce antibiotic compounds. The antibacterial activity of microalgae is attributed to the presence of volatile compounds such as phenols and fatty acids [10].

2.2 Neuroprotective Products

Neuroprotective products help the protection or slow progression of diseases related to the nervous system. Microalgae strains are also known to produce these types of products beneficial to nerve cell survival. Among the various species, *Spirulina* sp. is considered a profound source for neuroprotective products. The neuroprotective abilities of *Spirulina plantensis* in neurodegenerative diseases like Alzheimer's or Parkinson's disease have been reported in the literature. *Spirulina maxima* has also been reported for its neurotoxicity of MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) prevention and oxidative stress [11].

2.3 Therapeutic Proteins

Cultivating therapeutic proteins in algae is a cost effective procedure which could reduce the treatment costs of cancer and other diseases. Recombinant protein expression in microalgae cultures facilitates the large-scale production of proteins. The green algae *Chlamydomonas reinhardtii* has been reported for large-scale production of VEGF (vascular endothelial growth factor), HMGB1 (high mobility group protein1), Domain 14 human fibronectin, and Domain 10 of human fibronectin. Also, certain studies indicate that human proinsulin has also been produced in lower levels by microalgae. Compared to mammalian cell cultures, this is a cost efficient method for the production at large scales [12].

2.4 Cosmetological Ingredients

Microalgae are also known to produce different types of ingredients for different cosmetological formulations with nutaceutical abilities. *Chlorella* and *Spirulina* sp. have the ability to produce such compounds [13]. They are also used as a thickening and water binding agent. Algal species like Irish moss are a rich source of carrageenan (a polysaccharide), minerals, vitamins, and proteins, which serve as probable candidates for different skin and hair product formulations. Some also have the ability to be used as skin irritants, e.g., phycocyanin in blue-green algae can cause allergy and dermatitis [14].

2.5 Drug Candidates

It has been reported that algae contain certain compounds which are used as drugs. Algal chemistry has been investigated to develop such drugs and make the process of manufacturing more cost efficient and applicable as a sustainable resource. Scientists have developed a class of anti-cancerous drugs which are extracted from microalgae. For example, a bioactive compound called cryptophycin 1 isolated from blue-green algae has shown anti-carcinogenic properties. Other species of microalgae have been studied for their potential to produce alkaloidal neurotoxins like saxitoxin as well as the polyketide neurotoxins like brevetoxins, which have anti-cancer potential [15]. Various carotenoids obtained from *Chlorella* species have been shown to suppress colon cancer development which. The reported pharmaceutical compounds from microalgae up to date are summarized in Tab. 1.

3 Nutraceutical Compounds

Microalgae can be vital untapped sources of new biological activities which can serve as functional compounds such as pharmaceutical and nutraceutical compounds under abiotic stress conditions. Polyunsaturated fatty acids (PUFAs), components of renewable bioactive lipids, have been used in the prevention/treatment of cardiovascular diseases. Derivatives of PUFAs, namely γ-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), have also been reported for the treatment of type 2 diabetes, inflammatory bowel disorders, skin disorders, and asthma. These compounds also have an application as additives in cosmetic formulations. *Dunaliella* sp., *Chlorella* sp., and *Spirulina* sp. are three major types that have been used successfully to produce high concentrations of valuable compounds such as lipids, protein and pigments. The companies Seambiotic and Aurora algae are operating pilot-scale plants producing products like EPA, whereas the leftover algal biomass serve as animal feed

Table 1. Important pharmaceutical compounds produced from microalgae.

Organism	Bioactive compound	Yield/effect	Ref.
Carotenoids			
Dunaliella salina, Dunaliella bardawil	β-Carotene	13.5 mg $LRV^{-1}d^{-1}$	[16, 17]
Haematococcus pluvalis	Astaxanthin, cantaxanthein, lutein	13 g $m^{-2}d^{-1}$	[18–20]
Chlorella vulgaris	Astaxanthin, cantaxanthein	13.3 % of dry weight	[21]
Coelastrella striolata var. multistriata	Astaxanthin	1.5 mg g^{-1} dry weight	[22]
	Cantaxanthein	47.5 mg g^{-1} dry weight	
	β-Carotene	7.0 mg g^{-1} dry weight	
Scenedesmus almeriensis	β-Carotene	0.945 $\pm$ 0.452 mg g^{-1} dry weight	[23]
	Lutein	0.026 $\pm$ 0.020 mg g^{-1} dry weight	
Muriellopsis sp.	Lutein	35 mg L^{-1}	[24, 25]
Chlorella zofingiensis	Lutein, astaxanthin	10.3 mg L^{-1}	[26]
Chlorococcum citriforme	Lutein	38 $\pm$ 3.6 mg L^{-1}	[24]
	Astaxanthin	1.3 $\pm$ 0.1 mg L^{-1}	
	Canthaxanthin	1.8 $\pm$ 0.2 mg L^{-1}	
	β-Carotene	6.1 $\pm$ 0.6 mg L^{-1}	
	Violaxanthin	7.9 $\pm$ 0.6 mg L^{-1}	
Neospongiococcus gelatinosum	Lutein	29.8 $\pm$ 2.8 mg L^{-1}	[24]
	β-Carotene	4.4 $\pm$ 0.4 mg L^{-1}	
	Violaxanthin	5.7 $\pm$ 0.5 mg L^{-1}	
Nannochloropsis gaditana	Lutein	0.343 µg mg^{-1} dry weight	[23]
Synechococcus sp.	Zeaxanthin	0.39 $\pm$ 0.02 µg ml^{-1}	[27]
Cllorella saccharophila	Zeaxanthin	11.32 $\pm$ 0.64 mg g^{-1}	[28]
Phaeodactylum tricornutum	Fucoxanthin	15.71 mg g^{-1} freeze-dried sample weight	[29, 30]
Isochrysis sp.		17 mg g^{-1}	
Anticancer agents			
Lyngbya majuscule	8-Epi-malyngamide C, lyngbic acid	Cytotoxic to HT29 colon cancer cells	[31]
Calothrix sp.	Calothrixin B, N-substituted calothrixin B	Antiproliferative activity against HCT-116 and HL 60 cells	[32]
Arthrospira platensis	Extracellular polysaccharide	Cytotoxic against kidney and colon cancer cell line	[33]
Isochrysis galbana, Gyrodinium impudicum	(1→3, 1→6)-β-D-glucan sulfated exopolysaccharide	Cytotoxicity against lymphoma cells	[34, 35]
Chlorella pyrenoidosa	CPAP (*C. pyreniodosa* antitumor polypeptide)	Inhibitory activity on human liver cancer cell HepG2	[36]
Anti-inflammatory agents			
Tetraselmis suecica	Sulfated polysaccharide	Inhibition of NO, TNF-α, IL-6	[37]
Nannochloropsis oculata	Docosapentaenoic acid (DPA)	Inhibition of pro-inflammatory prostaglandin E2 (PGE 2)	[38]
Tetraselmis sp.	Docosahexaenic acid (DHA)	Inhibition of IL-6, IL-β	[39]

Table 1. Continued.

Organism	Bioactive compound	Yield/effect	Ref.
Phaeodactylum tricornutum	Sulfated extracellular polysaccharide	Immunostimulant	[40]
Chlorella stigmatophora	Sulfated polysaccharide	Immunosuppressant	[41]
Porphyridium sp.	Sulfated polysaccharides	Inhibition of the migration of polymorphonuclear leukocytes (PMN)	[41]
Antioxidants			
Spirulina maxima, Chlorella ellipsoidea, Nannochloropsis sp.	Phenolic compounds	Presence of radical scavenging activity	[42, 43]
Gymnodinium mikimotoi, Pavlova lutheri	Monogalactosyl diacylglycerol (MGDG)		[44, 45]
Stephanodiscus sp.	Digalactosyldiacylglycerol (DGDG)	Enhanced cell differentiation	[46, 47]
Antiviral agents			
Haematococcus pluvialis, Dunaliella salina	Pressurized liquid extraction against Herpes simplex virus type 1	IC_{50} = 189.58 ± 3.18 μg ml^{-1}, 168.81 ± 5.25 μg ml^{-1}	[48]
Gyrodinium, Impudium	Sulfated polysaccharide against influenza virus	EC_{50} = 0.19–0.48 μg ml^{-1}	[49]
Navicula directa	Polysaccharide against HSV 1and 2 and influenza virus	IC_{50} = 240 ± 42 μg ml^{-1}	[50]
Gyrodinium impudicum	p-KG03 exopolysaccharides against encephalomyocarditis virus	EC_{50} = 26.9 μg ml^{-1}	[51]
Antibacterial agents			
Anabaena sp.	Ethanol extract against *Staphylococcus aureus, E.coli* 3702	*MIC* = 0.39 mg ml^{-1}	[52]
Synechocystis sp.	Ethanol extract against *S. aureus, E.coli* 3702	*MIC* = 2.5–1.25 mg ml^{-1}	[52]
Porphyridium cruentum	Sulfated exopolysaccharide against HSV virus, vaccinia virus, vesicular somatitis virus		[53]

LRV = $\log_{10}$ reduction value; IC_{50} = concentration causing 50 % inhibition of the desired activity; EC_{50} = concentration of a drug that gives half-maximal response; *MIC* = minimal inhibitory concentration.

or fuel oil [54, 55]. Other microalgae lack the level of production needed for such important biomolecules when produced at large scale, e.g., heterokont algae only produce 3–5 % long chain PUFAs [56]. Nutraceuticals from microalgae have generally been classified into single cell protein, polyunsaturated fatty acids, carotenoids and pigments as well as bioactive compounds (Fig. 1).

