[Frontiers In Bioscience, Scholar, 9, 319-342, June 1, 2017]

High-value compounds from microalgae with industrial exploitability – A review

Muhammad Bilal[1], Tahir Rasheed[2], Ishtiaq Ahmed[3], Hafiz M.N. Iqbal[4]

[1]State Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China, [2]The School of Chemistry and Chemical Engineering, State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, Shanghai 200240, China, [3]School of Medical Science, Gold coast campus, Griffith University, Southport QLD 4222, Australia, [4]School of Engineering and Science, Tecnologico de Monterrey, Campus Monterrey, Ave. Eugenio Garza Sada 2501, Monterrey, N.L., CP 64849, Mexico

TABLE OF CONTENTS

1. ABSTRACT

From the past several years, algal biotechnology has gained noticeable interests among research-based organizations and industrial sectors. Recent studies have provided considerable evidence that microalgae-derived bioactive compounds could play a vital role in bio- and non-bio sectors of the modern world. Microalgae-based industrial exploitability ranges from basic biomass-based food and feeds nutraceuticals to high-value pharmaceuticals, cosmeceuticals, ecological and biomedical applications. With ever increasing scientific knowledge, social and environmental awareness, bio-inspired synthesis of microalgae-based green products, and recent advancements in algal biotechnology will extend the utilization of microalgae into new areas. Microalgae offer high biodiversity with an enormous potential to produce structurally complex yet bioactive compounds which are either impossible or difficult to produce via synthetic routes. In this paper, a range of value-added bioactive compounds from microalgae with industrial potentialities has been reviewed. The contribution ended with a critical description of the main research gaps and envisioned with future considerations to progress further in this exciting era of research.

2. INTRODUCTION

Recently, microalgae are gaining considerable attention as a promising source for the sustainable production of biotechnologically- pertinent compounds including fatty acids, carotenoids, vitamins, and others (1, 2). Microalgae-derived secondary metabolites have a great perspective for industrial growth and development, since they synthesise bioactive molecules, such as antioxidant, antiviral, antibacterial, antifungal, anti-inflammatory, antitumor, and antimalarial (3). Despite, numerous obvious advantages offered by microalgae over plants, including rapid growth rates, and lack of competition for resources used for food crops (use of fresh water and arable lands), the isolation of natural products from microalgae remain largely unexplored as compared with terrestrial plants (4). The GRAS (Generally recognized as safe) status granted by the United States Food Drug Administration (FDA) unlocks the path wide for the potential application of microalgae as an attractive cell factory. This "safe to consume" status is of great importance for industrial perspective since it trims downstream purification costs of proteins or compounds (5, 6).

In the past five decades, industrial-scale cultivation of microalgae has dramatically increased worldwide, and most of the applications are commercialized into different sectors (1, 7). To date, although genetically unmodified microalgae are exploited for synthesizing specific metabolites, it is believed that strain improvement is mandatory to upgrade the feasibility of industrial throughput. Therefore, there is a need to develop novel strains with desirable features including elevated yield, faster growth as well as low liability to lights and heat, etc. Different approaches such as mutagenesis, adaptive laboratory evolution, and genetic engineering have been envisioned as strategies for deciphering algal cell factories in the production of bioactive compounds. Moreover, synthetic and systems biology perspectives are appearing with increased algal productivity of value added products (8, 9).

3. MICROALGAE AND HIGH-VALUE COMPOUNDS

3.1. Microalgae and Pigments – carotenoids and phycobilins

Natural pigments contribute a vital part in the photosynthesis processes along with other pigmentation-based activities in microalgae. Likewise, other biological sources in nature, microalgae also offer numerous bioactive functionalities including neuroprotective, anticancer, antiangiogenic, antioxidant, anti-obesity, and anti-inflammatory, etc. (10, 11). Chlorophylls, phycobilins, and carotenoids are the three classes of pigments that occur in microalgae. Chlorophylls are fat-soluble greenish pigments with a porphyrin ring that are responsible for converting solar energy into chemical energy during photosynthesis (12). Most microalgae possess chlorophyll a. However, some other classes of algae like Dinophyta (dinoflagellates) contain chlorophyll b and c (13). Chlorophyllins is a derivative of chlorophyll in which the magnesium is replaced by sodium or copper (14). Chlorophyllins have been used for multi-purposes, such as a dietary supplement and to regulate body odor of geriatric patients, etc. (15). Many studies revealed that chlorophyll and chlorophyllin also exhibit antimutagenic and anticarcinogenic functionality (16–18).

Phycobilins are water-soluble proteins. They can be easily isolated and purified in a high proportion. During photosynthesis, phycobilins play a vital role in capturing light. Four classes of phycobilins namely (1) allophycocyanin (green-bluish in color), (2) phycocyanin (blue in color), (3) phycoerythrin (purple in color), and (4) phycoerythrocyanin (orange in color) are mainly produced by red algae (Rhodophyta and Glaucophytes); allophycocyanin (green-bluish), phycocyanin (blue), phycoerythrin (purple), and phycoerythrocyanin (orange) (12, 19). Phycobilins are widely used in industrial sectors particularly in immunology laboratories due to their characteristic absorption properties. Phycobilins are frequently employed as fluorescent markers in molecular biology and immune assays and as fluorescent dyes for microscopy purposes (20). Considerably, most of the patents published by the United States of America on these substances are primarily based on fluorescent applications, while Japanese has published patents regarding production, purification, and therapeutic as well as diagnostic purposes (19). The use of fluorescent probes as labels in immunoassay has increased dramatically in current years. Fluorescence immunoassay would be of even greater value, however, if probes were available with properties like high solubility, etc. Furthermore, they can easily be conjugated to specific molecules so as to be useful in the context of biological assay procedures. Figure 1 illustrates fluorescent based applications of Phycobiliproteins.

Carotenoids are fat-soluble in nature with multi-color appearances i.e. varying form brown, red, and orange to yellow. Together with providing the photoprotection to the photosynthetic systems, the carotenoids also perform the function of absorbing light in the UV-visible region, where chlorophyll does not absorb efficiently during photosynthesis. This photoprotection mechanism eliminates the formation of reactive oxygen species (ROS) making the carotenoids as an excellent antioxidant (21). The *β*-carotene, lycopene, astaxanthin, zeaxanthin, violaxanthin and lutein are the main carotenoids of microalgae. Amongst these, the β-carotene, lutein, and astaxanthin are the most studied ones (22). The *β*-carotene is a precursor of vitamin A (retinol) with orange-yellowish color and widely used as a colorant for food or nutritional supplement. Natural carotenoids exhibit remarkable properties as compared to synthetic one. Therefore the demand for natural carotenoids is increasing day by day. For example, Jayappriyan

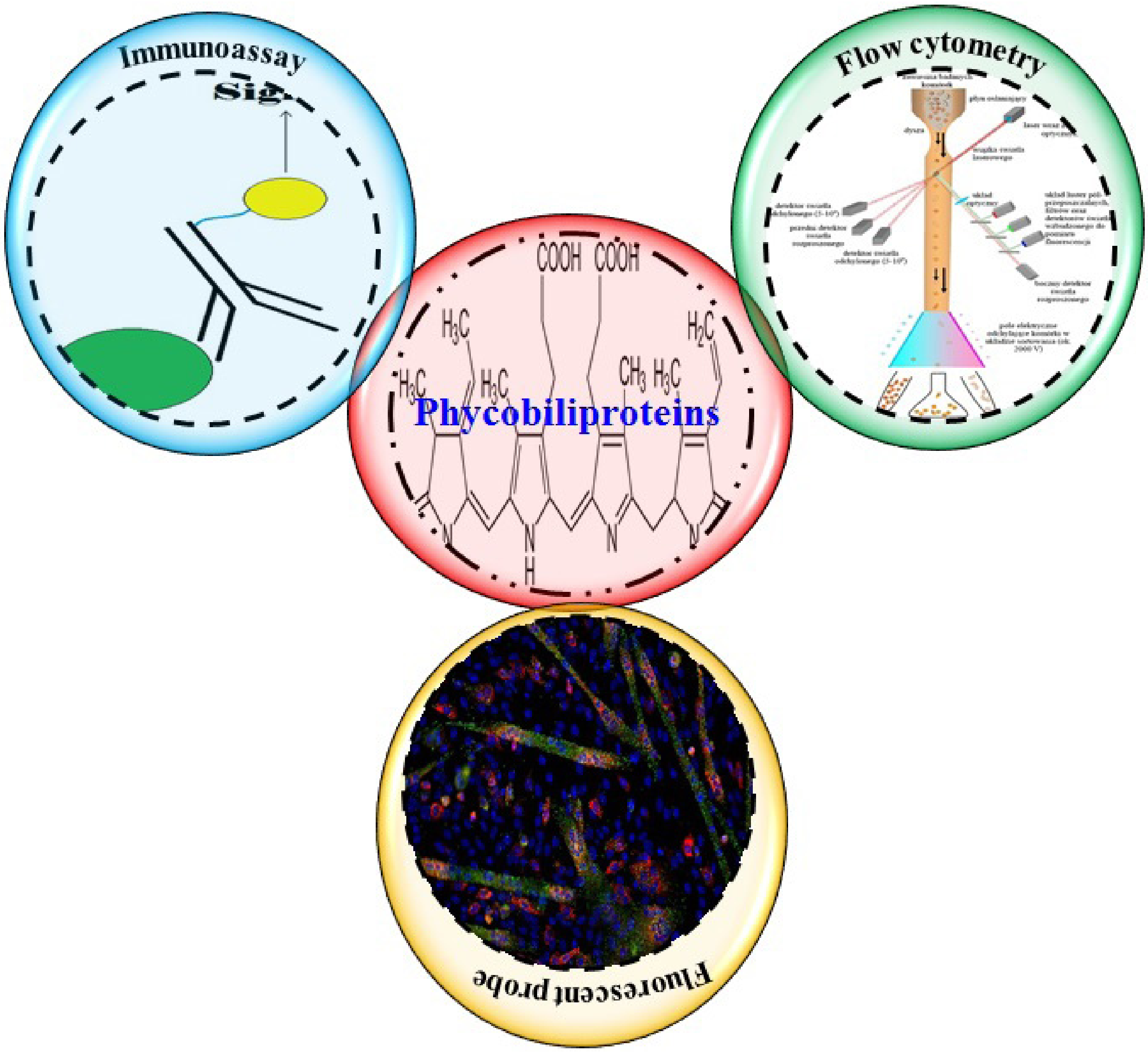

Figure 1. Phycobiliproteins and utility in the fluorescent based application.

et al. (23) reported that *Dunaliella salina* extracted *β*-carotene causes a high apoptosis rate in prostate cancer cells than the synthetic one. In the laboratory, Alencar and co-workers, (24) quantified β-carotene by cultivating cyanobacteria *Spirulina* and *Spirulina platensis* and found 14 times higher β-carotene in the bacteria. In contrast with other carotenoids, Astaxanthin has greater ability to sequester reactive oxygen free radicals and therefore, it can be used for various diseases, such as diabetes, heart, and chronic inflammatory diseases as well as in the prevention of some cancers (25, 26). Increased concentration of secondary carotenoids in the microalgae pointed out the improved cell survivability under oxidative stress generated by certain light conditions, UV-B and nutrients (27). Lutein, a pigment yellowish at lower concentrations and reddish-orange at higher concentrations, protects the tissues from free radicals (28). Lutein can also prevent atherosclerosis, cataracts, diabetic retinopathy, and retinal degeneration (29). Several researchers have been investigated the synthesis of lutein by different microalgae, such as *Muriellopsis sp., Chlorella zofingensis, Chlorella protothecosis, Scenedesmus almeriensi* (30–32).

