Chapter 3
Microalgal Promise to the Next Generation: A Dual Potential Perspective as Cosmeceuticals and Biofuels

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Abstract The gradual rise in the human population and an ever-increasing demand for high-value products and alternative fuels has pushed industries into discovering new bioactive compounds and process technologies. Microalgae, an emerging bioresource with distinctive metabolite composition and an efficient growth rate, thus offer a unique platform to meet these demands of sustainable and alternative sources of food and energy. Till date several species of microalgae have been evaluated for their application as cosmeceuticals, pharmaceuticals, nutraceuticals, and biofuels owing to the major bioactive profiles including proteins, polysaccharides, polyunsaturated fatty acids (PUFAs), functional pigments, and vitamins. In addition, the prospect of genetically modified microalgae holds a greater promise to the future for high-value products and third-generation biofuels.

Keywords Cosmeceuticals · Biofuels · Bioactive ingredients · Genetically modified microalgae

Abbreviations

PUFAs	polyunsaturated fatty acids
CAGR	compound annual growth
US	United States of America
MAAs	mycosporine-like amino acids
HA	hydroxy acids
PMNS	polymorphonuclear leukocyte
mg	milligram
ml	milliliter
kDa	kilodaltons
EPA	eicosapentaenoic acid
DHA	docosahexaenoic acid

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TAGs	triacylglycerols
MUFAs	monounsaturated fatty acids
SFAs	saturated fatty acids
MGDG	monogalactosyl diacylglycerol
DGDG	digalactosyldiacylglycerol
MW	molecular weight
UV	ultraviolet radiation
FADD	Fas-associated death domain
MMPs	matrix metalloproteinases
CDP	*Chlorella*-derived peptide
ACE	angiotensin-converting enzyme
CaO	calcium oxide
CO_2	carbon dioxide
CME	*Caulerpa microphysa* extract
FAME	fatty acid methyl ester
V/V	volume per volume
Mg/gm	milligram per gram
cc/g	cubic centimeter per gram
AFDW	ash-free dry weight
MJ/kg	megajoules per kilogram
SEM	scanning electron microscope
PSII	photosystem II
CRISPR	clustered regularly interspaced short palindromic repeats
SSF	saccharification and fermentation
SHF	separate hydrolysis and fermentation
CH_4	methane

3.1 Introduction

The cosmetic industry is predicted to exceed more than US$ 390 billion by 2025, with a compound annual growth rate (CAGR) of 4.3% from 2016 to 2022. Changing lifestyles, the rising economy, and the ineluctable health benefits from natural ingredients are the major factors impacting the thriving trend in the global cosmetics market. Growing awareness about the ill effects of toxic constituents and the developing need for a green lifestyle has further shifted the oversight towards natural products derived from cosmetics. Cosmetics and cosmeceuticals are increasingly using active ingredients from natural sources which present great biodiversity of desired and safe components. Microalgae have come a long way as a unique potential with several primary bioactive compounds of cosmeceutical, pharmaceutical, and nutraceutical importance such as proteins, polysaccharides, pigments, lipids, and vitamins (Agrawal and Verma 2022; Andrade 2018; Morais et al. 2015). Owing to a range of bioactive compounds displaying antioxidant,

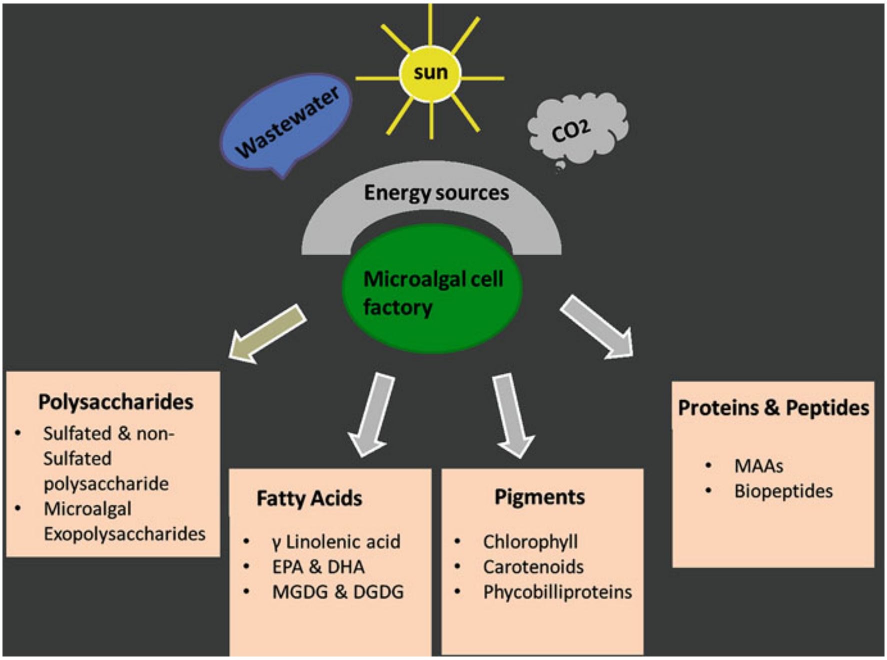

Fig. 3.1 Bioactive components from microalgae with cosmeceutical potentials

immunomodulatory, and anticancer activities, microalgae are currently explored for utilizing these properties in cosmetic application including anti-aging, sunscreens, wound healing, and anti-acne treatments (Mehariya et al. 2021; Yarkent et al. 2020; Pangestuti and Kim 2011). Numerous microalgae have prospected for cosmeceutical properties, for example, *Spirulina platensis* extracts showed wound healing activities in HS2 keratinocyte cells (Gunes et al. 2017), and *Spirulina* extract loaded PCL nanofiber improved skin regeneration by increasing fibroblasts viability and modulating (reactive oxygen species) ROS levels. Furthermore, antiproliferative and anticancer properties of *Spirulina* and *Chlorella* are also widely reported (Cha et al. 2008; Czerwonka et al. 2018; El-fayoumy et al. 2021; Fayyad et al. 2019; Kyadari et al. 2013). Notably, application of microalgae in thalassotherapy as a marine cosmetic for the therapeutic and revitalizing schemes are novel frontiers in microalgal biotechnology (Mourelle et al. 2017) (Fig. 3.1).