3.1 Polyunsaturated Fatty Acids

Microalgae are considered as a predominant production sources for PUFAs that have to be supplied to the human diet [58, 59]. PUFAs play a major role in the treatment of arthritis, obesity, Parkinson's disease, and heart disease [60, 61]. EPA and DHA are the main derivatives of omega-3 fatty acids (PUFA n-3) and play a role in lowering the blood cholesterol and in the fetal brain development, respectively [62, 63]. The major algal producers of EPA are *Porphyridium cruentum, Monodus subterrans*, and *Nitzschia inconspicua* [64]. Arachidonic acid (AA) is a derivative of omega-6 fatty acid [65] and is considered as a precursor of prostaglandin and leucotriene synthesis, which play a major role in circulatory and CNS functions [66]. *Isochrysis galbana, Phaeodactylum tricornutum, Porphyridium cruentum*, and *Crypthecodinium cohnii* have been reported for improved production of AA. Along with the algal species mentioned above, other reported microalgal species for PUFA production are *Monoraphidium* sp., *Scenedesmus* sp., and *Nannochloropsis* sp. [67, 68].

3.2 Carotenoids and Pigments

Carotenoids and pigments are the main constituents of microalgal-based food supplements. The health benefits of microalgal carotenoids and pigments range from antioxidant activities to neuroprotective action and protection against chronic diseases [69]. Some of the noteworthy nutraceutical abilities of microalgae compounds include the antioxidant potential of carotenoid pigments, higher provitamin A activity of β-Carotene and lipid peroxidation activity of Astaxanthin [70]. Fucoxanthin is an-

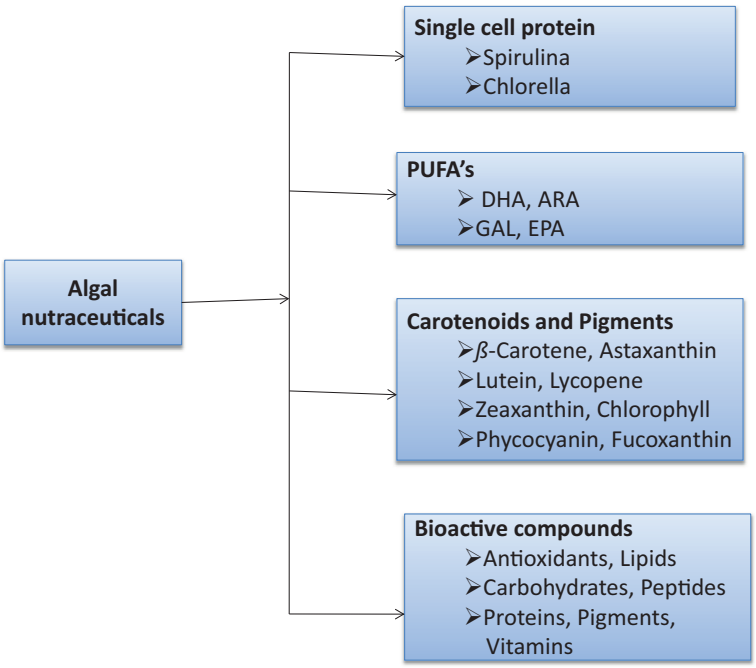

Figure 1. Classification of nutraceuticals from microalgae [57]

other microlagal pigment with nutraceutical abilities towards the weight loss management [71].

3.3 Proteins, Lipids, and Carbohydrates

Proteins from microalgae also are a substantial part of the nutraceutical abilities of microalgal supplements due to their therapeutic potential in the treatment of various chronic diseases. Along with the neutraceutical abilities, microalgal proteins also play an important role in replacement of damaged tissues. The rich protein content and amino acid profiles of *Chlorella* and *Spirulina* as part of functional foods are also well acknowledged by different researchers in the prevention of various diseases [71, 72]. Phytosterols are the lipid components of microalgae which have been reported for their role in the inhibition of cholesterol absorption in the intestines [51]. Microalgal polysaccharides are also a part of prebiotic supplements towards the promotion of gut microflora growth and regulation of blood glucose [73]. Some of the important microalgae-based nutraceuticals and its applications along with the producers have been listed in Tab. 2 [74].

Table 2. Important microalgae-based nutraceuticals and their applications along with the producers; modified from [74].

Type of Nutraceutical	Product name	Producing microalgae	Industrial application
PUFA's	EPA	*Pavlova* sp., *Nannochloropsis* sp., *Monodus* sp., *Phaeodactylum* sp.	Nutritional supplement and aquaculture feed constituent
	DHA	*Crypthecodiuimu* sp., *Schizochytrium* sp.	Nutritional supplement and constituent of infant and aquaculture feeds
	GLA (γ-linolenic acid)	*Spirulina* sp.	Nutritional supplement
	AA	*Porphyridium* sp.	Nutritional supplement
Phycobiliproteins	Phyocyanin	*Spirulina platensis*	Natural colorant for food and cosmetological products, anti-oxidant
	Phycoerythrin	*Porphyridium cruentum*	Diagnostic fluorescence agent
Carotenoids	β-Carotene	*Dunaliella salina*	Natural food colorant, antioxidant, anti-cancer properties
	Astaxantin	*Haematococcus pluvialis*	Pigment and antioxidant
	Echinenone, Zeaxanthin	*Dunaliella* sp.	Food colorant
	Lutein	*Chlorella* sp., *Chlamydomonas* sp.	Food colorant
	Phycocyanobilin	*Synechocystis* sp., *Cyanidioschyzon* sp.	Food colorant
Aminoacids	MAA (mycosporine-like amino acids)	*Aphanizomenon flos-aquae*	Sunscreen agent
Polysaccharides	Carragenan/aliginate	*Porphyridium cruentum*	Viscosifier, lubricant, flocculant, antiviral agent
Phycotoxins	Okadaic acid, gonyautoxins, yessotoxins	*Amphidinium* sp., *Prorocentrum* sp., *Dinophysis* sp.	Diagnostic agent for neurodegenerative diseases

4 Microalgae Cultivation

Microalgae can be cultivated either in open systems or closed systems. Open systems are the most studied designs for large-scale cultivation of microalgae due to their low investment and maintenance costs. They include shallow ponds, raceway ponds, tanks, and circular ponds. These open systems suffer from uncontrollable parameter regulation of the microbial growth (illumination, temperature, pH, nutrient levels) as well as contamination with predators [75]. On other hand, closed systems, i.e., photo bioreactors, have controllable machinery for adjusting the growth parameters and have overcome the contamination problems associated with the open-systems. The typical designs of closed photoreactor systems fall into three categories: flat plate, tubular, and vertical column type. A foresight of advantages and disadvantages of open (raceway pond) and closed-type photobioreactors are given in Tab. 3 [76–78].

4.1 Important Growth Parameters for Production of Secondary Metabolites from Microalgae

Abiotic stresses such as light, temperature, nutrient starvation, use of certain metals, and UV-radiation play a significant role in the production of secondary metabolites in microalgae. Little changes in the levels of these as well as the presence or absence of these abiotic stresses during microlagal cultivation result in lesser metabolite yields [79, 80].

4.1.1 Effect of Light

Light plays an important factor in the growth of algae since it is their energy source. Thus, a change in light intensity or light quality affects their growth. Algae have photoprotective mechanisms to cope with extreme light intensities, which include the formation of pigments which may not be a desired product. For example, EPA concentration was found to be significantly higher with low light intensity while DHA concentrations were found to be higher with higher light intensity in *Pavlova lutheri* [81, 82].

4.1.2 Role of UV Radiation

The presence of UV radiation stimulates the intracellular reactive oxygen species production which triggers the antioxidative defense. This defense includes the formation of antioxidant compounds such as ascorbate, carotenoids, tocopherol, etc., which are important bioactive compounds. In various studies, it has been shown that UV exposure increases carotenoid production. In another study, it has been found that PUFA, EPA, and DHA contents were reduced during 8-day exposure of UV light in *Pavlova lutheri* [83].