3.2. Microalgae – polysaccharides

Economically, algal polysaccharides are the most important products obtained from algae (33). Figure 2 illustrates metabolic pathways for lipid and carbohydrate synthesis (34). A lot of reviews have been presented for the biochemical functioning and structural behavior of the polysaccharides (35–38). The alginic acid which is also referred to as Alginate is

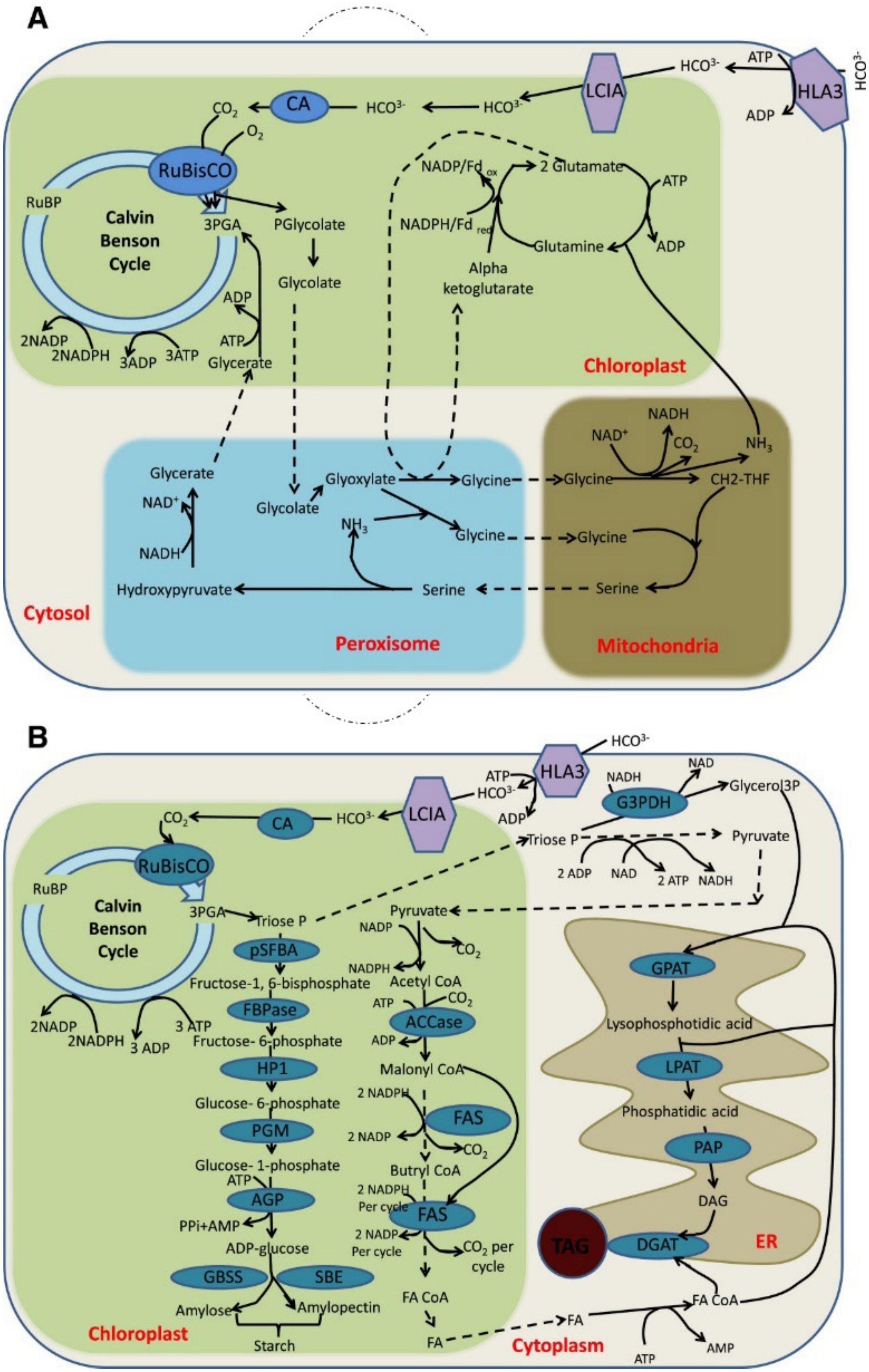

Figure 2. Metabolic pathways for lipid and carbohydrate synthesis. A. Outline of the Calvin-Benson and photorespiration cycles. B. Description of storage carbohydrate and TAG synthesis “Reproduce with permission from” (34).

a natural polysaccharide that is obtained mainly from 30 to 60% of brown algae on a dry weight basis. It accumulates in the plant as gel bodies after it combines with some minerals from the seawater. Several extraction techniques are used for the extraction of algal polysaccharides, considered as “green” such as MAE, and EAE (39, 40). Various properties like antitumor, antiviral, antioxidative, antibacterial, and anti-inflammatory are used for the characterization. The extraction of algal polysaccharides and their advance identification have proposed various applications in different sectors of the modern world including pharmaceutical, cosmeceuticals, nutraceuticals, and biomedical. Many of the polysaccharides obtained from algae including agar, alginate, and carrageenan, etc. have been commonly employed as functional ingredients or stabilizers in pharmaceutical and nutraceutical industries. Furthermore, many other polysaccharides from seaweeds have potent features for prebiotic exploitability. The algal polysaccharides are advantageous for human, animals as well as for plants. In an earlier study, Vera *et al.* (41) investigated various seaweed polysaccharides that includes ulvans from green algae (*Chlorophyta*), alginates, fucans, laminarin from brown algae (*Phaeophyta*), and carrageenans, porphyrin from red algae (*Rhodophyta*). In the same study, Vera and co-workers have observed stimulatory defense response along with anti-pathogen, in the plant, the potential of some algae-derived oligosaccharides.

Other potential applications of algal-based polysaccharides have also been reported as good metal ion chelators. It has been shown that seaweed polysaccharides are rich in functional groups that can bind microelement ions important in plant nutrition. The chelating properties of polysaccharides from seaweeds can be used in the production of new formulations-carriers of microelements in fertilizers. In textile printing, alginic acid is used to thicker the production paste which is then used by fabrics in a roller printing or a screen printing equipment. They are mostly used for reactive dyes because they combine chemically well with the cellulose of the fabrics. There are other thickeners such as starch that react with reactive dyes, but this causes lower color yields, and secondary products are not removed easily from the fabric, and since alginates do not react with them they can be removed or washed easily from the textiles. Although alginate is much more expensive than starch, however, it is a most convenient choice.

3.3. Microalgae – lipids

Lipids exist as polar and neutral molecules with poor solubility in water but are readily soluble in most of the organic solvents. Phospholipids and glycolipids are referred as polar lipids, whereas neutral lipids include acyl-glycerides (tri, di- and mono-glycerides) and free fatty acids (FFA). Neutral lipids act as a source of energy for microalgae, while polar lipids are used for the synthesis of the cell membrane. Nevertheless, there are some fatty-acid free components, i.e., pigments and steroids which are not transformed into biodiesel (42). Chlorophyta most studied for biodiesel production comprises *Auxenochlorella protothecoides, Chlorella vulgaris, Chlamydomonas rheinhardii*, and *Dunaliella salina.* These species accumulated the elevated production of lipids than that of other divisions presumably due to faster growth. Strains such as *Euglenophyta* and *Dinophyta* have the potential to be used for the production of biodiesel since their lipid production ability is greater than some *Chlorophyta*. Zanchett *et al.* (43) also evaluated the biodiesel production aptitude of *potentla Cyanophyta* because of their greater cell growth rate. Nonetheless, some toxic and carcinogenic substances (microcystin) are produced during the process. Since fatty acids influence the quality of biodiesel, oxidative stability, and contents of mono, di, and triglycerides, therefore evaluating the type of fatty acids is of great importance (44). The lipid metabolism starts with a common initial pool of molecules consisting of three carbons, such as 3-phosphoglycerate (3PG) and glyceraldehyde 3-phosphate (GAP) (45) (Figure 3).

3.4. Microalgae – fatty acids

The carboxylic acids with 4.0.-36 carbon atom chain length are known as fatty acids. Various researchers in microalgae have studied their composition. By nature, the fatty acids are categorized as saturated and unsaturated fatty acids. The fatty acids lacking multiple bonds are denoted as saturated (SFA), whereas the acids are exhibiting multiple bonds are termed as unsaturated fatty acid (USFA). The majority of the fatty acids of microalgae are mono-saturated (46). As the fatty acids greatly influence the quality of biodiesel, so their composition plays a vital role in the production of biodiesel. The presence of a lot of polyunsaturated fatty acids (PUFA) have a positive impact on the outflow properties, especially in cold weathers, but may negatively affect the oxidative stability (47). The latter problem can be resolved by using antioxidants (48). On the other hand, the large amounts of SFA possess remarkable combustion properties but can cause problems in the cold outflow (49). Various factors such as light intensity, nutrient concentration, carbon source, high salinity, and temperature may influence the lipid accumulation. However, stress in microalgae cultivation increases lipid accumulation but reduce growth rate, affecting lipid productivity (50). The cultivation of *Chlorella sp.* and *Scenedesmus sp.* in the media containing varying nutrients quantities was investigated by Zhan *et al.* (51). Enhanced lipid accumulation was noted in the media containing lesser nutrients in contrasted with the media having more nutrients for both microalgae.

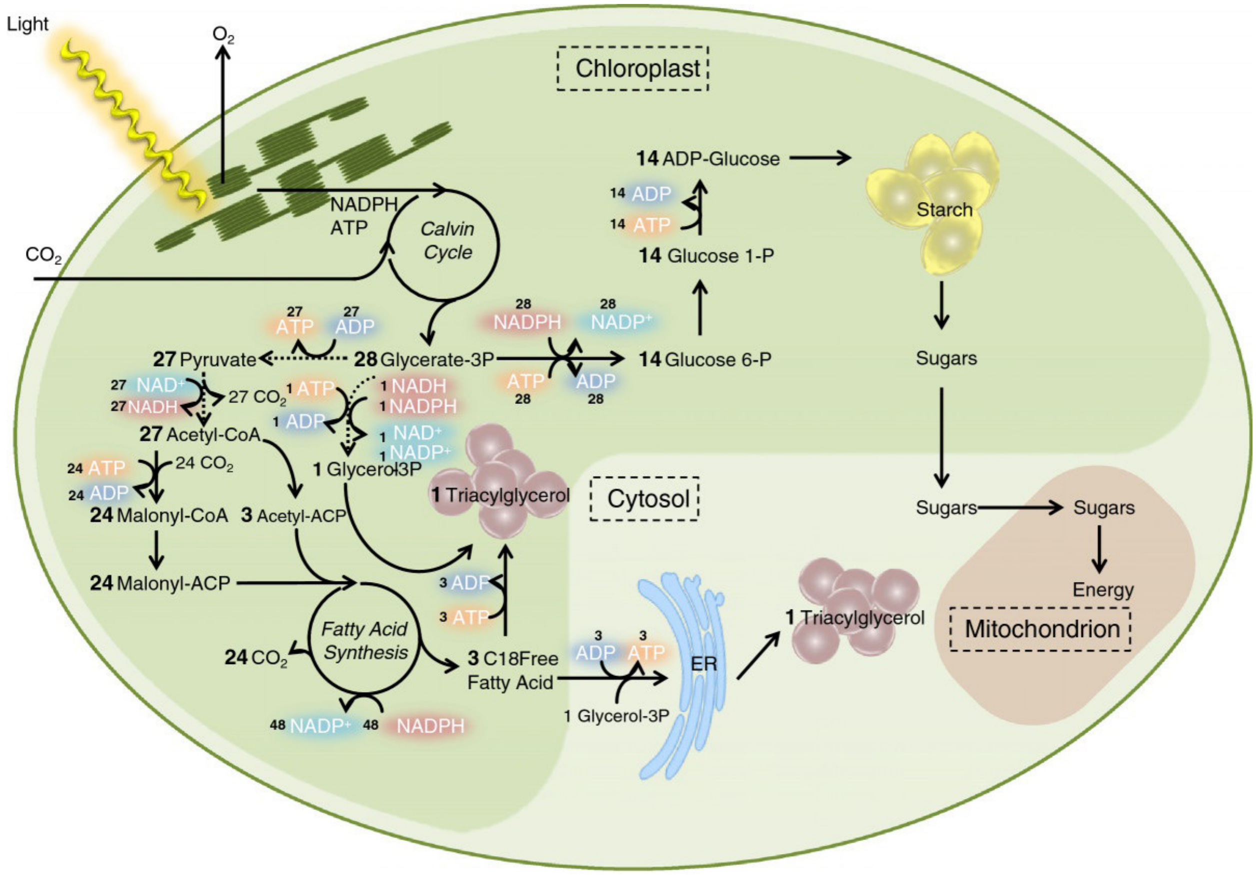

Figure 3. Simplified triacylglycerol and starch metabolism in green microalgae. The dashed lines are reactions that take place in the cytosol. Two possible ways for the formation of TAG molecules are shown following the postulated route in the chloroplasts or over the ER membranes in the cytosol. 3PG, 3-phosphoglycerate; ER, endoplasmic reticulum; TAG, triacylglycerol "Reproduce with permission from" (45).

Grama *et al.* (52) found an increase in lipid productivity of *Acutodesmus sp.* with increasing light intensity and determined the best conditions to be 600uE m^2/s with optimal lipid productivity of 85 mg/L/day. Earlier studies have also shown that mixed photoautotrophic cultures are more valuable and low cost, eliminating the need to add organic carbon sources. Therefore, mixed photoautotrophic cultures are more economically viable to produce biodiesel than autotrophic or heterotrophic cultures (53).

4. MICROALGAE – BIOTECHNOLOGICAL POTENTIALITIES

Algae have a great potential to produce a wide range of important biochemical for food, medical research and other uses and many exciting and important biochemical are yet to be discovered from microalgae. Figure 4 illustrates various biotechnological potentialities of microalgae.