4.1.3 Nutrient Starvation

There are several micronutrients essential for microalgae growth and changes in their concentration can create stress in them. For example, enhanced β-carotene production with increased levels of nitrogen (1 mM) and salinity (30 %) in *Dunaliella salina*, as well as nitrogen limitation in *Haematococcus pluvialis* leads to an increased accumulation of astaxanthin [84].

Table 3. Advantages and disadvantages of open- and closed-type cultivation systems for microalgae [76–78].

Cultivation system	Advantages	Disadvantages
Open type, Raceway pond	– Easy construction and operation – Low energy input and low cost	– Water loss due to evaporation – Difficult to control the growth parameters – Contamination problem with predators
Closed type, Photobioreactors		
Flat plate type	– Large illumination surface area for solar energy – Lower concentration of dissolved oxygen – Possible inclination to face the solar energy – Low power consumption	– Tedious for scale-up studies – Uncontrollable temperature
Tubular type	– Large illumination surface area – Relatively higher biomass productivity – Possibility of minimizing cell damage by using airlift system	– Requires large area – Susceptible higher oxygen concentration in case of long tubes usage – Possibility for decreased Co_2 concentration, which deprives the carbon source for algae – Mixing problem in case of long tubes usage
Vertical column type	– Higher mass-transfer rates with better mixing conditions – Easy to operate – Relatively low cost – Lower power consumption	– Small illumination surface area – Possibility of sedimentation problem if airlift system is not used

4.1.4 Influence of Metals

Certain metals are essential for the cellular metabolic reactions of microalgae, e.g., in photosynthesis, respiration, transport of molecules, etc. The presence of metals like copper, zinc, and magnesium in increased concentrations leads to the generation of reactive oxygen species and results in the formation of anti-oxidative compounds. Many microalgae release certain exopolysaccharides to absorb excess metals present in the medium. These polysaccharides can be depolymerized to obtain different compounds having substantial therapeutic value [85]. The effect of some important parameters on the production of nutraceutical and pharmaceutical compounds has been summarized in Tab. 4.

5 Large-scale Algal Production Systems for Pharmaceutical and Nutraceutical Compounds

Although various compounds from microalgae have been found and studies on their potential pharmaceutical have been done, still very few commercial setups are present for large-scale production of these compounds. The possible reasons which hinder scale-up of the operations can be non-availability of a proper photobioreactor with automated parameters, contamination of the water source, choice of light system, choice of the strain, etc. Three strategies are currently being used to produce algae on a large scale, based on feeding strategies and described below [99].

5.1 Photoautotrophic Production Systems

Photoautotrophic production systems use light and carbon dioxide as the energy and carbon sources for algae. They are

Table 4. Effect of cultivation parameters on the production of nutraceutical and pharmaceutical compounds.

Cultivation parameter	Produced nutraceutical/ pharmaceutical compound	Yield enhancement [%]	Ref.
Chlorella zofingiensis			
High light intensity	Astaxanthin	1.5	[86]
0.2 M NaCl	Astaxanthin	>4	[86]
N deficiency	Lipids	65.1	[87]
P deficiency	Lipids	47.7	[87]
Chlorococcum sp.			
Addition of H_2O_2	Astaxanthin	0.71	[88]
N deficiency	Carbohydrates	39.8–41	[89]
Haematococcus pluvialis			
Nutrient starvation	Astaxanthin	4	[90]
High light intensity and non-aerated mixotrophic cultivation	Astaxanthin	10	[91]
N starvation	Carbohydrates	74	[92]
P starvation	Carbohydrates	48	[92]
0.8 % NaCl	Carbohydrates	48	[92]
P starvation	Lipids	43	[93]
Chlorella protothecoides			
High temperature (35 °C)	Lutein	4.6	[94]
Heterotrophy	Lipids	50	[95]
Dunaliella salina			
High light intensity	β-Carotene	3.1	[96]
N starvation	β-Carotene	2.7	[97]
N starvation	Carbohydrates	>55	[98]

mostly applied in open systems including raceways and thin layer reactors or closed systems including flat panels and tubular photobioreactors. Although open systems provide a better surface-to-volume ratio, which is essential for algal growth, the lack of automation and threat of contamination lead to the development of closed systems. These can provide higher biomass with better control of growth parameters, but closed systems are expensive compared to open ponds and raceways and also need better mass transfer capacity. Thus, despite being efficient, photobioreactors are still not being widely used, and thus, a lot of effort is required to decrease the expenses and make them economically feasible [100]. The commercial production of β-carotene by *Dunaliella salina* utilizes the open system. Currently, for commercial production of β-carotene production, industries are using open and closed cultivation processes. The Australian firms Betaten and Aquacaroten grow microalgae in unmixed open ponds resulting in a β-carotene production of about 13 t a^{-1} (approx. 510 ha of culture area). Nature Beta Technologies, a company from Israel, reported an annual production of β-carotene of 3 t a^{-1} by cultivating microalgae in raceway ponds [101]. Another study, in which β-carotene was produced from *Dunaliella salina* in a photobioreactor, showed 50 % higher output than open-pond cultivation [102].

5.2 Heterotrophic Production

Photobioreactors present the problem of less mass transfer capacity and decreased distribution of light with increasing reactor volume. This gave rise to the need for a heterotrophic production method. In this process, organic carbon sources such as sugars or organic acids replace light as the energy source. Till today, only a few studies (e.g., *Chlorella prototheocoides, Chlorella sorokiniana, Haematococcus pluvialis*) report the successful heterotrophical growth of microalgae [103–106]. Unlike photoautotrophic production, which depends on the area of illumination, heterotrophic cultures use organic carbon sources in the culture. Heterotrophic production facilitates the scale-up since the illumination of the photobioreactor does not have to be considered and it increases productivity as well. It also reduces production and maintenance costs. The only disadvantage of this system is that any contamination will out compete with the algae species for the organic carbon sources. Thus, the system and inoculum and even the organic carbon source will have to be free of any contaminants to ensure enhanced productivity. Examples include commercial production of heterotrophically grown *Chlorella* in the fermenter for aquaculture and health food applications, as is a common practice in Japan and Korea [107].

5.3 Mixotrophic Production

Mixotrophic production is a growth method for algae, combining both phototrophic and heterotrophic methods. Algae can easily utilize light and carbon dioxide for nutrition, but at night, productivity will be reduced due to respiration. Mixotrophic microalgae can utilize organic energy and both inorganic and organic carbon substrates by concurrently driving phototrophy and heterotrophy, thus, enhancing the culture productivity, while the diminished utilization of light sources decreases the cost of the production [108]. BioReal (Sweden) was the first company applying mixotrophy cultures in indoor closed photobioreactors for the commercial production of Astaxanthin (30 t a^{-1}) [101]. The high cost of the organic carbon source is the major drawback of this method, which led to the utilization of industrial dairy wastes and molasses as cheap sources [109, 110]. The recent trends in microalgae cultivation are depicted in Fig. 2.

6 Constraints in Large-Scale Production of Algal Compounds

The commercial production of pharmaceutical compounds from microalgae is still in an infant stage due to the unavailability of proper strains and optimal growth parameters for enhanced biomass yields. The lack of extensive phenotypic and genetic characterization also presents a difficulty in finding a suitable strain for research [111]. Common cultivation methods have to be designed to support the existing and new microalgae strains growth for optimal results. Growth parameters such as contamination, the amount of sunlight as well as harvesting and extraction of algae are also critical parameters to consider. Microalgae production at a large scale requires the optimization of many parameters discussed above. But these parameters are not easy to control and to be optimized. These major con-

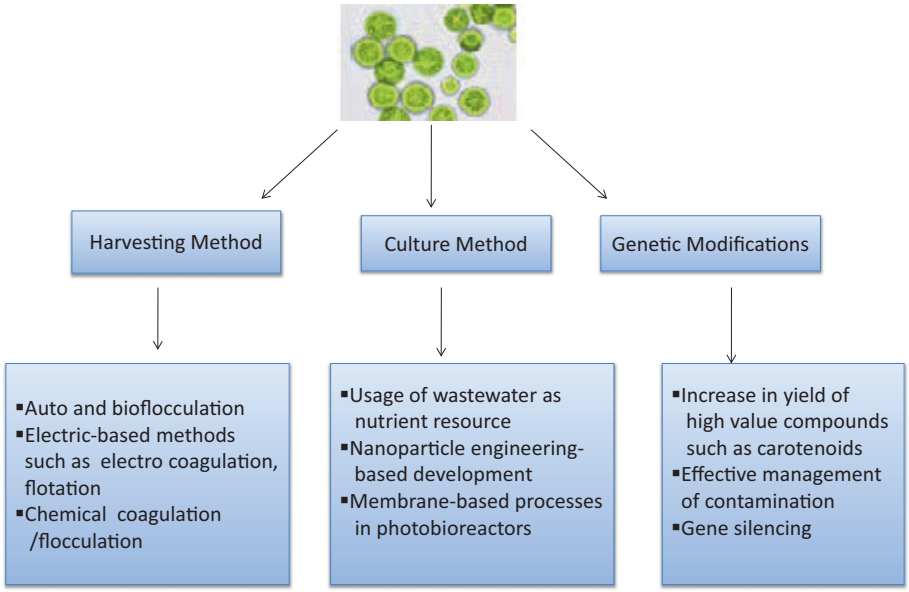

Figure 2. Recent trends in microalgae cultivation.

straints in the cultivation of microalgae on a large-scale are summarized in the sections below.

6.1 Temperature

The optimal temperature for algal growth is 15–25 °C. Below the optimal temperatures, the enzyme activities of the Calvin cycle will decrease, which eventually leads to a negative effect on photosynthesis and cell division. Above the optimum temperature, the activity of algae decreases and may even reach zero in some cases. Thus, the temperature beyond the optimum might slow the growth or kill the algae. Microalgae can be screened or acclimatized for temperature tolerance [112].

6.2 Light

Setups utilizing natural light sources suffer from issues like daily or seasonal fluctuation in light intensity. Also, the quality of light penetration might be detrimental to the surface layer of algae, but the reduced intensity of light might be insufficient for the growth of algae. Artificial lighting is important when the continuous growth of algae is required, but it increases the expenses [113].

6.3 Size

Due to the small size of microalgae, it is hard to harvest them in a conventional manner. High shear processes like centrifuging add the problem of cell wall damage in shear sensitive cells. The common harvesting methods include the concentration of biomass by centrifugation, flocculation, membrane filtration, and ultrasonic separation. These methods are cost intensive and add to the cost of entire process [113].

6.4 Water

Small-scale systems in open and sunny conditions suffer from loss of water due to evaporation. Change in climate conditions is also a matter of concern. An alternative source of water like wastewater is being considered as a possible option and has been used for microalgae growth [114].

6.5 Contamination

Bacteria and other lower metazoans are the common contaminants. Their growth can surpass the growth of algae or release certain toxic products which can hinder algal growth. On the other hand, reports of certain bacteria with growth-promoting factors have also been reported [115].

7 Latest Advances in Microalgae-based Pharmaceuticals

The use of microalgae is not restricted to anti-carcinogenic, antiviral, antibacterial, or antifungal agents. Many new possibilities have been tried. In a recent example, production of HIV vaccine from microalgae has been reported. A defective cell technique was used in this process to inhibit the release of transgenic material into the environment. Thus, the algae can be used as edible vaccines. The fact that individual algal components such as sulfated polysaccharides and bioactive peptides inhibit the entry of the HIV also provided the groundwork for the use of algae as a potential source of vaccine. The same process can be used for other diseases such as smallpox, polio, tuberculosis, and others like anti-hypertensive and anti-coagulant conditions. But still there are a lot of hurdles for the commercial production of this vaccine and extensive studies regarding its impact on human health would have to be done. Despite all these obstacles, the production of algae presents a cost effective method as they are easy to grow with minimum requirements [79].

Also, the concept of using algae to produce personalized drugs has also been studied by researchers. Producing the drugs in algae would enable the provision of designer drugs in days instead of months. Microalgae are also being used to produce monoclonal antibodies and single-chain fragment antibodies. *Chlamydomonas reinhardtii* has been reported for the production of human IgA single chain fragment antibody, an immunotoxin in the form of αCD22. The produced human IgA single-chain antibody in combination with exotoxin A (an enzymatic domain from *Pseudomonas aeruginosa*) is a likely therapeutic candidate against human B cell tumors and for the production of *Plasmodium falciparum* surface protein 25, a vaccine candidate for Malaria [80–82]. Metabolic engineering in microalgae is an essential tool to introduce the desired characteristics and produce valuable compounds. It is still in a starter phase, which could be due to the lack of information about metabolic pathways and genetic characterization. But recent advancements have helped to develop easier tools to perform such experiments. The major advancement in using metabolic engineering for microalgae-based pharmaceuticals is the enhanced yields of carotenoid production. Recent studies revealed that utilization of trophic conversion, improvement in photosynthesis, and management of contamination techniques results in better growth of microalgae. Enhancement of carotenoid biosynthesis through metabolic engineering has been reported for *Chlamydomonas reinhardtii* by nuclear transformation using a phytoene synthase gene isolated from *Chlorella zofingiensis* [116]. The sequence of steps utilized in nuclear transformation is depicted in Fig. 3. Metabolic engineering efforts to enhance the pharmaceutical compounds from microalgae are summarized in Tab. 5.

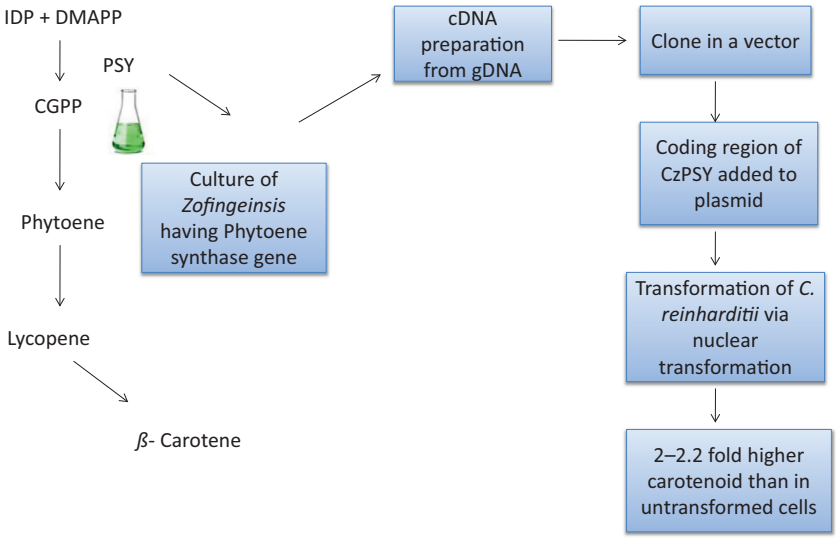

Figure 3. Sequence of steps for nuclear transformation in microalgae.

8 Safety and Toxicity Issues of Microalgal Nutraceuticals

Microalgae-based nutraceuticals are highly demanded to provide the active ingredients for combating the malnutrition-associated problems throughout the world [125]. These unknown nutraceutical compounds have to overcome the socio-ethnological and toxicity-related issues before consumer acceptance as safer food ingredients can follow. Naturally occurring toxins, heavy metal contamination, and the presence of pathogenic microorganisms are some of the critical points to evaluate [126]. The reports on allergenicity of microalgal nutraceutical are very scarce. Some of them reported the development of anaphylaxis after consumption of *Spirulina* tablets [127] and human poisoning episodes after the intake of wild-harvested *Spirulina* due to the production of neurotoxins by contaminated species of Microcystis and other freshwater cyanobacteria [128]. The higher doses of amino acids from red algae, namely, kainic acid and domoic acid, showed the neurotoxic effects in disease models in mice and other animals [129]. Illness-associated deaths have been reported in association with the sea vegetables *Gracilaria* sp. and *Caulerpa* sp. in the western Pacific region, where by mistake toxic species were picked [130, 131].

In order to prevent these safety and toxicity issues, Cyanotech, Earthrise Farms [132], and Solazyme, Inc. [133] supply

Table 5. Metabolic engineering approaches of microalgae for pharmaceutical compounds.