4.1. Microalgae and proteomics

More recently developed sophisticated biotechnological approaches such as transcriptomics, metabolomics, nutrigenomics, proteomics and metagenomic profiling are now being applied to microalgae to elucidate their genomic and pharmacological interactions with bacteria (54–57). Transcriptomic-based studies on microalgae furnish valuable genomic data that may be used to ascertain algal species with significant antibiotic potential. Sequencing the transcriptomic data enables the identification and comparison of differentially-expressed genes in distinct cell populations, or in response to different environmental factors (58, 59). Recently, Hovde and coworkers, (60) described the sequencing of microalgae (*Chrysochromulina tobin*) transcriptomic data at seven different time points over a 24 h light/dark circadian cycle. A considerable difference in gene expression was recorded at all investigated time points for biological processes such as fatty acid biosynthesis. Among the genes identified, the defense-related genes were found to encode novel antibacterial peptides, potential antibiotics, and antibiotic extrusion proteins. The transcriptomic-based sequencing findings may provide budding routes for the biosynthesis of therapeutics and valued novel metabolites. Algal-extracted compounds with pronounced pharmacological

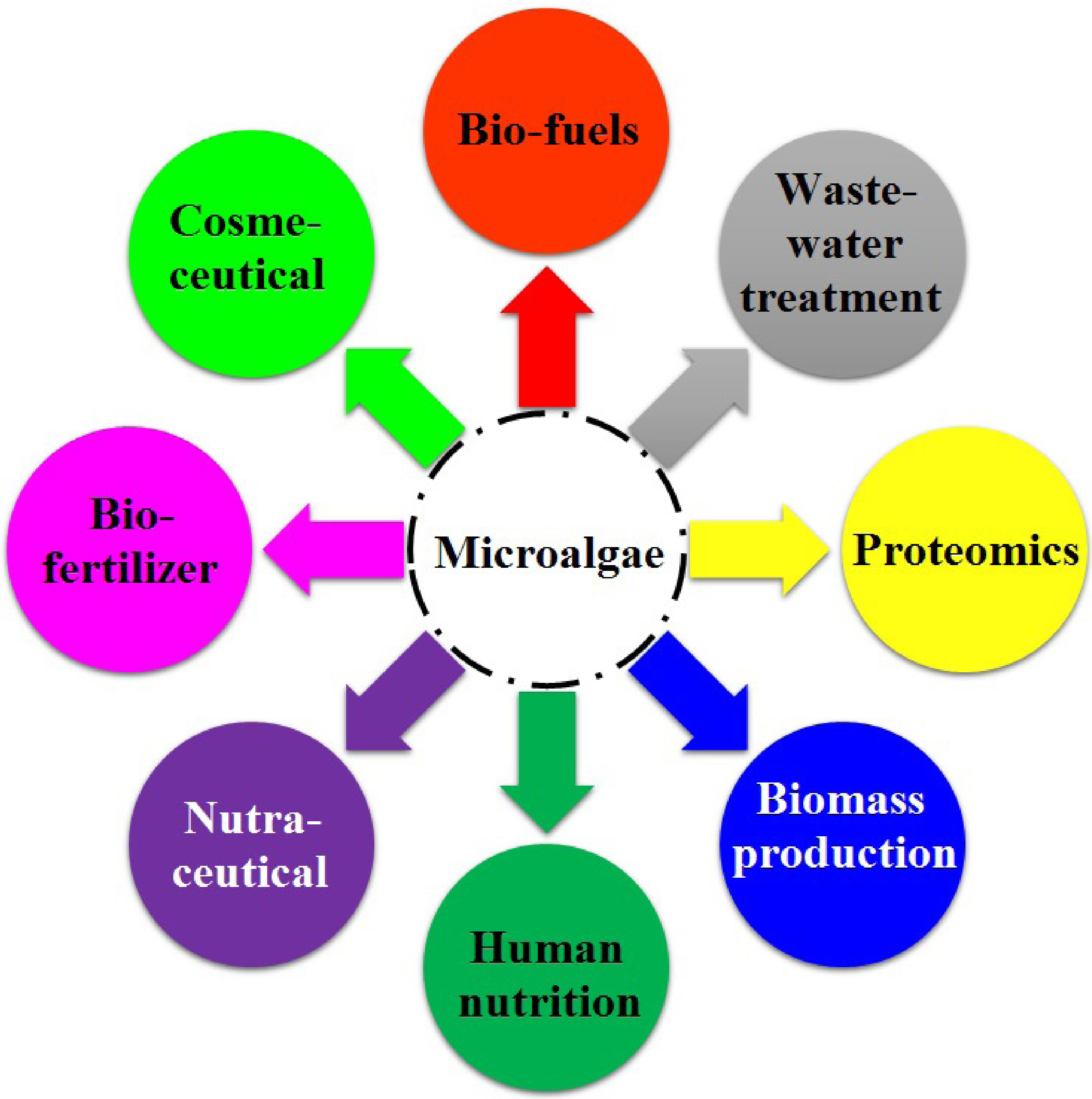

Figure 4. Biotechnological application of microalgae in different sectors.

interest were also documented by de Oliveira *et al.* (61) from the transcriptomic sequencing of the red seaweed, *Laurencia dendroidea*. Explicating the genomic mechanisms of pharmacologically bioactive metabolites from microalgae offers a potential for industrial and biotechnological interventions.

Algal biotechnology entails the application of microalgae as a platform to produce valuable products for specific purposes (62). This may implicate manipulating microalgae cells into overexpressing a secondary metabolite, or some compound of pharmacological significance by exploring environmental parameters such as pH, temperature or food accessibility. Algal biotechnology may also involve the insertion of a foreign gene into organisms to produce a new biomolecule or expression product having profound antibacterial potentialities such as terpenes or phlorotannins. The chloroplast transformation in *Chlamydomonas* is the first and best-studied model system in green algae since its genome has been completely sequenced (63). To date, numerous microalgal species have been transformed in a stable manner. For instance, a luciferase (*Luc*) and a homologous nitrate reductase gene have successfully been turned into Chlorella (64). The recombinant proteins have been produced stably by transforming five heterologous genes into the chloroplast of *D. tertiolecta* (65). Of most recent, Guzman-Zapata *et al.* (66) carried out the inheritable genetic transformation of chloroplasts into green microalga (*Chlamydomonas reinhardtii*) to potentially secrete useful recombinant proteins.

Literature survey revealed that most of the recent advances in genetically engineered microalgae had been endowed in *C. reinhardtii* using both chloroplast and nuclear transformation. The *C. reinhardtii* has been demonstrated to be a robust cell factory for the elevated synthesis of many important recombinant proteins, including antibodies, vaccines, protein therapeutics, enzymes, and additives, such as viral protein 28 (VP28), erythropoietin and phytase, etc. A detailed summary of information on the production of recombination proteins in *C. reinhardtii* and some other biotechnologically important microalgae is listed in Table 1. Apart from *C. reinhardtii*, several other algae (including species of *Chlorella*, *Scenedesmus,* and *Dunaliella*) have also been considered more productive and certified as safe. Though the information regarding recombinant protein synthesis lacks in these species, in some cases, however, they can produce recombinant proteins at the identical levels as *C. reinhardtii*. For instance, five chloroplast industrial enzymes and recombinant protein in *D. tertiolecta* and *Scenedesmus dimorphus* were expressed as same amounts as in *C. reinhardtii* (65, 67). Likewise, a monoclonal human IgG antibody against hepatitis B was expressed in the *P. tricornutum* (68, 69). Moreover, successful chloroplast transformation in *Porphytridium* spp., *Euglena gracilis* and *Haematococcus pluvialis* have also been reported in earlier studies (70, 71).

4.2. Microalgae and human nutrition

Microalgae are an abundant source of carbohydrates, protein, enzymes and fiber. In addition to many vitamins and minerals like vitamin A, C, B1, B2, B6, niacin, iodine, potassium, iron, calcium and magnesium are amply found in microalgae. Given a rich source of essential nutrients, they are a major source of food in Asian countries, in particular like China, Japan, and Korea. Green microalgae have also been used as a food source or nutritional supplement in Asiatic countries for hundreds of years. At present, the consumption of microalgal-based biomass is restricted to very few taxa, e.g., *Spirulina* (Arthrospira), *Chlorella*, *Dunaliella* and *Aphanizomenon* with *Chlorella* and *Spirulina* being dominant in the microalgal market, across the globe. During the past decades, microalgal biomass was extensively used in the health food market, with more than 75% of the annual microalgal biomass production being utilized for the manufacturing of powders, tablets, capsules, or pastilles. Moreover, numerous mixtures of microalgae with other health foods can also be found on the market. Microalgae are also incorporated into pasta, snack foods or drinks either as natural food colorants or nutritional food supplements (72). Functional food oil, rich in fatty acids and antioxidants, extracted through supercritical CO_2 from the microalga *Chlorella* vulgaris have its use in food industry especially for derived seafood (73). Amongst many efforts dedicated to explicating the health-promoting properties of microalgal biomass, a generalized immune-modulating effect appears to be the most responsible (74). At contemporary, most of the bio-products launched to serve the health food market are provided as tablets and powder. Nonetheless, algal extracts in various product forms are also developing a new market sector for microalgal products.

4.3. Microalgae and nutraceutical

Compared with algal powders, functional food or nutraceuticals produced with microalgal biomass are amazingly much more beneficial and variable, thus consolidating health benefits with allurement to customers. The market of nutraceuticals/functional foods is postulated to be the most dynamic sector of the food industry and could constitute up to 20% of the whole food market within the next few years. Food supplemented with microalgal biomass might have other positive influences, e.g., prebiotic effects or mineral fortification. *Spirulina* biomass, as an extract or processed in pasta, biscuits, and other functional food products, provokes the functionality of the digestive tract, e.g., assists in maintaining healthy intestinal bacteria. The supplementation of *Spirulina* biomass and aqueous extract led to a 10-folds increase in the growth rate of the lactobacilli compared with the control. In Germany, food production and distribution companies (i.e., pasta, bread, yogurt and soft drinks) have started serious activities to market functional foods together with the amalgamation of cyanobacteria and microalgae. Analogous progress can be observed in many other developed countries like France, Japan, USA, China, and Thailand (75).

It is estimated that the global nutraceutical market was valued at around $250 billion in 2014 and is predicted to reach around $385 billion by 2020 due to rapidly increasing consumers demand for nutraceuticals (76). The global market is dominated by the USA, Europe, and Japan who contribute more than 85% of the nutraceutical market. It is anticipated that these three regional markets will remain at the forefront of the nutraceutical industry both as producers and consumers because of increasing awareness, higher income levels, and a preference for nutraceutical products, preventive medicine, and self-treatment. It has also been found that amongst the consumers worldwide, 72% were women aged 35 to 49 years with a higher educational level and greater adherence to the Mediterranean diet pattern than non-consumers (77). The intake of nutraceutical products also fluctuates with lifestyle; the individuals with healthy lifestyles are

Table 1. Detailed biosynthetic summary of recombinant proteins in several important microalgae

Microalgae	Transformation method	Expressed genes	Products	Expression location (Chloroplast/Nucleus)	References
C. reinhardtii	Particle bombardment	lsc	Anti-HSV glycoprotein D lsc	Nucleus	(108)
C. reinhardtii	Particle bombardment	gelonin	Anti-CD22-gelonin sc	Chloroplast	(109)
C. reinhardtii	Particle bombardment	Exotoxin A	Anti-CD22-ETA sc	Chloroplast	(110)
C. reinhardtii	Particle bombardment	VP1, CTB	VP1-CTB	Chloroplast	(111)
C. reinhardtii	Glass beads method	E7GGG	E7GGG	Chloroplast	(112)
C. reinhardtii	Particle bombardment	VEGF	VEGF	Chloroplast	(113)
C. reinhardtii	Particle bombardment	HMGB1	HMGB1	Chloroplast	(113)
C. reinhardtii	Particle bombardment	14FN3	14FN3	Chloroplast	(113)
C. reinhardtii	Particle bombardment	metallothionein-2	Metallothionein-2	Chloroplast	(114)
C. reinhardtii	Particle bombardment	Strail	TRAIL	Chloroplast	(115)
C. reinhardtii	Particle bombardment	apcA and apcB	Allophycocyanin	Chloroplast	(116)
C. reinhardtii	Particle bombardment	IgG1 lc, hc	Anti-PA 83 anthrax IgG1	Chloroplast	(117)
C. reinhardtii	Glass beads method	crEpo	Erythropoietin	Nucleus	(118)
C. ellipsoidea	Electroporation	mNP-1	mNP-1	Nucleus	(119)
C. reinhardtii	Particle bombardment	m-saa	M-SAA (bovine mammary-associated serum amyloid)	Chloroplast	(120)
C. reinhardtii	Particle bombardment	E2	Swine fever virus (CSFV) structural protein	Chloroplast	(121)
C. reinhardtii	Particle bombardment	VP28	VP28	Chloroplast	(122)
C. reinhardtii	Particle bombardment	hGAD65	hGAD65	Chloroplast	(123)
C. reinhardtii	Particle bombardment	CTB, D2	CTB-D2	Chloroplast	(124)
C. reinhardtii	Glass beads method	PfMSP1–19	GBSS-PfMSP1–19	Nucleus	(107)
C. reinhardtii	Glass beads method	PbAMA1-C	GBSS-PbAMA1-C	Nucleus	(107)
C. reinhardtii	Particle bombardment	Pfs25	Pfs25	Chloroplast	(125)
C. reinhardtii	Particle bombardment	Pfs28	Pfs28	Chloroplast	(125)
C. reinhardtii	Particle bombardment	c.r.pfs48/45	c.r.Pfs48/45	Chloroplast	(126)
C. reinhardtii	Particle bombardment	Cr.ctxB-pfs25	Cr.CtxB-Pfs25	Chloroplast	(127)
C. reinhardtii	Particle bombardment	appA	AppA phytase	Chloroplast	(128)
C. reinhardtii	Electroporation	xyn1	β-1,4-endoxylanase	Nucleus	(129)
C. reinhardtii	LiAc/PEG	fGH	Flounder growth hormone	Nucleus	(130)
C. reinhardtii	Glass beads method	human Sep15	Human Sep15 protein	Nucleus	(131)
D. salina	Electroporation	HBsAg	HBsAg	Nucleus	(132)
D. salina	Glass beads method	VP28	VP28	Nucleus	(133)
D.tertiolecta	Particle bombardment	xylanase/phytase	xylanase/phytase	Chloroplast	(65)
L. oebiformis	Particle bombardment	RbcS	Rubisco small subunit (RbcS) protein	Nucleus	(134)
Porphyridium sp.	Particle bombardment	AHAS (W492S)	acetohydroxyacid synthase	Chloroplast	(135)
P.tricornutum	Particle bombardment	IgG LC, HC	Monoclonal human IgG antibody against HBsAg	Chloroplast	(68)
S.microadriaticum	SiC whiskers	Gus	β-glucoronidase	Nucleus	(136)
C. reinhardtii	Agrobacterium	HBcAgII	HBcAg-GS-AgII-GS-HBcAg	Chloroplast	(137)
C. reinhardtii	Glass beads method	P24	subunit of HIV-1 viral particles	Nucleus	(138)
C. reinhardtii	Particle bombardment	CTB:p210	p210 epitope from ApoB100	Chloroplast	(139)

C. reinhardtii - Chlamydomonas reinhardtii; C. ellipsoidea - Chlorella ellipsoidea; D. salina - Dunaliella salina; S. microadriaticum - Symbiodinium microadriaticum; L. amoebiformis - Lotharella amoebiformis; P. tricornutum - Phaeodactylum tricornutum; TSP - total soluble proteins.

more commonly users of the nutraceutical products (78). Marine-based nutraceutical products represent a significant portion of the global market and are derived from a diverse range of sources providing a myriad of biologically active molecules. These sources, as well as the constituent bioactive molecules and their potential applications, are summarized in Table 2.