Strain	Method	Result	Ref.
Chlamydomonas reinhardtii	*psy* gene from *Chlorella zofingeinsis* was transformed into *C. reinhardtii*	2–2.2fold increase in astaxanthin	[116]
Chlamydomonas reinhardtii	Introduction of the *ptxD* gene from *Pseudomonas stutzeri* WM88 into *C. reinhardtii*	Engineered strains were able to use phosphite as main phosphorus source, making them a dominant species and reducing the chance of contamination	[117]
Chlamydomonas reinhardtii	Single amino acid mutation L505F in the phytoene desaturase gene	29 % increase in PDS activity, resistance to norflurazon, and increased amount of zeta carotene	[118]
Chlamydomonas reinhardtii	Plastid transformation of the *psy* gene from *D. salina* to *C. reinhardtii*	Increase in various carotenoids from 125–260 %	[119]
Chlamydomonas reinhardtii	Introduction of the *HUP1* gene (hexose uptake protein 1) from *Chlorella kessleri*	Engineered strain was able to uptake glucose and survive in the dark	[120]
Chlorella zofingiensis	L516F change in the *PDS* gene	33 % increase in desaturase activity, 32.1 % increase in total carotenoids, and 54.1 % increase in astaxanthin content	[121]
Dunaleilla bardawil	Calvin cycle enzyme SBP was introduced in *D. bardawil* from *C. reinhardtii*	Improvement in photosynthesis and increase in organic carbon content	[122]
Haematococcus pluvialis	Side directed mutagenesis of L504R in the *PDS* gene	Enhanced accumulation of astaxanthin up to 26 % and resistance to norflurazon	[123]
Phaeodaclylurn tricornutum	Introduction of the glucose transporter genes *glut1* from humans	The essentially autotroph organism was able to use organic carbon source and survive in the dark	[124]

large quantities of GRAS (generally regarded as safe) spirulina and GRAS Algalin protein to the nutraceutical markets. These results are showcasing the importance of safety and toxicity-related issues of microalgal nutraceuticals which have to be tackled by developing controlled large-scale cultivation methods coupled with routine quality control analysis [134].

9 Market Potential of Algal Nutraceuticals

The consumer's awareness of the nutraceutical's role in combating the incidence of chronic and lifestyle diseases paved the way for increased demands for algal-based nutraceuticals. Based on the overwhelming response from customers, the Transparency Market Research (TMR) projects, the global nutraceuticals market, is set to cross $US 278.96 billion at a compound annual growth rate of 7.3 % by the end of 2021 [135]. Various companies are operating in the global nutraceuticals market including Royal DSM N.V., BASF SE, Groupe Danone S.A., E. I. du Pont de Nemours, Nestle S.A., etc. The global market of nutraceuticals revolves around three production strains, i.e., *Spirulina* (cyanobacteria), *Chlorella* (green-algae) and *Aphanizomenon* (Klamath algae, eukaryotic, name originated from the source lake). The advantage of Klamath algae is that it naturally grows without contaminants [134, 136]. The market size of EPA/DHA is expected to increase over 11 % and will surpass to reach sales of $US 4 billion by 2022. Algal species of *Nannochloropsis* and *Chlorella vulgaris* are the primary formulation ingredients for the sports nutrition industry and are priced at about $US 18 000–36 000 t^{-1} [137].

10 Future Prospects

The approach to algae-based pharmaceutical compounds is still growing in the industry and the coming years promise a boom for the pharmaceutical compounds derived from algae resources. Compounds like astaxanthin, β-carotene, and *Spirulina* seem to be demanding in countries where people understand the importance of nutraceuticals from such sources. Techno-economic studies suggested that the harvesting (flocculation, centrifugation, and solvent extraction) costs of microalgae accounts for 20–30 % of the entire production costs [138, 139]. Hence, to enhance the techno-economic feasibility of the algal technology, stringent measures like optimization of the process parameters and strain selection have to be implemented. For cost intensification, leftover biomass after harvesting has to be put back into the production process, and penultimate biomass could be marketed as animal feed or valorization to biofuel products. Moreover, the high-value product status of pharmaceuticals requires the utilization of stringent safety regulations and process measures in the production process. Researchers examining the production in microalgae also look with a sense of economic viability and environmental feasibility. The potential "saviors of the world climate" are just a few micrometers in size, can be multi-purposely be used and, thus, help in reducing the load on non-renewable resources. Due to massive production of compounds from microalgae, it surely will not away soon from the pharmaceutical biotechnology.

11 Conclusions

Over the ages, there has been significant research into the utilization of microalgae as sustainable resource for the production of high-value products such as nutraceuticals and therapeutic compounds. Due to their sustainability, microalgae also help to preserve the environment as they have the ability for carbon dioxide sequestration. Microalgae mainly have been exploited for various uses, and they promise a lot of medicinal and nutritional value. Different species of microalgae have proven significant for producing different components like pharmaceuticals, nutraceuticals, and cosmotologicals compounds. The bioactive compounds from microalgae also play a role in combating different types of carcinomas and neurodegenerative diseases.

The polysaccharides, pigments and lipids produced through microalgae were also reported for their nutraceutical properties. A little change in the cultivation parameters along with the changed stress conditions will have profound effects on the overall production of different dietary compounds such as astaxanthin, β-carotene, fatty acids (PUFA), antioxidants, DHA, EPA, etc. Microalgae-based bio-active components as dietary supplements can serve as an essential elements for fulfilling the human dietary requirements. Microalgae also serve as a food for different marine biota.

Not only do microalgae potential for pharmaceutical and nutraceutical components, but also as a feed supplement for various animals, the use as biofertilizers, and they have been studied for their potential to produce biofuels. Since there are so many fields to profit, the microalgae undeniably fulfil the need for various sustainable industries. Microalgae species like *Chlorella* and *Spirulina* are the most researched species for the production of bioactive compounds. The microalgae-based production platform has grown over the years and has more potential to decipher the compounds necessary for a healthy living in today's world. Fortunately, the demand for food and medicines is will not diminish but rather increase, so that the research of microalgal technology will continue to meet the demanding market potential.

Acknowledgements

The authors gratefully acknowledge JUIT, India, for providing library resource facilities.

Durga Jha received her dual degree (B.Tech and M.Tech) in Biotechnology from the Jaypee University of Information Technology, India, in 2016. She is currently working as a Project Associate at the Ministry of Human Resource Development – Centre for Excellence in Biomedical Applications (MHRD-CEMA), Anna University, India. Her research interest lies in the utilization of single nucleotide polymorphisms as diagnostic markers in personalized medicine.

Vishakha Jain received her dual degree (B.Tech and M.Tech). in Biotechnology from Jaypee University of Information Technology, Waknaghat, India. In her M.Sc. research, she worked on expression analysis of transcription factor genes related to medicinally important secondary metabolites. Currently she is working as a Project Fellow at the Center of Innovation and Applied Bioprocessing under Department of Biotechnology, Mohali, India. Her research is focused on developing the enzymes from biomass and optimizing their activity for industrial purposes.

Brinda Sharma is currently pursuing the final semester of her B.Tech. in Biotechnology at the Jaypee University of Information Technology, Waknaghat, India. As a part of her academic career, she did an internship with Invictus Oncology, New Delhi, working on qualitative aspects of targeted nanodrug-based anti-tumor treatment as well as with the National Institute of Pathology, ICMR, New Delhi, where she learned molecular biology techniques for diagnosis and treatment of Leishmaniasis. Presently, her project is studying algae-based nano-particle synthesis.

Anil Kant received his Ph.D. in Biotechnology from Dr. YS Parmar University Solan, India, in 2002. Currently, he is working as Assistant Professor Senior Grade in the Department of Biotechnology and Bioinformatics at Jaypee University of Information Technology, Waknaghat, India. His research interests include molecular markers, functional genomics, plant and algal cell culture, and recombinant DNA technology. He is also working on the selection of microalgae as biofuel feedstock.

Vijay Kumar Garlapati received his Ph.D. from IIT Kharagpur, India, in 2011. He then started his postdoc career as an Assistant professor at Jaypee University of Information Technology (JUIT), India. Later on, he moved to Universitat Autonoma de Barcelona (UAB), Spain, as a part of a A4U Post-Doctoral Fellowship until 2013. Afterwards, he joint JUIT, taking on teaching and research responsibilities in the "Bioprocess Engineering" discipline. His primary research interests include the exploitation of extremophiles and green chemistry practices in the bioenergy and biocatalysis sectors as well as modeling and optimization of biological processes.

Abbreviations

AA	arachidonic acid
ALA	γ-linolenic acid
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
GRAS	generally regarded as safe
PUFA	polyunsaturated fatty acid

References

[1] I. Priyadarshini, B. Rath, *J. Algal. Biomass Utln.* **2012**, *3*, 89–100.

[2] V. Mimouni, L. Ulmann, V. Pasquet, *Curr. Pharm. Biotechnol.* **2012**, *13*, 2733–2750. DOI: 10.2174/138920112804724828

[3] O. Pulz, W. Gross, *Appl. Microbiol. Biotechnol.* **2004**, *65*, 635–648. DOI: 10.1007/s00253-0041647-x
[4] R. H. Wijffels, M. J. Barbosa, *Biof. Bioprod. Bioref.* **2010**, *4*, 287–295. DOI: 10.1002/bbb.215
[5] S. Boussiba, *Physiol. Planta.* **2010**, *108*, 111–117. DOI: 10.1034/j.1399-3054.2000.108002111.x
[6] M. A. Borowitzka, *J. Appl. Phycol.* **2013**, *25*, 743–756. DOI: 10.1007/s10811-013-9983-9
[7] S. Boussiba, C. Aflalo C, *Innov. Food. Technol.* **2005**, *28*, 37–39.
[8] www.oilgae.com/blog/2010/09/algae-as-a-source-of-pharmaceuticals-nutraceuticals.html (Accessed on October 11, 2015)
[9] H. Katircioglu, *Int. J. Microbiol.* **2006**, *2*, 2.
[10] Z. Kamenarska, J. Serkedjieva, H. Najdenski, *Bot. Mar.* **2009**, *52*, 80–86. DOI: 10.1515/BOT.2009.030
[11] D. Q. Jin, C. S. Lim, J. Y. Sung, *Neurosci. Lett.* **2006**, *402*, 154–158. DOI: 10.1016/j.neulet.2006.03.068
[12] K. McDonald, *Study shows potential for using algae to produce human therapeutic proteins, University of California*, Press release, San Diego News, **2010**. www.ucsdnews.ucsd.edu/archive/newsrel/science/03-08TherapeuticProteins.asp
[13] P. Spolaore, C. Joannis-Cassan, E. Duran, *J. Biosci. Bioeng.* **2006**, *101*, 87–96. DOI: 10.1263/jbb.101.87
[14] B. A. Neilan, *Curr. Iss. Mol. Biol.* **2002**, *4*, 1–11.
[15] H. Kharkawal, D. D. Joshi, P. Panthari, M. K. Pant, *Asian. J. Pharm. Clin. Res.* **2012**, *5*, 1–4.
[16] D. M. M. Kleinegris, M. Janssen, W. A. Brandenburg, R. H. Wijffels, *Enzyme Microb. Technol.* **2011**, *48*, 253–259. DOI: 10.1016/j.enzmictec.2010.11.005
[17] S. Rabbani, P. Beyer, J. Von Lintig, P. Hugueney, H. Kleinig, *Plant. Physiol.* **1998**, *116*, 1239–1248. DOI: 10.1007/s002530100702
[18] M. C. García-Malea, F. G. Acién, E. del Río, J. M. Fernández, M. C. Cerón, M. G. Guerrero, E. Molina-Grima, *Biotechnol Bioeng.* **2009**, *102*, 651–657. DOI: 10.1002/bit.22076
[19] A. R. Domínguez-Bocanegra, L. I. Guerrero, F. M. Jerónimo, A. T. Campocosio, *Biores. Technol.* **2004**, *92*, 209–214. DOI: 10.1016/j.biortech.2003.04.001
[20] M. Olaizola, *J. Appl. Phycol.* **2000**, *12*, 499–506. DOI: 10.1023/A:1008159127672
[21] R. L. Mendes, H. L. Fernandes, J. P. Coelho, E. C. Reis, J. M. S. Cabral, J. M. Novais, A. F. Palabra, *Food. Chem.* **1995**, *53*, 99–103. DOI: 10.1016/0308-8146(95)95794-7
[22] K. Abe, H. Hattor, M. Hiran, *Food. Chem.* **2005**, *100*, 656–661. DOI: 10.1016/j.foodchem.2005.10.026
[23] M. D. Macías-Sánchez, M. Fernandez-Sevilla, F. G. Acién-Fernández, M. C. Cerón-Garcia, E. Molina-Grima, *Food. Chem.* **2010**, *123*, 928–935.DOI: 10.1016/j.foodchem.2010.04.076
[24] J. A. Del Campo, J. Moreno, H. Rodríguez, M. A. Vargas, J. Rivas, M. G. Guerrero, *J. Biotechnol.* **2000**, *76*, 51–59.
[25] J. A. Del Campo, H. Rodríguez, J. Moreno, M. A. Vargas, J. Rivas, M. G. Guerrero, *J. Biotechnol.* **2001**, *85*, 289–295. DOI: 10.1016/S0168-1656(99)00178-9
[26] P. F. Ip, F. Chen, *Process. Biochem.* **2005**, *40*, 733–738. DOI: 10.1016/j.procbio.2004.01.039
[27] D. Lagarde, L. Beuf, W. Vermaas, *Appl. Environ. Microbiol.* **2000**, *66*, 64–72. DOI: 10.1128/AEM.66.1.64-72.2000
[28] D. Singh, M. Puri, S. Wilkens, A. S. Mathur, D. K. Tuli, C. J. Barrow, *Bioresour. Technol.* **2013**, *143*, 308–314. DOI: 10.1016/j.biortech.2013.06.006
[29] S. M. Kim, Y. J. Jung, O. N. Kwon, K. H. Cha, B. H. Um, D. Chung, C. H. Pan, *Appl. Biochem. Biotechnol.* **2012**, *166*, 1843–1855. DOI: 10.1007/s12010-012-9602-2
[30] P. Crupi, A. T. Toci, S. Mangini, F. Wrubl, L. Rodolfi, M. R. Tredici, A. Coletta, D. Antonacci, *Rapid Commun. Mass Spectrom.* **2013**, *27*, 1027–1035. DOI: 10.1002/rcm.6531
[31] J. C. Kwan, M. Teplitski, S. P. Gunasekera, V. J. Paul, H. Luesch, *J. Nat. Prod.* **2010**, *73*, 463–466. DOI: 10.1021/np900614n
[32] N. Hatae et al., *Med. Chem. Res.* **2014**, *23*, 4956–4961. DOI: 10.1007/s00044-014-1061-6
[33] R. Challouf, L. Trabelsi, R. B. Dhieb, O. El Abed, A. Yahia, K. Ghozzi, J. B. Ammar, H. Omran, H. B. Ouada, *Braz. Arch. Biol. Technol.* **2011**, *54*, 831–838. DOI: 10.1590/S1516-89132011000400024
[34] I. Sadovskaya, A. Souissi, S. Souissi, T. Grard, P. Lencel, C. M. Greene, S. Duin, P. S. Dmitrenok, A. O. Chizhov, A. S. Shashkov, *Carbohydr. Polym.* **2014**, *111*, 139–148. DOI: 10.1016/j.carbpol.2014.04.077
[35] S. Y. Bae, J. H. Yim, H. K. Lee, S. Pyo, *Int. Immunopharmacol.* **2006**, *6*, 473–484. DOI: 10.1016/j.intimp.2005.09.009
[36] X. Wang, X. Zhang, *Biotechnol. Prog.* **2013**, *29*, 681–687. DOI: 10.1002/btpr.1725
[37] W. S. Jo, Y. J. Cho, H. J. Kim, B. Y. Nam, S. H. Hong, G. A. Lee, S. W. Lee, S. Y. Seo, M. H. Jeong, *Food. Sci. Biotechnol.* **2010**, *19*, 1519–1528. DOI: 10.1007/s10068-010-0216-6
[38] J. M. Nauroth, Y. C. Liu, M. Van Elswyk, R. Bell, E. B. Hall, G. Chung, L. M. Arterburn, *Lipids* **2010**, *45*, 375–384. DOI: 10.1007/s11745-010-3406-3
[39] R. C. Robertson, F. Guiheneuf, B. Bahar, M. Schmid, *Mar. Drugs* **2015**, *13*, 5402–5424. DOI: 10.3390/md13085402
[40] S. Guzmán, A. Gato, M. Lamela, M. Freire-Garabal, J. M. Calleja, *Phytother. Res.* **2003**, *17*, 665–670. DOI: 10.1002/ptr.1227
[41] S. M. Matsui, N. Muizzudin, S. M. Arad, K. Marenus, *Appl. Biochem. Biotechnol.* **2003**, *104*, 13–22. DOI: 10.1385/ABAB:104:1:13
[42] H. H. Abd El-Baky, F. K. El-Baz, G. S. El Baroty, *J. Sci. Food. Agric.* **2010**, *90*, 299–303. DOI: 10.1002/jsfa.3815
[43] T. S. Cha, C. F. Chen, W. Yee, A. Aziz, S. H. Loh, *J. Microbiol. Methods* **2011**, *84*, 430–434. DOI: 10.1016/j.mimet.2011.01.005
[44] L. A. Meirless, A. C. Guedes, F. X. Malcata, *J. Agric. Food. Chem.* **2003**, *51*, 2237–2241. DOI: 10.1021/jf025952y
[45] C. C. Parrish, G. Bodennec, P. Gentien, *Phytochem.* **1998**, *47*, 783–787. DOI: 10.1016/S0031-9422(97)00661-4
[46] Z. Hossain, H. Kurihara, M. Hosokawa, K. Takahashi, *In Vitro Cell. Dev. Biol.: Anim.* **2005**, *41*, 154–159. DOI: 10.1290/0409058.1
[47] N. Maeda, Y. Kokai, S. Ohtani, T. Hada, H. Yoshida, Y. Mizushina, *Food. Chem.* **2009**, *112*, 205–210. DOI: 10.1016/j.foodchem.2008.05.059
[48] S. Santoyo et al., *J. Appl. Phycol.* **2012**, *24*, 731–741. DOI: 10.1007/s10811-011-9692-1
[49] M. Kim et al., *Antiviral Res.* **2012**, *93*, 253–259. DOI: 10.1016/j.antiviral.2011.12.006