4.4. Microalgae and bio-fertilizer

Microalgae are frequently employed in agriculture as bio-fertilizers and soil conditioners. The majorities of cyanobacteria exhibit atmospheric nitrogen fixing capability and thus, are effectively used as bio-fertilizers. Cyanobacteria play a fundamental role in the maintenance and building up of soil fertility and consequently enhance crops productivity by acting as a natural bio-fertilizer. Nitrogen is a crucial limiting factor for plants growth, and deficiency of this element could be encountered by the addition of an appropriate level of fertilizers (39). A diversity of free-living cyanobacteria has now been recognized as efficient components of cyanobacterial-based bio-fertilizers. Apart from contributing nitrogen, cyanobacteria also positively assist crop plants by generating various growth-promoting substances such as vitamin B_{12}, indole-3-acetic acid, indole-3-propionic acid or 3-methyl indole, etc. The thermochemical decomposition of biomass to bio-oil, syngas, and charcoal at elevated temperatures (350–700 °C) in the absence of oxygen is referred as pyrolysis (79). This thermal-driven conversion process results in the formation of a solid charcoal residue called ‘‘biochar’’ from algae that present promising agricultural applications as a bio-fertilizer and for carbon sequestration (80). Biochar can also be effectively used as process fuel in bioenergy conversion. It is deliberated a long-term sink in carbon sequestration process, which could be used to reduce carbon dioxide emissions. Moreover, the biochar sequestration bestows the perspective to generate a carbon-negative biofuel (81) potentially.

Historically, macroalgae are represented as soil fertilizer in coastal regions, across the globe. The rational thought behind this exciting exploitation of macroalgae or macroalgae-extracted residues is the increase in water-binding capacity and mineral composition of the soil (82). These interesting properties are exploited nowadays using liquid biofertilizers produced from microalgae with an objective to avoid erosion and to initiate floral succession (75). Previously, the important role of microalgae in the soil ecosystem has often been abandoned. The beneficial effects originate not only from the production of polymers for particle adherence and water storage in soils or nitrogen-fixing but also from alga-derived bioactive compounds which influence higher plants (82). At present, microalgal biotechnologists pointed out the soil microalgae as a promising research area to explore new species with unexpected properties. In the past decade, plant growth regulators obtained from microalgae attracted considerable researcher’s attention. Further, the discovery of bioactive substances or extracts which promote germination, leaf or stem growth or flowering is also of profound importance. A future trend appears to be the investigation of the biological potential of microalgal-derived bioactive products against plant diseases caused by bacteria or viruses. It is worth noting that microalgae could be a source of a novel class of plant-protecting biological substances shortly (75).

4.5. Microalgae and cosmeceutical

Microalgae are a biochemically diverse congregation of chlorophyll-containing microorganisms with the remarkable capability of oxygenic photosynthesis that is largely found in aquatic environments. The presence of some individual algal pigments, together with specific pigment concentration is a distinctive feature of each species of microalgae. Its evaluation reflects as an indirect measure of cell growth, and a parameter to check the trophic level of waters. Components of algae are frequently used in cosmetics as thickening agents, water-binding agents, and antioxidants (73). Several microalga species are exploited in cosmetics industries, particularly in the skin care market, the main ones being *Arthrospira* and *Chlorella* (83). Other typical species that also possess engrossment in cosmetics purposes are *Chondrus crispus*, *Mastocarpus stellatus*, *Ascophyllum nodosum*, *Alaria esculenta*, *Spirulina platensis*, *Nannochloropsis oculata*, *Chlorella vulgaris* and *Dunaliella salina* (73). The microalgae-derived extracts can be mainly found in face and skin care products (e.g., anti-aging cream, refreshing care products, emollient and as an anti-irritant in peelers). Microalgae are also exploited in hair care and sun protection bio-products. The commercially available products and their properties claimed by their companies are a) a protein-rich extract from *Arthrospira* repairs the signs of early skin aging, exerts a tightening effect and prevents stria formation (Protulines, Exsymol S.A.M., Monaco); b) an extract from *Chlorella vulgaris* stimulates collagen synthesis in skin, thereby supporting tissue regeneration and wrinkle reduction (Dermochlorella, Codif, St. Malo, France). Recently, two new products have also been launched by Penta-pharm (Basel, Switzerland) including, an ingredient from *Nannochloropsis oculata* with excellent skin-tightening properties (Pepha-Tight) and *D. salina*, which shows the ability to markedly stimulate cell proliferation and turn over and to positively influence the energy metabolism of skin (Pepha-Ctive) (83).

4.6. Microalgae and biomass

Innovativeness for microalgae-based products has recently developed numerous new technical systems

Table 2. Marine bioactive molecules: sources, applications and health perspectives (2)

Category	Bioactive Molecules	Applications	Major Marine Sources	Health Perspectives	References
Protein and Peptides	Collagen	Edible coating in meat industry (e.g., sausages)	Fish (albacore tuna, silver-line grunt, brown-backed toadfish, hake, trout, lingcod, catfish, rainbow trout, yellow sea bream and common horse mackerel, etc.)	Anti-oxidant, anti-hypertensive and anti-skin-aging activities.	(140, 141)
	Gelatin	Stabilizer, texturizer, or thickener in ice cream, jam, yogurt, cream cheese, margarine, confectionaries, utilized in low-fat foods and clarifiers	Fish, especially cold-water (Pollock, cod, haddock, hake and cusk)	It has been shown to prevent and treat chronic atrophic gastritis	(142)
	Albumin	Whipping, suspending or stabilizing agent	Mollusks, crustaceans, low-fat fish	Anticoagulant and Antioxidant Properties	(143)
Poly-Saccharides	Carrageenan	Gel formation and coatings in the meat and dairy industry	Macroalgae, e.g., K. alvarezii, E. denticulatum, and B. gelatinum	Anti-HIV activity and anticoagulant properties	(144)
	Agar agar	Gel formation and food gums	Red Alga is the largest source of agar like Gelidium, Gracilaria, Hypnea, and Gigartina	-	(145)
	Fucans and fucanoids	Nutraceutical supplements	Cell walls of brown algae, sea urchin eggs, sea cucumbers	Anticoagulant, antiviral, antithrombotic, proliferative and anti-inflammatory	(146)
	Chitin, chitosan, and derivatives	Gelling agents, edible protective films, clarification and de-acidification of fruits	Shrimp, crab, lobster, prawn and krill	Increase dietary fiber, reduce lipid absorption, antitumor, bactericidal and fungicidal activities	(147)
Fatty acids	Omega-3 fatty acids	Nutraceuticals (fish oil and capsules), fortification of livestock, feed, and infant formula	Almost all marine sources	Numerous health benefits (e.g., visual and neurodevelopment, reduce risk of cardiovascular problems, ameliorate diseases such as arthritis and hypertension)	(148)
Phenolic compounds and other pigments	Phlorotannins	Active ingredients in the nutraceuticals	They are the most abundant polyphenols found in the marine brown algae	Antioxidant activity	(149)
	Carotenoids: β-carotene, and lutein	Natural food colorings, nutraceutical agents, farmed salmon pigmentation	Dunaliella salina, Sarcina maxima, Chlorella protothecoides, Chlorella vulgaris and Haematococcus pluvialis	Vitamin A precursors, antioxidants, anti-carcinogenic and anti-inflammatory	(150)
	Chlorophylls	Natural food and beverage colorants	S. platensis and A. flos-aquae	Anticancer activity, natural source of pigmentation	(151)
Marine enzymes	Gastric proteases; pepsins, gastricsins and chymosins	Cold renneting milk and fish feed digestion aid	Various fish body viscera like Atlantic cod, carp, harp seals, and tuna, etc.	-	(152)
	Serine and cysteine proteases	Preventing unwanted color changes in food products, meat tenderizing, curing of Herring, squid fermentation	Crustaceans, mollusks, and short-finned squid	-	(152)
	Lipases	Numerous uses in the fats and oils industry	Atlantic cod, seal, salmon, sardine, Indian mackerel and red sea bream	-	(152)
	Transglutaminase	Creates protein cross-links to improve rheological properties of gels, i.e., surimi, gelatin	Red sea bream, rainbow trout, Atka mackerel, walleye, Pollock liver, and scallop	-	(153)
Vitamins and Minerals	Fat and water soluble vitamins, iron, iodine, manganese, and zinc	Food, Pharma and nutraceutical industries	Almost all marine sources. Seaweeds are rich sources of vitamins and minerals	Vitamins and Minerals perform many essential functions in the body, for example, they provide transport inside cells and also serve as cofactors during metabolic processes	(154)

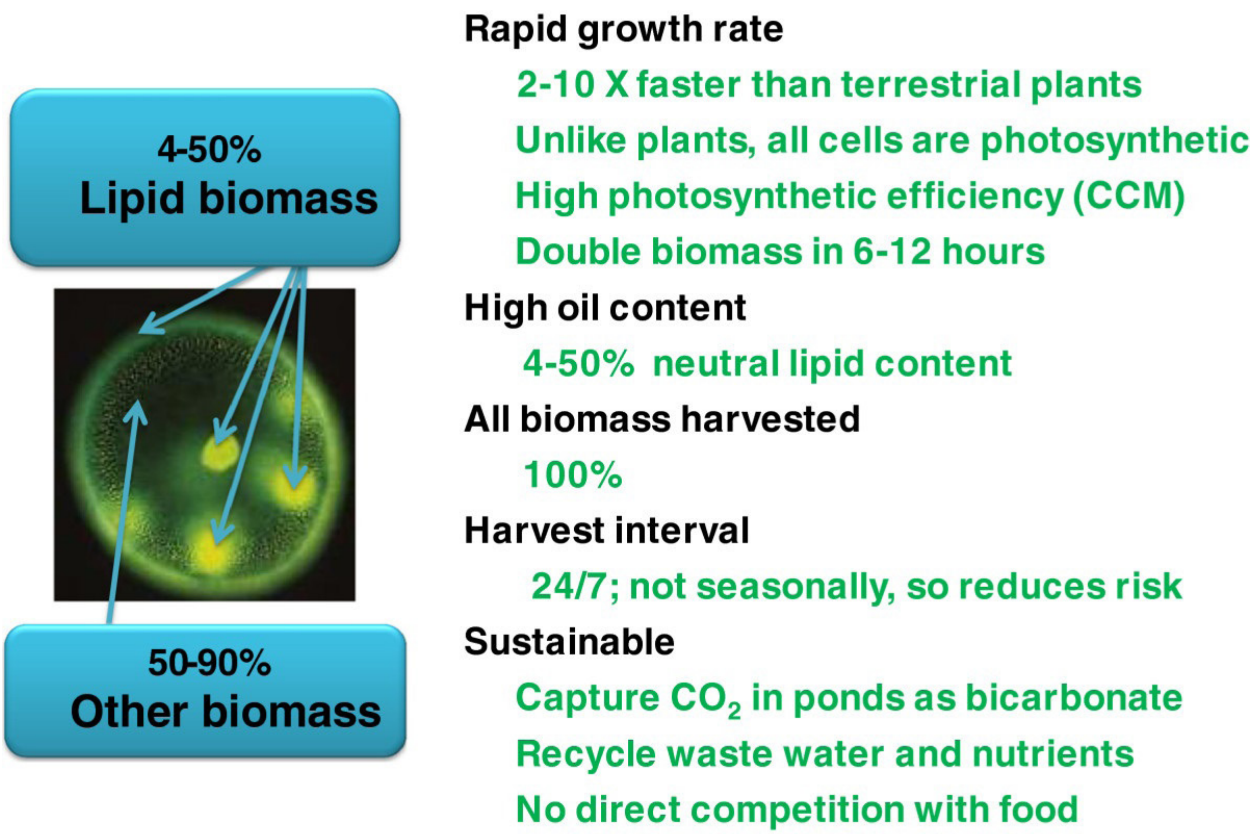

Figure 5. The advantages of algal biomass production systems. CCM; carbon concentrating mechanism "Reproduce with permission from" (34).

for biomass production and down-streamed this biomass to commercially valuable products such as functional food, feed additive, aquaculture, soil conditioner, phycocyanin, phycoerythrin, antioxidants, *β*-carotene, etc. For this standpoint, completely controllable and closed industrial-scale photobioreactors (PBRs) are fetching more importance in recent years. However, regardless of an invaluable array of commodity products from microalgal biotechnology, the most significant product in consideration of product quantity and economic impact is still the microalgal biomass itself (75). The microalgae-based biomass as a sun-dried or spray-dried powder or in compressed form as pastilles is regarded as the predominant product in microalgal biotechnology. This biomass is harvested from natural waters or cultures in artificial ponds with subsequent separation from the growth media and drying. Ultimate product of biomass production is usually a green- or orange-colored powder, which is used in the human health food or animal nutrition both aquaculture and for animal husbandry (75). Figure 5 illustrates advantages of algal biomass production systems. CCM; carbon concentrating mechanism (34).