[50] J. B. Lee et al., *Biol. Pharm. Bull.* **2006**, *29*, 2135–2139. DOI: 10.1248/bpb.29.2135
[51] J. H. Yim et al., *Mar. Biotechnol.* **2004**, *6*, 17–25. DOI: 10.1007/s10126-003-0002-z
[52] H. M. Najdenski et al., *Int. J. Food. Sci. Technol.* **2013**, *48*, 1533–1540. DOI: 10.1111/ijfs.12122
[53] M. F. Raposo, A. .M. de Morais, R. M. de Morais, *Life Sci.* **2014**, *101*, 56–63. DOI: 10.1016/j.lfs.2014.02.013
[54] W. M. Bishop, M. Z. Zubeck, *J. Nutr. Food. Sci.* **2012**, *2*, 5. DOI: 10.4172/2155-9600.1000147
[55] S. Leu, S. Boussiba, *Ind. Biotechnol.* **2014**, *10 (3)*, 169–183. DOI: 10.1089/ind.2013.0039
[56] K. Abe, N. Nishmura, M. Hirano, *J. Appl. Phycol.* **1999**, 11, 33–36. DOI: 10.1023/A:1008195710981
[57] www.mercola.com (Accessed on April 15, 2016)
[58] O. P. Ward, A. Singh, *Process Biochem.* **2005**, *40*, 3627–3652. DOI: 10.1016/j.procbio.2005.02.020
[59] N. A. Handayania, D. Ariyantib, H. Hadiyanto, *Int. J. Sci. Eng.* **2011**, *2 (1)*, 13–16.
[60] I. P. S. Fernando, J. W. Nah, Y. J. Jeon, *Environ. Toxicol. Pharmacol.* **2016**, *48*, 22–30. DOI: 10.1016/j.etap.2016.09.023
[61] S. Danielle, B. Robert, A. Shaker, A. Mousa, *Am. Soc. Adv. Nutr.* **2012**, *3*, 1–7.
[62] J. C. McCann, B. N. Ames, *Am. J. Clin. Nutr.* **2005**, *82 (2)*, 281–295.
[63] A. R. Medina, L. E. Cerdán, A. G. Giménez, B. C. Páez, M. J. I. González, E. M. Grima, *J. Biotechnol.* **1999**, *70*, 379–391.
[64] N. W. Kerby, W. D. P. Stewart, in *Biochemistry of the Algae and Cyanobacteria* (Eds: L. J. Rogers, J. R. Gallon), Oxford University Press, Oxford **1987**.
[65] N. Kalogeropoulos, A. Chiou, E. Gavala, M. Christea, N. K. Andrilkopoulos, *Food. Res. Int.* **2010**, *43*, 2006–2013.
[66] A. R. Medina, E. M. Grima, A. G. Giménez, M. J. I. González, *Biotechnol. Adv.* **1997**, *16*, 517–580.
[67] O. Ana, G. Digna, F. Jaime, *J. Appl. Phycol.* **1997**, *9*, 465–469.
[68] Y. Jiang, F. Chen, S. Z. Liang, *Process Biochem.* **1999**, *34*, 633–637.
[69] R. Pangestuti, S. K. Kim, *J. Funct. Foods* **2011**, *3 (4)*, 255–266.
[70] M. F. De Jesus Raposo, R. M. S. C. De Morais, A. M. M. B. De Morais, *Life Sci.* **2013**, *93 (15)*, 479–486.
[71] M. Abidov, Z. Ramazanov, R. Seifulla, S. Grachev, *Diabetes. Obes. Metab.* **2010**, *12 (1)*, 72–81.
[72] M. Plaza, M. Herrero, A. Alejandro Cifuentes, E. Ibáñez, *J. Agric. Food Chem.* **2009**, *57 (16)*, 7159–7170.
[73] E. Ibáñez, A. Cifuentes, *J. Sci. Food Agric.* **2013**, *93 (4)*, 703–709.
[74] W. L. Chu, *Int. e-J. Sci. Med. Edu.* **2012**, *6 (Suppl 1)*, S24–S37.
[75] A. L. Stephenson, E. Kazamia, J. S. Dennis, C. J. Howe, S. Scott, A. G. Smith, *Energy Fuels* **2010**, *24*, 4062–4077. DOI: 10.1021/ef1003123
[76] J. U. Grobbelaar, *J. Appl. Phycol.* **2009**, *21 (5)*, 519–522. DOI: 10.1007/s10811-008-9372-y
[77] R. Harun, M. Singh, G. M. Forde, M. K. Danquah, *Renewable Sustainable Energy Rev.* **2010**, *14*, 1037–1047. DOI: 10.1016/j.rser.2009.11.004
[78] J. C. M. Pires, M. C. M. Alvim-Ferraz, F. G. Martins, M. Simões, *Renewable Sustainaböe Energy Rev.* **2012**, *16*, 3043–3053. DOI: 10.1016/j.rser.2012.02.055
[79] T. S. Vo, S. K. Kim, *Mar. Drugs* **2010**, *8*, 2871–2892. DOI: 10.3390/md8122871
[80] S. P. Mayfield, S. E. Franklin, *Vaccine* **2005**, *23*, 1828–1832. DOI: 10.1016/j.vaccine.2004.11.013
[81] M. Tran, C. Van, D. J. Barrera, P. L. Petterson, C. D. Peinado, J. Bui, S. P. Mayfield, *Proc. Nat. Acad. Sci. USA* **2013**, *110 (1)*, E15–E22. DOI: 10.1073/pnas.1214638110
[82] N. Munjal, A. J. Garzon-Sanabria, K. W. Quinones, J. Gregory, Z. L. Nikolov, *Processes* **2014**, *2*, 625–638. DOI: 10.3390/pr2030625
[83] F. Guiheneuf, M. Fouqueray, V. Mimouni, L. Ulmann, B. Jacquette, G. Tremblin, *J. Appl. Phycol.* **2010**, *22*, 629–638. DOI: 10.1007/s10811-010-9503-0
[84] J. Riyahi, Y. Haouazine, R. Akallal, A. Mouradi, A. Creach, R. M. Givernaudi, *Bull. Soc. Pharm. Bordeaux* **2007**, *146*, 235–250.
[85] J. Courtois, *Curr. Opin. Microbiol.* **2009**, *12*, 261–273. DOI: 10.1016/j.mib.2009.04.007
[86] J. A. Del Campo, H. Rodríguez, J. Moreno, M. A. Vargas, J. Rivas, M. G. Guerrero, *Appl. Microbiol. Biotechnol.* **2004**, *64*, 848–854. DOI: 10.1007/s00253-003-1510-5
[87] P. Feng, Z. Deng, L. Fan, Z. Hu, *J. Biosci. Bioeng.* **2012**, *114 (4)*, 405–410. DOI: 10.1016/j.jbiosc.2012.05.007
[88] R. Y. N. Ma, F. Chen, *Process Biochem.* **2001**, *36*, 1175–1179. DOI: 10.1016/S0032-9592(01)00157-1
[89] G. Dragone, D. B. Fernandes, A. P. Abreu, A. A. Vicente, J. A. Teixeira, *Appl. Energ.* **2011**, *88*, 3331–3335. DOI: 10.1016/j.apenergy.2011.03.012
[90] S. Boussiba, W. Bing, J. P. Yuan, A. Zarka, F. Chen, *Biotechnol. Lett.* **1999**, *21*, 601–604. DOI: 10.1023/A:1005507514694
[91] A. R. Dominguez-Bocanegra, I. L. Guerrero, F. J. Martinez, A. C. Tomasini, *Bioresour. Technol.* **2004**, *92*, 209–214. DOI: 10.1016/j.biortech.2003.04.001
[92] S. Boussiba, A. Vonshak, *Plant Cell. Physiol.* **1991**, *32*, 1077–1082. DOI: 10.1093/oxfordjournals.pcp.a078171
[93] S. K. Saha, E. McHugh, J. Hayes, S. Moane, D. Walsh, P. Murray, *Bioresour. Technol.* **2013**, *128*, 118–124. DOI: 10.1016/j.biortech.2012.10.049
[94] X. Shi, Z. Wu, F. Chen, *Mol. Nutr. Food. Res.* **2006**, *50*, 763–768. DOI: 10.1002/mnfr.200600037
[95] M. C. Cerón-García, M. D. Macías-Sánchez, A. Sánchez-Mirón, F. García-Camacho, E. Molina-Grima, *Appl. Energy.* **2013**, *103*, 341–349. DOI: 10.1016/j.apenergy.2012.09.054
[96] P. P. Lamers et al., *Biotechnol. Bioeng.* **2010**, *106*, 638–648. DOI: 10.1002/bit.22725
[97] P. P. Lamers, M. Janssen, R. C. H. De Vos, R. J. Bino, R. H. Wijffels, *J. Biotechnol.* **2012**, *162*, 1–7. DOI: 10.1016/j.jbiotec.2012.04.018
[98] A. Ben-Amotz, T. G. Tornabene, W. H. Thomas, *J. Phycol.* **1985**, *21*, 72–81. DOI: 10.1111/j.0022-3646.1985.00072.x
[99] B. D. Fernandes, A. Mota, J. A. Teixeira, A. A. Vicente, *Biotechnol. Adv.* **2015**, *33*, 1228–1245. DOI: 10.1016/j.biotechadv.2015.03.004