4.7. Microalgae and biofuels

A diverse variety of biofuels such as bio-oil, bio-diesel, bio-ethanol, bio-methane, bio-hydrogen, syngas, and charcoal can be derived from algal biomass using some novel multidisciplinary bioconversion technologies. Algae are predominantly responsible for over 50% of photosynthetic throughput on earth and are promising sunlight cell factories for a wide-range of potentially useful biologically active compounds, but are seldom exploited for commercial purposes (84–86). A great variety of biofuel candidates were proposed to substitute fossil fuels and to meet the paucity of energy sectors. In this context, bioethanol and biodiesel production from terrestrial plants have received a lot of impetus worldwide as potential substitutes (84). However, limited availability of non-edible crops, food versus fuel competition as well as land consumption have criticized these biofuels and resultantly brought much disagreement and debate on their sustainability (87). Considering that crisis, it is of profound importance to explore new feedstock, suitable for biofuel production, which does not deplete the edible feedstock resource. Third generation biofuel technology based on algae or cyanobacteria is widely regarded as one of the most efficient alternative methods to the conventional crops-based feedstocks. The algae appear to represent the contemporary renewable source of biofuels and could encounter the global demand for transport fuels (88). It is confessing an economically feasible and environmentally sustainable, renewable biomass for biofuels production (89).

Advantageously, algae have the aptitude to transform almost all of the energy in biomass residuals and wastes to methane and hydrogen (90). Certain algae and cyanobacteria contain high carbohydrate and lipid contents that can be used as a potential raw material for bioethanol and biodiesel production, respectively (91). Moreover, the algal biofuel does not possess any conflict with food production and have the potential to satisfy the global transportation fuels demand. Given practical standpoint, algae are easy to cultivate, can grow with slight or even no effort using water (92).

For bioethanol production using algae as raw material, the biomass is first grounded, and the starch is converted to sugars followed by mixing with water and *Saccharomyces cerevisiae* yeast at the warm environment in fermenters (93). The yeast breaks down the sugars and converts them into bioethanol. Microalgae such as *C. vulgaris* have been regarded as a good source of ethanol due to the high starch content (37% dry weight) with up to 65% ethanol conversion efficiency (94). Ueno *et al.* (95) appraised ethanol production in dark fermentation by using green alga *Chlorococcum littorale*. Under dark anaerobic conditions, after 24 h of dark fermentation conditions at 25 °C, about 27% of the cellular starch was decomposed, which was further accelerated at the elevated reaction temperature. During the fermentation process, acetate, ethanol, hydrogen, and carbon dioxide were achieved as fermentation products (96).

4.8. Microalgae and wastewater treatment

The cultivation systems involving microalgae production and wastewater treatment (e.g. of amino acids, enzyme, or food industries wastewaters) seem to be the best options for microalgae growth together with alleviating the environmental burden (92). There is an inimitable prospect to carry out consolidated processing of wastewater treatment and nutrients supply to algal cultivation using nutrient-rich (nitrogen and phosphorus) effluent streams. Biofuel production from algal biomass leads to find out a pathway for the effective elimination of chemical/organic contaminants, heavy metals, and pathogens from wastewater (97). Wastewater treatment through algae offers the opportunity to recycle these nutrients into algae biomass as a bio-fertilizer and as a consequence can compensate treatment process cost. At the same time, microalgae can alleviate the effects of sewage effluent and nitrogenous industrial wastes (92).

Most of the used water turns into wastewater endangering the environment and causing severe health hazards as well. Opportunely, if 50% of this consumed water is made manageable for algae production, it could generate up to 247 and 37 million tons of algal biomass and bio-oil, respectively. Due to obvious variations in the composition of wastewater, only specific algae may bring about their potential (98). Thus, it is crucial to select strains with an incredible ability to grow in a variety of wastewaters and producing feedstock for renewable and environmentally-friendly biofuels (99).

To date, several examples of wastewater treatment by microalgae have been reported in the scientific literature. Martinez *et al.* (100) accomplished a considerable removal of nitrogen and phosphorus from urban wastewater using the microalgal *S. obliquus*. They succeeded to achieve 98% removal of phosphorus and a complete removal (100%) of ammonium in an agitated culture of 183 h at 25 °C, respectively. Similarly, Gomez-Villaetal *et al.* (101) investigated the cultivation of microalgae *S. obliquus* in artificial wastewater with a total nitrogen reduction of 47% and 79% in winter and summer, respectively. A 72% and 28% removal efficiency of nitrogen and phosphorus from wastewater was recorded by Aslan and Kapdan, (102) using *C. vulgaris*. Hodaifa *et al.* (103) achieved a 67.4% removal of BOD5 with *S. obliquus* cultured in olive-oil based industrial wastewater. Use of microalgae is advantageous to generate photosynthetic oxygen required by bacteria to biodegrade environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs), phenolic and organic solvents. The oxygen produced by microalgae eliminates the need for external mechanical aeration (97). The blue-green algae *Spirulina* sp. has been proved as a potential bio-sorbent of heavy metals ($Cr3^{+}$, $Cd2^{+}$, and $Cu2^{+}$) (104). In addition to aforementioned examples, the *Chlorella* sp., *Scenedesmus* sp., *Spirulina sp.*, *Nannochloris*, *B. brauinii* and cyanobacterium *Phormidium bohneri* have been extensively reported microalgae for the removal of contaminants or pollutants from wastewater (99, 100, 105, 106).

5. LIMITATIONS OF THE ALGAL BIOTECHNOLOGY

A plethora of information is available, as discussed above, about many potential aspects of algal biotechnology. Nevertheless, considerable critiques are still outstanding and need to be addressed in future studies. In spite of current scientific advancements in algal biotechnology, a substantial research with proven employability of algal sources is needed in this particular line of research. Similarly, many other unsolved questions are posing a big research gap that needs to be addressed comprehensively. Major limitations and research gaps in algal biotechnology includes but not limited to the, (1) microalgae cell fragility, (2) light, CO_2, pH and temperature intensity issues, (3) overall yield variation with different biological sources, (4) bioactivity and biostability variation with different algal species, (5) initial processing during biosynthesis, (6) size and shape-dependent efficiency of bioactive molecules, (7) stable and efficient *in vivo* profile, and (8) activity mechanisms and futuristic applications in human.

6. CONCLUDING REMARKS AND FUTURE CONSIDERATIONS/ RECOMMENDATIONS

Certainly, algal biotechnology belongs to an exciting era of research that has a great potential to offer new types of novel and biologically active molecules. In this context, a critical literature from recent studies has provided considerable evidence that microalgae-derived bioactive molecules play a vital role in bio- and non-bio sectors of the modern world. In conclusion, the above-discussed literature shows the potential of microalgae with proven advantages. However, there is a dire need to engineer multifunctional products of interests on a pilot scale. In this background, research investigators have directed or redirecting their interest to explore new dimensions in algal biotechnology in particular. The bio-inspired synthesis of various bioactive molecules through green routes using microalgae has following advantages among others i.e. (1) natural sources which are renewable, sustainable and environmentally friendly, (2) the synthesis process is easy to scale up, (3) overall cost-effective ratio is net positive, (4) carbon neutral, (5) stable formulations with adjustable sizes and shapes, (6) no or less consumption of harsh chemicals, and (7) no or less toxic contaminants/by-products, etc. Despite the biotechnological advances in scientific awareness, ever increasing social and ecological awareness, many challenges to improved or enhanced cultivation practices are still unsolved, which must be cost-effective, proficient and profitable. Although massive steps have already been taken in the past few years, however, in-depth, focused and genetically orientated research is necessary. Communally, the synchronization of several biotechnological practices including biological strategies, chemical-based methodologies, and informatics technologies is key to the success of algal bio-discovery. In summary, the present review work aimed at research that underpins the development of strategies to mitigate the effects e.g. through novel alternatives to unsustainable or synthetic routes.

7. ACKNOWLEDGEMENTS

The authors are grateful to the Shanghai Jiao Tong University, Shanghai 200240, China, and Tecnologico de Monterrey, Mexico for providing literature facilities. We do not have any conflicting, competing and financial interests in any capacity.

8. REFERENCES

1. M. F. d. J. Raposo, R. M. S. C. de Morais and A. M. M. Bernardo de Morais: Bioactivity and applications of sulphated polysaccharides from marine microalgae. *Marine drugs*, 11(1), 233–252 (2013)
DOI: 10.3390/md11010233

2. H. A. R. Suleria, S. Osborne, P. Masci and G. Gobe: Marine-based nutraceuticals: An innovative trend in the food and supplement industries. *Marine drugs*, 13(10), 6336–6351 (2015)
DOI: 10.3390/md13106336

3. L. Liu, G. Pohnert and D. Wei: Extracellular Metabolites from Industrial Microalgae and Their Biotechnological Potential. *Marine drugs*, 14(10), 191 (2016)
DOI: 10.3390/md14100191

4. I. Michalak and K. Chojnacka: Algae as production systems of bioactive compounds. *Engineering in Life Sciences*, 15(2), 160–176 (2015)
DOI: 10.1002/elsc.201400191

5. J. N. Rosenberg, G. A. Oyler, L. Wilkinson and M. J. Betenbaugh: A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. *Current opinion in Biotechnology*, 19(5), 430–436 (2008)
DOI: 10.1016/j.copbio.2008.07.008

6. J. A. V. Costa and M. G. de Morais: Microalgae for food production. In: *Fermentation Processes Engineering in the Food Industry*. CRC Press, (2013)
DOI: 10.1201/b14070-17

7. J. P. Berry, M. Gantar, M. H. Perez, G. Berry and F. G. Noriega: Cyanobacterial toxins as allelochemicals with potential applications as algaecides, herbicides and insecticides. *Marine drugs*, 6(2), 117–146 (2008)
DOI: 10.3390/md6020117

8. K. Wichuk, S. Brynjólfsson and W. Fu: Biotechnological production of value-added carotenoids from microalgae: Emerging technology and prospects. *Bioengineered*, 5(3), 204–208 (2014)
DOI: 10.4161/bioe.28720

9. W. Fu, K. Wichuk and S. Brynjólfsson: Developing diatoms for value-added

products: Challenges and opportunities. *New biotechnology*, 32(6), 547–551 (2015) DOI: 10.1016/j.nbt.2015.03.016

10. A. C. Guedes, H. M. Amaro and F. X. Malcata: Microalgae as sources of carotenoids. *Marine drugs*, 9(4), 625–644 (2011) DOI: 10.3390/md9040625

11. M. M. Ciccone, F. Cortese, M. Gesualdo, S. Carbonara, A. Zito, G. Ricci, F. De Pascalis, P. Scicchitano and G. Riccioni: Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care. *Mediators of inflammation*, 2013 (2013) DOI: 10.1155/2013/782137

12. S. P. Cuellar-Bermudez, I. Aguilar-Hernandez, D. L. Cardenas-Chavez, N. Ornelas-Soto, M. A. Romero-Ogawa and R. Parra-Saldivar: Extraction and purification of high-value metabolites from microalgae: essential lipids, astaxanthin and phycobiliproteins. *Microbial biotechnology*, 8(2), 190–209 (2015) DOI: 10.1111/1751-7915.12167

13. L. Barsanti and P. Gualtieri: Algae: anatomy, biochemistry, and biotechnology. CRC press, (2014) DOI: 10.1201/b16544

14. D. J. Castro, C. V. Löhr, K. A. Fischer, K. M. Waters, B.-J. M. Webb-Robertson, R. H. Dashwood, G. S. Bailey and D. E. Williams: Identifying efficacious approaches to chemoprevention with chlorophyllin, purified chlorophylls and freeze-dried spinach in a mouse model of transplacental carcinogenesis. *Carcinogenesis*, 30(2), 315–320 (2009) DOI: 10.1093/carcin/bgn280

15. T. Tumolo and U. M. Lanfer-Marquez: Copper chlorophyllin: A food colorant with bioactive properties? *Food Research International*, 46(2), 451–459 (2012) DOI: 10.1016/j.foodres.2011.10.031

16. K. Chimploy, G. D. Díaz, Q. Li, O. Carter, W. M. Dashwood, C. K. Mathews, D. E. Williams, G. S. Bailey and R. H. Dashwood: E2F4 and ribonucleotide reductase mediate S-phase arrest in colon cancer cells treated with chlorophyllin. *International journal of cancer*, 125(9), 2086–2094 (2009) DOI: 10.1002/ijc.24559

17. L. Du, N. Jiang, G. Wang, Y. Chu, W. Lin, J. Qian, Y. Zhang, J. Zheng and G. Chen: Autophagy inhibition sensitizes bladder cancer cells to the photodynamic effects of the novel photosensitizer chlorophyllin e4. *Journal of Photochemistry and Photobiology B: Biology*, 133, 1–10 (2014) DOI: 10.1016/j.jphotobiol.2014.02.010

18. M. G. Ferruzzi and J. Blakeslee: Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. *Nutrition Research*, 27(1), 1–12 (2007) DOI: 10.1016/j.nutres.2006.12.003

19. S. Sekar and M. Chandramohan: Phycobiliproteins as a commodity: trends in applied research, patents and commercialization. *Journal of Applied Phycology*, 20(2), 113–136 (2008) DOI: 10.1007/s10811-007-9188-1

20. R. Chandra, R. Parra-Saldivar, and H.M.N. Iqbal: Phycobiliproteins: a novel green tool from marine origin blue-green algae and red algae. *Protein and peptide letters*, 24(2), 118–125 (2017). DOI: 10.2174/0929866523666160802160222

21. J. C. Varela, H. Pereira, M. Vila and R. León: Production of carotenoids by microalgae: achievements and challenges. *Photosynthesis research*, 125(3), 423–436 (2015) DOI: 10.1007/s11120-015-0149-2

22. J. A. Del Campo, M. García-González and M. G. Guerrero: Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. *Applied microbiology and biotechnology*, 74(6), 1163–1174 (2007) DOI: 10.1007/s00253-007-0844-9

23. K. Jayappriyan, R. Rajkumar, V. Venkatakrishnan, S. Nagaraj and R. Rengasamy: *In vitro* anticancer activity of natural β-carotene from Dunaliella salina EU5891199 in PC-3 cells. *Biomedicine & Preventive Nutrition*, 3(2), 99–105 (2013) DOI: 10.1016/j.bionut.2012.08.003

24. D. B. d. Alencar, K. M. d. S. Pires-Cavalcante, J. P. d. S. Saboya, M. B. d. Sousa, W. R. L. Farias and S. Saker-Sampaio: Contents of β-carotene in supplements and biomass of Spirulina. *Ciência e Agrotecnologia*, 35(2), 386–391 (2011) DOI: 10.1590/s1413-70542011000200021

25. K. Uchiyama, Y. Naito, G. Hasegawa, N. Nakamura, J. Takahashi and T. Yoshikawa: Astaxanthin protects β-cells against glucose toxicity in diabetic db/db mice. *Redox Report*, 7(5), 290–293 (2002)
DOI: 10.1179/135100002125000811

26. M. A. Gammone, G. Riccioni and N. D'Orazio: Carotenoids: potential allies of cardiovascular health? *Food & nutrition research*, 59 (2015)
DOI: 10.3402/fnr.v59.26762

27. Y. Lemoine and B. Schoefs: Secondary ketocarotenoid astaxanthin biosynthesis in algae: a multifunctional response to stress. *Photosynthesis research*, 106(1–2), 155–177 (2010)
DOI: 10.1007/s11120-010-9583-3

28. D. Andrade, A. Colozzi-Filho, C. Guedes, F. Lima, G. Machineski and M. Matos: Principais produtos da biomassa algal e suas aplicações biotecnológicas. *Microalgas de águas continentais: potencialidades e desafios do cultivo, IAPAR, Londrina*, 265–343 (2014)
DOI: 10.5433/1679-0367.2014v35n2p125

29. L. Ma and X. M. Lin: Effects of lutein and zeaxanthin on aspects of eye health. *Journal of the Science of Food and Agriculture*, 90(1), 2–12 (2010)
DOI: 10.1002/jsfa.3785

30. X. M. Shi and F. Chen: High-Yield Production of Lutein by the Green Microalga Chlorella protothecoidesin Heterotrophic Fed-Batch Culture. *Biotechnology progress*, 18(4), 723–727 (2002)
DOI: 10.1021/bp0101987

31. J. Sánchez, J. Fernández, F. Acién, A. Rueda, J. Pérez-Parra and E. Molina: Influence of culture conditions on the productivity and lutein content of the new strain Scenedesmus almeriensis. *Process Biochemistry*, 43(4), 398–405 (2008)
DOI: 10.1016/j.procbio.2008.01.004

32. J. M. Fernández-Sevilla, F. A. Fernández and E. M. Grima: Biotechnological production of lutein and its applications. *Applied microbiology and biotechnology*, 86(1), 27–40 (2010)
DOI: 10.1007/s00253-009-2420-y

33. S. Kraan: Algal polysaccharides, novel applications and outlook. INTECH Open Access Publisher, (2012)
DOI: 10.5772/51572

34. S. Subramanian, A. N. Barry, S. Pieris and R. T. Sayre: Comparative energetics and kinetics of autotrophic lipid and starch metabolism in chlorophytic microalgae: implications for biomass and biofuel production. *Biotechnology for biofuels*, 6(1), 150 (2013)
DOI: 10.1186/1754-6834-6-150

35. K. H. Cardozo, T. Guaratini, M. P. Barros, V. R. Falcão, A. P. Tonon, N. P. Lopes, S. Campos, M. A. Torres, A. O. Souza and P. Colepicolo: Metabolites from algae with economical impact. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 146(1), 60–78 (2007)
DOI: 10.1016/j.cbpc.2006.05.007

36. S. Gupta and N. Abu-Ghannam: Bioactive potential and possible health effects of edible brown seaweeds. *Trends in Food Science & Technology*, 22(6), 315–326 (2011)
DOI: 10.1016/j.tifs.2011.03.011

37. E. Ibañez, M. Herrero, J. A. Mendiola and M. Castro-Puyana: Extraction and characterization of bioactive compounds with health benefits from marine resources: macro and micro algae, cyanobacteria, and invertebrates. In: *Marine Bioactive Compounds*. Springer, (2012)
DOI: 10.1007/978-1-4614-1247-2_2

38. S. U. Kadam, B. K. Tiwari and C. P. O'Donnell: Application of novel extraction technologies for bioactives from marine algae. *Journal of agricultural and food chemistry*, 61(20), 4667–4675 (2013)
DOI: 10.1021/jf400819p

39. R. M. Rodriguez-Jasso, S. I. Mussatto, L. Pastrana, C. N. Aguilar and J. A. Teixeira: Microwave-assisted extraction of sulfated polysaccharides (fucoidan) from brown seaweed. *Carbohydrate Polymers*, 86(3), 1137–1144 (2011)
DOI: 10.1016/j.carbpol.2011.06.006

40. S.-C. Ko, S.-H. Lee, G. Ahn, K.-N. Kim, S.-H. Cha, S.-K. Kim, B.-T. Jeon, P.-J. Park, K.-W. Lee and Y.-J. Jeon: Effect of enzyme-assisted extract of Sargassum coreanum on induction of apoptosis in HL-60 tumor cells. *Journal of applied phycology*, 24(4), 675–684 (2012)
DOI: 10.1007/s10811-011-9685-0

41. J. Vera, J. Castro, A. Gonzalez and A. Moenne: Seaweed polysaccharides and

derived oligosaccharides stimulate defense responses and protection against pathogens in plants. *Marine drugs*, 9(12), 2514–2525 (2011)
DOI: 10.3390/md9122514

42. R. Halim, M. K. Danquah and P. A. Webley: Extraction of oil from microalgae for biodiesel production: A review. *Biotechnology advances*, 30(3), 709–732 (2012)
DOI: 10.1016/j.biotechadv.2012.01.001

43. G. Zanchett and E. C. Oliveira-Filho: Cyanobacteria and cyanotoxins: from impacts on aquatic ecosystems and human health to anticarcinogenic effects. *Toxins*, 5(10), 1896–1917 (2013)
DOI: 10.3390/toxins5101896

44. G. Knothe, J. Krahl and J. Gerpen: The Biodiesel Handbook. 2nd. *Urbana: AOCS press* (2010)
DOI: 10.1016/b978-1-893997-62-2.50003-6

45. L. de Jaeger, R. E. Verbeek, R. B. Draaisma, D. E. Martens, J. Springer, G. Eggink and R. H. Wijffels: Superior triacylglycerol (TAG) accumulation in starchless mutants of Scenedesmus obliquus:(I) mutant generation and characterization. *Biotechnology for biofuels*, 7(1), 69 (2014)
DOI: 10.1186/1754-6834-7-69

46. E. B. D'Alessandro and N. R. Antoniosi Filho: Concepts and studies on lipid and pigments of microalgae: A review. *Renewable and Sustainable Energy Reviews*, 58, 832–841 (2016)
DOI: 10.1016/j.rser.2015.12.162

47. G. Knothe: Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel processing technology*, 86(10), 1059–1070 (2005)
DOI: 10.1016/j.fuproc.2004.11.002

48. G. Knothe: "Designer" biodiesel: optimizing fatty ester composition to improve fuel properties. *Energy & Fuels*, 22(2), 1358–1364 (2008)
DOI: 10.1021/ef700639e

49. G.-T. Jeong, J.-H. Park, S.-H. Park and D.-H. Park: Estimating and improving cold filter plugging points by blending biodiesels with different fatty acid contents. *Biotechnology and Bioprocess Engineering*, 13(4), 505 (2008)
DOI: 10.1007/s12257-008-0144-y

50. A. Converti, A. A. Casazza, E. Y. Ortiz, P. Perego and M. Del Borghi: Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production. *Chemical Engineering and Processing: Process Intensification*, 48(6), 1146–1151 (2009)
DOI: 10.1016/j.cep.2009.03.006

51. K. Zhang, B. Sun, X. She, F. Zhao, Y. Cao, D. Ren and J. Lu: Lipid production and composition of fatty acids in Chlorella vulgaris cultured using different methods: photoautotrophic, heterotrophic, and pure and mixed conditions. *Annals of microbiology*, 64(3), 1239–1246 (2014)
DOI: 10.1007/s13213-013-0766-y

52. B. S. Grama, S. N. Agathos and C. S. Jeffryes: Balancing photosynthesis and respiration increases microalgal biomass productivity during photoheterotrophy on glycerol. *ACS Sustainable Chemistry & Engineering*, 4(3), 1611–1618 (2016)
DOI: 10.1021/acssuschemeng.5b01544

53. Q. Zhang and Y. Hong: Comparison of growth and lipid accumulation properties of two oleaginous microalgae under different nutrient conditions. *Frontiers of Environmental Science & Engineering*, 8(5), 703–709 (2014)
DOI: 10.1007/s11783-014-0649-x

54. M. T. Guarnieri and P. T. Pienkos: Algal omics: unlocking bioproduct diversity in algae cell factories. *Photosynthesis research*, 123(3), 255–263 (2015)
DOI: 10.1007/s11120-014-9989-4

55. D. B. Stengel and S. Connan: Marine algae: A source of biomass for biotechnological applications. *Natural Products From Marine Algae: Methods and Protocols*, 1–37 (2015)
DOI: 10.1007/978-1-4939-2684-8_1

56. M. Kumar, U. Kuzhiumparambil, M. Pernice, Z. Jiang and P. J. Ralph: Metabolomics: An emerging frontier of systems biology in marine macrophytes. *Algal Research*, 16, 76–92 (2016)
DOI: 10.1016/j.algal.2016.02.033

57. D. Agyei, I. Ahmed, Z. Akram, H. Iqbal and M. K. Danquah: Protein and Peptide Biopharmaceuticals: An overview. *Protein and peptide letters* (2016)
DOI: 10.2174/0929866523666161222150444

58. G. Piganeau: Genomic insights into the biology of algae. Academic Press, (2012) DOI: 10.1016/b978-0-12-391499-6.22001-x

59. D. Prakash and G. Sharma: Phytochemicals of nutraceutical importance. CABI, (2014) DOI: 10.1079/9781780643632.0000

60. B. T. Hovde, C. R. Deodato, H. M. Hunsperger, S. A. Ryken, W. Yost, R. K. Jha, J. Patterson, R. J. Monnat Jr, S. B. Barlow and S. R. Starkenburg: Genome sequence and transcriptome analyses of Chrysochromulina tobin: metabolic tools for enhanced algal fitness in the prominent order Prymnesiales (Haptophyceae). *PLoS Genet*, 11(9), e1005469 (2015) DOI: 10.1371/journal.pgen.1005469

61. L. S. de Oliveira, G. B. Gregoracci, G. G. Z. Silva, L. T. Salgado, G. Amado Filho, M. Alves-Ferreira, R. C. Pereira and F. L. Thompson: Transcriptomic analysis of the red seaweed Laurencia dendroidea (Florideophyceae, Rhodophyta) and its microbiome. *BMC genomics*, 13(1), 487 (2012) DOI: 10.1186/1471-2164-13-487

62. M. A. Borowitzka: Algal Biotechnology. In: *The Algae World*. Springer, (2015) DOI: 10.1007/978-94-017-7321-8_11

63. J. Shrager, C. Hauser, C.-W. Chang, E. H. Harris, J. Davies, J. McDermott, R. Tamse, Z. Zhang and A. R. Grossman: Chlamydomonas reinhardtii genome project. A guide to the generation and use of the cDNA information. *Plant Physiology*, 131(2), 401–408 (2003) DOI: 10.1104/pp.016899

64. H. N. Dawson, R. Burlingame and A. C. Cannons: Stable transformation of Chlorella: rescue of nitrate reductase-deficient mutants with the nitrate reductase gene. *Current microbiology*, 35(6), 356–362 (1997) DOI: 10.1007/s002849900268