[100] A. A. Tsygankov, *Appl. Biochem. Microbiol.* **2013**, *37*, 333–341. DOI: 10.1023/A:1010266116747
[101] J. Del Campo, M. Garcia-Gonzalez, M. Guerrero, *Appl. Microbiol. Biotechnol.* **2007**, *74*, 1163–1174. DOI: 10.1007/s00253-007-0844-9
[102] Y. H. Zhu, J. G. Jiang, *Eur. Food. Res. Technol.* **2008**, *227*, 953–959. DOI: 10.1007/s00217-007-0789-3
[103] O. Pulz, *Appl. Microbiol. Biotechnol.* **2001**, *57*, 287–293. DOI: 10.1007/s002530100702
[104] X. M. Shi, F. Chen, *Biotechnol. Progress* **2002**, *18*, 723–727. DOI: 10.1021/bp0101987
[105] F. Chen, M. R. Johns, *J. Appl. Phycol.* **1991**, *3*, 203–209. DOI: 10.1007/BF00003578
[106] C. D. Kang, J. S. Lee, T. H. Park, S. J. Sim, *Appl. Microbiol. Biotechnol.* **2005**, *68*, 237–241. DOI: 10.1007/s00253-005-1889-2
[107] O. Perez-Garcia, F. M. E. Escalante, L. E. de-Bashan, Y. Bashan, *Water. Res.* **2011**, *45*, 11–36. DOI: 10.1016/j.watres.2010.08.037
[108] M. C. C. Garcia, A. S. Miron, J. M. F. Sevilla, M. Grima, E. Garcia, F. Camacho, *Process. Biochem.* **2005**, *40*, 297–305. DOI: 10.1016/j.procbio.2004.01.016
[109] A. P. Abreu, B. Fernandes, A. .A. Vicente, J. Teixeira, G. Dragone, *Bioresour. Technol.* **2012**, *118*, 61–66. DOI: 10.1016/j.biortech.2012.05.055
[110] M. R. Andrade, J. A. V. Costa, *Aquaculture* **2007**, *264*, 130–134. DOI: 10.1016/j.aquaculture.2006.11.021
[111] U. Emeka, G. I. Ndukwe, K. B. Mustapha, R. I. Ayo, *J. Algal. Biomass. Utln.* **2012**, *3*, 14–32.
[112] W. K. W. Li, in *Primary Productivity in the Sea* (Ed: P. G. Falkaowski), Plenum Press, New York **1980**, 259–279.
[113] G. I. Ndukwe, K. B. Mustapha, R. I. Ayo, *J. Algal Biomass Utln.* **2012**, *3*, 14–32.
[114] Y. Wang, T. Chen, S. Quin, *J. Chem. Technol. Biotechnol.* **2013**, *88*, 651–657. DOI: 10.1002/jctb.3881
[115] H. J. Kim, Y. K. Choi, J. J. Hyeon, S. K. Bhatia, Y. H. Kim, Y. G. Kim, K. W. Choi, H. J. Kim, S. H. Lee, Y. K. Lee, Y. H. Yang, *Biomass. Bioenergy.* **2015**, *74*, 213–219. DOI: 10.1016/j.biombioe.2015.01.012
[116] B. F. Cordero, I. Couso, R. León, H. Rodríguez, M. A. Vargas, *Appl. Microbiol. Biotechnol.* **2011**, *91*, 341–351. DOI: 10.1007/s00253-011-3262-y
[117] M. M. Loera-Quezada, L. A. Leyva-González, G. Velázquez-Juárez, L. Sanchez-Calderón, M. D. Nascimento, D. López-Arredondo, L. Herrera-Estrella, *Plant. Biotechnol. J.* **2016**, *14*, 2066–2076. DOI: 10.1111/pbi.12564
[118] J. Liu, H. Gerken, J. Huang, F. Chen, *Process. Biochem.* **2013**, *48*, 788–795. DOI: 10.1016/j.procbio.2013.04.020
[123] J. Steinbrenner, G. Sandmann, *Appl. Environ. Microbiol.* **2006**, *72*, 7477–7484. DOI: 10.1128/AEM.01461-06
[119] I. Couso, M. Vila, H. Rodriguez, M. A. Vargas, R. Leon, *Biotechnol. Progress* **2011**, *27*, 54–60. DOI: 10.1002/btpr.527
[121] J. Liu, Z. Sun, H. Gerken, J. Huang, Y. Jiang, F. Chen, *Appl. Microbiol. Biotechnol.* **2014**, *98*, 5069–5079. DOI: 10.1007/s00253-014-5593-y
[124] K. E. Apt, F. T. Allnutt, D. J. Kyle, J. C. Lippmeier, *US Patent 7939710*, **2011**.
[120] A. Doebbe, J. Rupprecht, J. Beckmann, J. H. Mussgnug, A. Hallmann, B. Hankamer, O. Kruse, *J. Biotechnol.* **2007**, *131*, 27–33. DOI: 10.1016/j.jbiotec.2007.05.017
[122] L. Fang, H. X. Lin, C. S. Low, M. H. Wu, Y. Chow, Y. K. Lee, *Plant Biotechnol. J.* **2012**, *10*, 1129–1135. DOI: 10.1111/pbi.12000
[125] E. W. Becker, *Biotechnol. Adv.* **2007**, *25*, 207–210. DOI: 10.1016/j.biotechadv.2006.11.002
[126] B. R. Draaisma, H. R. Wijffels, P. M. E. Slegers, L. B. Brentner, A. Roy, J. M. Barbosa, *Curr. Opin. Biotechnol.* **2013**, *24*, 169–177. DOI: 10.1016/j.copbio.2012.09.012
[127] T. M. Le, A. C. Knulst, H. Rockmann, *Food. Chem. Toxicol.* **2014**, *74*, 309–310. DOI: 10.1016/j.fct.2014.10.024
[128] K. W. Gellenbeck, *J. Appl. Phycol.* **2012**, *24*, 309–313. DOI: 10.1007/s10811-011-9722-z
[129] O. G. Mouritsen, *Seaweeds: Edible, Available, and Sustainable*, University of Chicago Press, Chicago **2013**.
[130] C. de Gaillande, C. Payri, G. Remoissenet, M. Zubia, *J. Appl. Phycol.* **2016**, 1–18. DOI: 10.1007/s10811-016-0912-6
[131] D. Cheney, in *Seaweeds in Health and Disease* (Eds: J. Fleurence, I. Levine), Elsevier, Amsterdam **2016**.
[132] A. Belay, in *Spirulina in Human Nutrition and Health* (Eds: M. E. Gershwin, A. Belay), CRC Press, Boca Raton, FL **2008**.
[133] N. J. Szabo, R. A. Matulka, T. Chan, *Food. Chem. Toxicol.* **2013**, *59*, 34–45. DOI: 10.1016/j.fct.2013.05.035
[134] M. Nicoletti, *Foods* **2016**, *5*, 54. DOI: 10.3390/foods5030054
[135] www.transparencymarketresearch.com/global-nutraceuticals-product-market.html (Accessed on March 04, 2017)
[136] W. W. Carmichael, C. Drapeau, D. M. Anderson, *J. Appl. Phycol.* **2000**, *12*, 585–595.
[137] www.cleantick.com/users/amandaleah/pages/high-value-end-products-from-algae/updates/10407 (Accessed on March 04, 2017)
[138] C. Gudin, C. Thepenier, *Adv. Biotechnol. Process.* **1986**, *6*, 73–110.
[139] P. J. K. Samudrala, V. K. Garlapati, A. Dash, R. Banerjee, P. Scholz, *Algal Res.* **2017**, *21*, 138–147. DOI: 10.1016/j.algal.2016.11.014