65. D. R. Georgianna, M. J. Hannon, M. Marcuschi, S. Wu, K. Botsch, A. J. Lewis, J. Hyun, M. Mendez and S. P. Mayfield: Production of recombinant enzymes in the marine alga Dunaliella tertiolecta. *Algal Research*, 2(1), 2–9 (2013) DOI: 10.1016/j.algal.2012.10.004

66. D. Guzmán-Zapata, K. S. Macedo-Osorio, A. L. Almaraz-Delgado, N. Durán-Figueroa and J. A. Badillo-Corona: Production of recombinant proteins in the chloroplast of the green alga Chlamydomonas reinhardtii. *Recombinant Proteins from Plants: Methods and Protocols*, 69–85 (2016) DOI: 10.1007/978-1-4939-3289-4_5

67. B. A. Rasala and S. P. Mayfield: Photosynthetic biomanufacturing in green algae; production of recombinant proteins for industrial, nutritional, and medical uses. *Photosynthesis research*, 123(3), 227–239 (2015) DOI: 10.1007/s11120-014-9994-7

68. F. Hempel, J. Lau, A. Klingl and U. G. Maier: Algae as protein factories: expression of a human antibody and the respective antigen in the diatom Phaeodactylum tricornutum. *PloS one*, 6(12), e28424 (2011) DOI: 10.1371/journal.pone.0028424

69. F. Hempel and U. G. Maier: An engineered diatom acting like a plasma cell secreting human IgG antibodies with high efficiency. *Microbial cell factories*, 11(1), 126 (2012) DOI: 10.1186/1475-2859-11-126

70. N. A. Doetsch, M. R. Favreau, N. Kuscuoglu, M. D. Thompson and R. B. Hallick: Chloroplast transformation in Euglena gracilis: splicing of a group III twintron transcribed from a transgenic psbK operon. *Current genetics*, 39(1), 49–60 (2001) DOI: 10.1007/s002940000174

71. C. L. Gutierrez, J. Gimpel, C. Escobar, S. H. Marshall and V. Henríquez: Chloroplast genetic tool for the green microalgae Haematococcus pluvialis (Chlorophyceae, Volvocales) *Journal of phycology*, 48(4), 976–983 (2012) DOI: 10.1111/j.1529-8817.2012.01178.x

72. W. Becker: 18 Microalgae in Human and Animal Nutrition. *Handbook of microalgal culture: biotechnology and applied phycology*, 312 (2004) DOI: 10.1002/9781118567166.ch25

73. I. Priyadarshani and B. Rath: Commercial and industrial applications of micro algae–A review. *J algal biomass utln*, 3(4), 89–100 (2012) DOI: 10.1787/9789264213562-9-en

74. J. Burgess, R. Osinga and R. Wijffels: Marine Bioprocess Engineering. Elsevier, (1999) DOI: 10.1016/s0079-6352(99)80091-5

75. O. Pulz and W. Gross: Valuable products from biotechnology of microalgae. *Applied microbiology and biotechnology*, 65(6), 635–648 (2004)
DOI: 10.1007/s00253-004-1647-x

76. M. Intelligence: Global Telemedicine Market—Growth, Trends and Forecasts (2015–2019). In, (2015)
DOI: 10.1002/9781119208082.ch18

77. M.-A. Rovira, M. Grau, O. Castañer, M.-I. Covas, H. Schröder and R. Investigators: Dietary supplement use and health-related behaviors in a Mediterranean population. *Journal of nutrition education and behavior*, 45(5), 386–391 (2013)
DOI: 10.1016/j.jneb.2012.03.007

78. J. A. Foote, S. P. Murphy, L. R. Wilkens, J. H. Hankin, B. E. Henderson and L. N. Kolonel: Factors associated with dietary supplement use among healthy adults of five ethnicities the multiethnic cohort study. *American journal of epidemiology*, 157(10), 888–897 (2003)
DOI: 10.1093/aje/kwg072

79. H. Goyal, D. Seal and R. Saxena: Bio-fuels from thermochemical conversion of renewable resources: a review. *Renewable and sustainable energy reviews*, 12(2), 504–517 (2008)
DOI: 10.1016/j.rser.2006.07.014

80. E. Marris: Putting the carbon back: Black is the new green. *Nature*, 442(7103), 624–626 (2006)
DOI: 10.1038/442624a

81. J. Lehmann: A handful of carbon. *Nature*, 447(7141), 143–144 (2007)
DOI: 10.1038/447143a

82. V. Ördög, J. Szigeti and O. Pulz: Proceedings of the Conference on" Progress in Plant Sciences from Plant Breeding to Growth Regulation": 17–19 June 1996, Mosonmagyaróvár, Hungary. Pannon University of Agricultural Sciences, (1997)
DOI: 10.1007/bf00024408

83. P. STOLZ and B. OBERMAYER: Manufacturing microalgae for skin care. *Cosmetics and toiletries*, 120(3), 99–106 (2005)
DOI: 10.1007/978-94-011-2268-9_3

84. Y. Chisti: Biodiesel from microalgae beats bioethanol. *Trends in biotechnology*, 26(3), 126–131 (2008)
DOI: 10.1016/j.tibtech.2007.12.002

85. M. Gavrilescu and Y. Chisti: Biotechnology—a sustainable alternative for chemical industry. *Biotechnology advances*, 23(7), 471–499 (2005)
DOI: 10.1016/j.biotechadv.2005.03.004

86. R. H. Wijffels: Potential of sponges and microalgae for marine biotechnology. *Trends in Biotechnology*, 26(1), 26–31 (2008)
DOI: 10.1016/j.tibtech.2007.10.002

87. C. S. Goh and K. T. Lee: A visionary and conceptual macroalgae-based third-generation bioethanol (TGB) biorefinery in Sabah, Malaysia as an underlay for renewable and sustainable development. *Renewable and Sustainable Energy Reviews*, 14(2), 842–848 (2010)
DOI: 10.1016/j.rser.2009.10.001

88. X. Miao and Q. Wu: Biodiesel production from heterotrophic microalgal oil. *Bioresource technology*, 97(6), 841–846 (2006)
DOI: 10.1016/j.biortech.2005.04.008

89. R. P. John, G. Anisha, K. M. Nampoothiri and A. Pandey: Micro and macroalgal biomass: a renewable source for bioethanol. *Bioresource technology*, 102(1), 186–193 (2011)
DOI: 10.1016/j.biortech.2010.06.139

90. J. Spiertz and F. Ewert: Crop production and resource use to meet the growing demand for food, feed and fuel: opportunities and constraints. *NJAS-Wageningen Journal of Life Sciences*, 56(4), 281–300 (2009)
DOI: 10.1016/s1573-5214(09)80001-8

91. E. C. Petrou and C. P. Pappis: Biofuels: a survey on pros and cons. *Energy & Fuels*, 23(2), 1055–1066 (2009)
DOI: 10.1021/ef800806g

92. T. M. Mata, A. A. Martins and N. S. Caetano: Microalgae for biodiesel production and other applications: a review. *Renewable and sustainable energy reviews*, 14(1), 217–232 (2010)
DOI: 10.1016/j.rser.2009.07.020

93. A. Demirbaş: Biomass resource facilities and biomass conversion processing for

fuels and chemicals. *Energy conversion and Management*, 42(11), 1357–1378 (2001)
DOI: 10.1016/s0196-8904(00)00137-0

94. A. Hirano, R. Ueda, S. Hirayama and Y. Ogushi: CO2 fixation and ethanol production with microalgal photosynthesis and intracellular anaerobic fermentation. *Energy*, 22(2–3), 137–142 (1997)
DOI: 10.1016/s0360-5442(96)00123-5

95. Y. Ueno, N. Kurano and S. Miyachi: Ethanol production by dark fermentation in the marine green alga, Chlorococcum littorale. *Journal of fermentation and bioengineering*, 86(1), 38–43 (1998)
DOI: 10.1016/s0922-338x(98)80031-7

96. S. Amin: Review on biofuel oil and gas production processes from microalgae. *Energy conversion and management*, 50(7), 1834–1840 (2009)
DOI: 10.1016/j.enconman.2009.03.001

97. R. Munoz and B. Guieysse: Algal–bacterial processes for the treatment of hazardous contaminants: a review. *Water research*, 40(15), 2799–2815 (2006)
DOI: 10.1016/j.watres.2006.06.011

98. S. Chinnasamy, A. Bhatnagar, R. W. Hunt and K. Das: Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresource technology*, 101(9), 3097–3105 (2010)
DOI: 10.1016/j.biortech.2009.12.026

99. T. Suganya, M. Varman, H. Masjuki and S. Renganathan: Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: A biorefinery approach. *Renewable and Sustainable Energy Reviews*, 55, 909–941 (2016)
DOI: 10.1016/j.rser.2015.11.026

100. M. Martınez, S. Sánchez, J. Jimenez, F. El Yousfi and L. Munoz: Nitrogen and phosphorus removal from urban wastewater by the microalga Scenedesmus obliquus. *Bioresource technology*, 73(3), 263–272 (2000)
DOI: 10.1016/s0960-8524(99)00121-2

101. H. Gomez-Villa, D. Voltolina, M. Nieves and P. Pina: Biomass production and nutrient budget in outdoor cultures of Scenedesmus obliquus(Chlorophyceae) in artificial wastewater, under the winter and summer conditions of Mazatlan, Sinaloa, Mexico. *Vie et milieu*, 55(2), 121–126 (2005)
DOI: 10.1016/s0960-8524(00)00183-8

102. S. Aslan and I. K. Kapdan: Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecological Engineering*, 28(1), 64–70 (2006)
DOI: 10.1016/j.ecoleng.2006.04.003

103. G. Hodaifa, M. E. Martínez and S. Sánchez: Use of industrial wastewater from olive-oil extraction for biomass production of Scenedesmus obliquus. *Bioresource technology*, 99(5), 1111–1117 (2008)
DOI: 10.1016/j.biortech.2007.02.020

104. K. Chojnacka, A. Chojnacki and H. Gorecka: Biosorption of Cr 3+, Cd 2+ and Cu 2+ ions by blue–green algae Spirulina sp.: kinetics, equilibrium and the mechanism of the process. *Chemosphere*, 59(1), 75–84 (2005)
DOI: 10.1016/j.chemosphere.2004.10.005

105. K. Lee and C.-G. Lee: Effect of light/dark cycles on wastewater treatments by microalgae. *Biotechnology and Bioprocess Engineering*, 6(3), 194–199 (2001)
DOI: 10.1007/bf02932550

106. E. J. Olguín, S. Galicia, G. Mercado and T. Pérez: Annual productivity of Spirulina (Arthrospira) and nutrient removal in a pig wastewater recycling process under tropical conditions. *Journal of Applied Phycology*, 15(2–3), 249–257 (2003)
DOI: 10.1023/a:1023856702544

107. D. Dauvillée, S. Delhaye, S. Gruyer, C. Slomianny, S. E. Moretz, C. d'Hulst, C. A. Long, S. G. Ball and S. Tomavo: Engineering the chloroplast targeted malarial vaccine antigens in Chlamydomonas starch granules. *PLoS One*, 5(12), e15424 (2010)
DOI: 10.1371/journal.pone.0015424

108. S. P. Mayfield, S. E. Franklin and R. A. Lerner: Expression and assembly of a fully active antibody in algae. *Proceedings of the National Academy of Sciences*, 100(2), 438–442 (2003)
DOI: 10.1073/pnas.0237108100

109. M. Tran, R. E. Henry, D. Siefker, C. Van, G. Newkirk, J. Kim, J. Bui and S. P. Mayfield:

Production of anti-cancer immunotoxins in algae: Ribosome inactivating proteins as fusion partners. *Biotechnology and bioengineering*, 110(11), 2826–2835 (2013)
DOI: 10.1002/bit.24966

110. M. Tran, C. Van, D. J. Barrera, P. L. Pettersson, C. D. Peinado, J. Bui and S. P. Mayfield: Production of unique immunotoxin cancer therapeutics in algal chloroplasts. *Proceedings of the National Academy of Sciences*, 110(1), E15-E22 (2013)
DOI: 10.1073/pnas.1214638110

111. M. Sun, K. Qian, N. Su, H. Chang, J. Liu and G. Shen: Foot-and-mouth disease virus VP1 protein fused with cholera toxin B subunit expressed in Chlamydomonas reinhardtii chloroplast. *Biotechnology letters*, 25(13), 1087–1092 (2003)
DOI: 10.1016/j.colsurfb.2006.11.043

112. O. C. Demurtas, S. Massa, P. Ferrante, A. Venuti, R. Franconi and G. Giuliano: A Chlamydomonas-derived Human Papillomavirus 16 E7 vaccine induces specific tumor protection. *PLoS One*, 8(4), e61473 (2013)
DOI: 10.1371/journal.pone.0061473

113. B. A. Rasala, M. Muto, P. A. Lee, M. Jager, R. M. Cardoso, C. A. Behnke, P. Kirk, C. A. Hokanson, R. Crea and M. Mendez: Production of therapeutic proteins in algae, analysis of expression of seven human proteins in the chloroplast of Chlamydomonas reinhardtii. *Plant biotechnology journal*, 8(6), 719–733 (2010)
DOI: 10.1111/j.1467-7652.2010.00503.x

114. Y. K. ZHANG, G. F. SHEN and B. G. RU: Survival of Human Metallothionein-2 Transplastomic Chlamydomonas reinhardtii to Ultraviolet B Exposure. *Acta biochimica et biophysica Sinica*, 38(3), 187–193 (2006)
DOI: 10.1111/j.1745-7270.2006.00148.x

115. Z. Yang, y. Li, F. Chen, D. Li, Z. Zhang, Y. Liu, D. Zheng, Y. Wang and G. Shen: Expression of human soluble TRAIL in Chlamydomonas reinhardtii chloroplast. *Chinese Science Bulletin*, 51(14), 1703–1709 (2006)
DOI: 10.1007/s11434-006-2041-0

116. Z. L. SU, K. X. QIAN, C. P. TAN, C. X. MENG and S. Qin: Recombination and heterologous expression of allophycocyanin gene in the chloroplast of Chlamydomonas reinhardtii. *Acta biochimica et biophysica Sinica*, 37(10), 709–712 (2005)
DOI: 10.1111/j.1745-7270.2005.00092.x

117. M. Tran, B. Zhou, P. L. Pettersson, M. J. Gonzalez and S. P. Mayfield: Synthesis and assembly of a full-length human monoclonal antibody in algal chloroplasts. *Biotechnology and bioengineering*, 104(4), 663–673 (2009)
DOI: 10.1002/bit.22446

118. A. Eichler-Stahlberg, W. Weisheit, O. Ruecker and M. Heitzer: Strategies to facilitate transgene expression in Chlamydomonas reinhardtii. *Planta*, 229(4), 873–883 (2009)
DOI: 10.1007/s00425-008-0879-x

119. L.-L. Bai, W.-B. Yin, Y.-H. Chen, L.-L. Niu, Y.-R. Sun, S.-M. Zhao, F.-Q. Yang, R. R.-C. Wang, Q. Wu and X.-Q. Zhang: A new strategy to produce a defensin: stable production of mutated NP-1 in nitrate reductase-deficient Chlorella ellipsoidea. *PLoS One*, 8(1), e54966 (2013)
DOI: 10.1371/journal.pone.0054966

120. A. L. Manuell, M. V. Beligni, J. H. Elder, D. T. Siefker, M. Tran, A. Weber, T. L. McDonald and S. P. Mayfield: Robust expression of a bioactive mammalian protein in Chlamydomonas chloroplast. *Plant biotechnology journal*, 5(3), 402–412 (2007)
DOI: 10.1111/j.1467-7652.2007.00249.x

121. D.-M. He, K.-X. Qian, G.-F. Shen, Z.-F. Zhang, L. Yi-Nü, Z.-L. Su and H.-B. Shao: Recombination and expression of classical swine fever virus (CSFV) structural protein E2 gene in Chlamydomonas reinhardtii chroloplasts. *Colloids and surfaces B: Biointerfaces*, 55(1), 26–30 (2007)
DOI: 10.1016/j.colsurfb.2006.10.042

122. R. Surzycki, K. Greenham, K. Kitayama, F. Dibal, R. Wagner, J.-D. Rochaix, T. Ajam and S. Surzycki: Factors effecting expression of vaccines in microalgae. *Biologicals*, 37(3), 133–138 (2009)
DOI: 10.1016/j.biologicals.2009.02.005

123. X. Wang, M. Brandsma, R. Tremblay, D. Maxwell, A. M. Jevnikar, N. Huner and S. Ma: A novel expression platform for the production of diabetes-

associated autoantigen human glutamic acid decarboxylase (hGAD65). *Bmc Biotechnology*, 8(1), 87 (2008)
DOI: 10.1186/1472-6750-8-87

124. I. A. Dreesen, G. Charpin-El Hamri and M. Fussenegger: Heat-stable oral alga-based vaccine protects mice from Staphylococcus aureus infection. *Journal of biotechnology*, 145(3), 273–280 (2010)
DOI: 10.1016/j.jbiotec.2009.12.006

125. J. A. Gregory, F. Li, L. M. Tomosada, C. J. Cox, A. B. Topol, J. M. Vinetz and S. Mayfield: Algae-produced Pfs25 elicits antibodies that inhibit malaria transmission. *PloS one*, 7(5), e37179 (2012)
DOI: 10.1371/journal.pone.0037179

126. C. S. Jones, T. Luong, M. Hannon, M. Tran, J. A. Gregory, Z. Shen, S. P. Briggs and S. P. Mayfield: Heterologous expression of the C-terminal antigenic domain of the malaria vaccine candidate Pfs48/45 in the green algae Chlamydomonas reinhardtii. *Applied microbiology and biotechnology*, 97(5), 1987–1995 (2013)
DOI: 10.1007/s00253-012-4071-7

127. J. A. Gregory, A. B. Topol, D. Z. Doerner and S. Mayfield: Alga-produced cholera toxin-Pfs25 fusion proteins as oral vaccines. *Applied and environmental microbiology*, 79(13), 3917–3925 (2013)
DOI: 10.1128/aem.00714-13

128. S.-M. Yoon, S. Y. Kim, K. F. Li, B. H. Yoon, S. Choe and M. M.-C. Kuo: Transgenic microalgae expressing Escherichia coli AppA phytase as feed additive to reduce phytate excretion in the manure of young broiler chicks. *Applied microbiology and biotechnology*, 91(3), 553–563 (2011)
DOI: 10.1007/s00253-011-3279-2

129. B. A. Rasala, P. A. Lee, Z. Shen, S. P. Briggs, M. Mendez and S. P. Mayfield: Robust expression and secretion of Xylanase1 in Chlamydomonas reinhardtii by fusion to a selection gene and processing with the FMDV 2A peptide. *PloS one*, 7(8), e43349 (2012)
DOI: 10.1371/journal.pone.0043349

130. D.-H. Kim, Y. T. Kim, J. J. Cho, J.-H. Bae, S.-B. Hur, I. Hwang and T.-J. Choi: Stable integration and functional expression of flounder growth hormone gene in transformed microalga, Chlorella ellipsoidea. *Marine Biotechnology*, 4(1), 63–73 (2002)
DOI: 10.1007/s1012601-0070-x

131. Q. Hou, S. Qiu, Q. Liu, J. Tian, Z. Hu and J. Ni: Selenoprotein-transgenic Chlamydomonas reinhardtii. *Nutrients*, 5(3), 624–636 (2013)
DOI: 10.3390/nu5030624

132. D. Geng, Y. Wang, P. Wang, W. Li and Y. Sun: Stable expression of hepatitis B surface antigen gene in Dunaliella salina (Chlorophyta). *Journal of Applied Phycology*, 15(6), 451–456 (2003)
DOI: 10.1023/b:japh.0000004298.89183.e5

133. S. Feng, W. Feng, L. Zhao, H. Gu, Q. Li, K. Shi, S. Guo and N. Zhang: Preparation of transgenic Dunaliella salina for immunization against white spot syndrome virus in crayfish. *Archives of virology*, 159(3), 519–525 (2014)
DOI: 10.1007/s00705-013-1856-7

134. Y. Hirakawa and K. i. Ishida: Internal plastid-targeting signal found in a RubisCO small subunit protein of a chlorarachniophyte alga. *The Plant Journal*, 64(3), 402–410 (2010)
DOI: 10.1111/j.1365-313x.2010.04334.x

135. M. Lapidot, D. Raveh, A. Sivan, S. M. Arad and M. Shapira: Stable Chloroplast Transformation of the Unicellular Red AlgaPorphyridium Species. *Plant physiology*, 129(1), 7–12 (2002)
DOI: 10.1104/pp.011023

136. M. R. Te and D. J. Miller: Genetic transformation of dinoflagellates (Amphidinium and Symbiodinium): expression of GUS in microalgae using heterologous promoter constructs. *The Plant Journal*, 13(3), 427–435 (1998)
DOI: 10.1046/j.1365-313x.1998.00040.x

137. R. E. Soria-Guerra, J. I. Ramírez-Alonso, A. Ibáñez-Salazar, D. O. Govea-Alonso, L. M. Paz-Maldonado, B. Bañuelos-Hernández, S. S. Korban and S. Rosales-Mendoza: Expression of an HBcAg-based antigen carrying angiotensin II in Chlamydomonas reinhardtii as a candidate hypertension vaccine. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 116(2), 133–139 (2014)
DOI: 10.1007/s11240-013-0388-x

138. R. Barahimipour, J. Neupert and R. Bock: Efficient expression of nuclear transgenes in the green alga Chlamydomonas: synthesis of an HIV antigen and development of a new selectable marker. *Plant molecular biology*, 90(4–5), 403–418 (2016)
DOI: 10.1007/s11103-015-0425-8

139. J. I. Beltrán-López, A. Romero-Maldonado, E. Monreal-Escalante, B. Bañuelos-Hernández, L. M. Paz-Maldonado and S. Rosales-Mendoza: Chlamydomonas reinhardtii chloroplasts express an orally immunogenic protein targeting the p210 epitope implicated in atherosclerosis immunotherapies. *Plant cell reports*, 35(5), 1133–1141 (2016)
DOI: 10.1007/s00299-016-1946-6

140. G. Lai, Y. Li and G. Li: Effect of concentration and temperature on the rheological behavior of collagen solution. *International Journal of Biological Macromolecules*, 42(3), 285–291 (2008)
DOI: 10.1016/j.ijbiomac.2007.12.010

141. P. Noitup, W. Garnjanagoonchorn and M. T. Morrissey: Fish skin type I collagen: Characteristic comparison of albacore tuna (Thunnus alalunga) and silver-line grunt (Pomadasys kaakan). *Journal of aquatic food product technology*, 14(1), 17–28 (2005)
DOI: 10.1300/j030v14n01_03

142. M. Gómez-Guillén, J. Turnay, M. Fernández-Dıaz, N. Ulmo, M. Lizarbe and P. Montero: Structural and physical properties of gelatin extracted from different marine species: a comparative study. *Food Hydrocolloids*, 16(1), 25–34 (2002)
DOI: 10.1016/s0268-005x(01)00035-2

143. J. Nicholson, M. Wolmarans and G. Park: The role of albumin in critical illness. *British journal of anaesthesia*, 85(4), 599–610 (2000)
DOI: 10.1093/bja/85.4.599

144. P. Vlieghe, T. Clerc, C. Pannecouque, M. Witvrouw, E. De Clercq, J.-P. Salles and J.-L. Kraus: Synthesis of New Covalently Bound κ-Carrageenan− AZT Conjugates with Improved Anti-HIV Activities. *Journal of medicinal chemistry*, 45(6), 1275–1283 (2002)
DOI: 10.1021/jm010969d

145. Y. Freile-Pelegrın and E. Murano: Agars from three species of Gracilaria (Rhodophyta) from Yucatán Peninsula. *Bioresource Technology*, 96(3), 295–302 (2005)
DOI: 10.1016/j.biortech.2004.04.010

146. O. Berteau and B. Mulloy: Sulfated fucans, fresh perspectives: structures, functions, and biological properties of sulfated fucans and an overview of enzymes active toward this class of polysaccharide. *Glycobiology*, 13(6), 29R-40R (2003)
DOI: 10.1093/glycob/cwg058

147. F. Shahidi and R. Abuzaytoun: Chitin, chitosan, and co-products: chemistry, production, applications, and health effects. *Advances in food and nutrition research*, 49, 93–135 (2005)
DOI: 10.1016/s1043-4526(05)49003-8

148. L. Sijtsma and M. De Swaaf: Biotechnological production and applications of the ω-3 polyunsaturated fatty acid docosahexaenoic acid. *Applied microbiology and biotechnology*, 64(2), 146–153 (2004)
DOI: 10.1007/s00253-003-1525-y

149. J. Arct and K. Pytkowska: Flavonoids as components of biologically active cosmeceuticals. *Clinics in dermatology*, 26(4), 347–357 (2008)
DOI: 10.1016/j.clindermatol.2008.01.004

150. H. Maeda, Y. Sakuragi, D. A. Bryant and D. DellaPenna: Tocopherols protect Synechocystis sp. strain PCC 6803 from lipid peroxidation. *Plant Physiology*, 138(3), 1422–1435 (2005)
DOI: 10.1104/pp.105.061135

151. S. Bhattacharya and M. Shivaprakash: Evaluation of three Spirulina species grown under similar conditions for their growth and biochemicals. *Journal of the Science of Food and Agriculture*, 85(2), 333–336 (2005)
DOI: 10.1002/jsfa.1998

152. F. Shahidi and Y. J. Kamil: Enzymes from fish and aquatic invertebrates and their application in the food industry. *Trends in Food Science & Technology*, 12(12), 435–464 (2001)
DOI: 10.1016/s0924-2244(02)00021-3

153. T. Chen, H. D. Embree, E. M. Brown, M. M. Taylor and G. F. Payne: Enzyme-catalyzed gel formation of gelatin and chitosan: potential for *in situ* applications. *Biomaterials*, 24(17), 2831–2841 (2003)
DOI: 10.1016/s0142-9612(03)00096-6

154. R. Parr, N. Aras and G. Iyengar: Dietary intakes of essential trace elements: Results from total diet studies supported by the IAEA. *Journal of radioanalytical and nuclear chemistry*, 270(1), 155–161 (2006) DOI: 10.1007/s10967-006-0323-2

Key Words: Microalgae, Value-Added Compounds, Industrial Exploitation, Review

Send correspondence to: Hafiz M. N. Iqbal, School of Engineering and Science, Tecnologico de Monterrey, Campus Monterrey, Ave. Eugenio Garza Sada 2501, Monterrey, N.L., CP 64849, Mexico, Tel: 5218183582000 Ext. 5561 Sub-ext. 115, E-mail: hafiz.iqbal@my.westminster.ac.uk