Another distinct application of microalgal bioresource includes the demanding area of biofuels. In the coming years, depletion of fossil fuels as well as concerns of global warming further necessitates an urgent requirement for alternative energy sources (Kotasthane 2017). In comparison to crop-based biofuels, Microalgal derived biofuels have several advantages as follows: (1) It reduces pressure on fertile agricultural land which can be instead used for agricultural practices to nourish the growing population. (2) Unlike lignocellulose-rich crop biomass which requires several pretreatment processes, microalgae with low levels of lignocellulose provide a cost-effective raw material for biofuel extraction. (3) Adaptability of microalgae in extreme conditions and wastewater provides feasible bioenergy source as well as scope for greener technological advancements. (4) Finally the prospect of genetically modified microalgae and strain optimization studies can further enhance the bio-products development in area of biodiesel, bioethanol, biogas, and bio-oil (Bhardwaj et al. 2020; Molinuevo-Salces et al. 2019; Adegboye et al. 2021; Goswami et al. 2021b; Hannon et al. 2010). In this chapter we briefly discuss the wide range of bioactive compounds with potential role in cosmeceuticals as well as capture the overview of different microalgal species involved in cosmeceuticals and biofuels generation. Additionally, we also discuss numerous bio-products that have potential role in biofuel development.

3.2 Microalgae as Cosmeceuticals

In view of the changing “greener” lifestyles and the growing awareness for natural ingredients, microalgae emerge as the popular choice for the cosmeceutical industry. The potential of microalgae as a rich source of pigments, proteins, vitamins, minerals, polysaccharides, and fatty acids provides a direct benefit for human health, especially as a cosmetic product. Microalgal bioresource offers a unique platform for novel bioactive molecules in the cosmeceutical industry, a combination of cosmetics and pharmaceuticals, i.e., possessing health benefits. Microalgae represent a diverse group of unicellular prokaryotic (Prochlorophyta and Cyanophyta) and eukaryotic (Chlorophyta, Bacillariophyta, Phaeophyta, Rhodophyta, and Chrysophyta) microorganisms characterized by a wide range of bioactive metabolites with antioxidant, anti-inflammatory, and immune-modulatory properties. Extracts from microalgae such as *Arthrospira*, *Chlorella*, *Nannochloropsis*, *Spirulina*, and *Dunaliella* have already been established as an ingredient in skincare compositions (Stolz and Obermayer 2005). Exsymol (Monaco) used protein-rich extract from *Spirulina platensis* with anti-aging properties. *Spirulina* firming masks produced by Optimum Derma Aciditate (Lithuania) improve skin moisture balance and immunity (Chakdar et al. 2012). Dermochlorella D, an amino acid concentrate from *Chlorella vulgaris* produced by Codif (France), promotes activation of collagen synthesis and reduces stretch marks and vascular imperfections (Ryu et al. 2015). The cosmeceutical value of microalgae is conferred due to the feasibility of growing them in extreme conditions and also due to the wide range of chemicals with favorable biological activity (Mobin and Chowdhury 2019). Although a plethora of microalgae have been identified and utilized in industrial operations for cosmeceuticals, yet these represent only a percentage of their substantial diversity. To date more than 25,000 novel bioactive compounds have been discovered from different oceanic regions of the world (Blunt et al. 2016). These bioactive metabolites are categorized into different major groups, viz., polysaccharides, fatty acids, pigments, proteins, peptides, etc. (Table 3.1).

Table 3.1 The cosmetic activity of major microalgae with their unique bioactive composition

Microalgal species	Cosmetic activity	Active ingredients	References
Spirulina platensis	Anti-aging, anti-acne, wound healing	α- and γ-linolenic acid, C-phycocyanin	Ragusa et al. (2021), Gunes et al. (2017)
Chlorella fusca, *Chlorella minutissima*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Chlorella ellipsoidea*	Anti-aging, control of inflammatory process and pigmentation, protection against sun damage	Chlorophyll, carotenoids, sporopollenin, and mycosporine-like amino acids	Agustina et al. (2021), Chatzikonstantinou et al. (2017), de Andrade et al. (2017), Kwang et al. (2008), Caicedo et al. (2020)
Nannochloropsis gaditana, *Nannochloropsis oceanica*	Anti-aging, oxidative stress response	Violaxanthin, fucosterol, antityrosinase, zeaxanthin	Kim (2014), Ma et al. (2015), Letsiou et al. (2017), Hyun Min Kim et al. (2019), Yarkent et al. (2020), Park et al. (2021)
Dunaliella salina	Antioxidants	β-Carotene	Kane et al. (2016), Silva et al. (2021), Tran et al. (2014), Yarkent et al. (2020)
Haematococcus pluvialis	Antioxidant, skin conditioning, and protection	Astaxanthin	Vieira et al. (2021), Guerin et al. (2003), Zakaria et al. (2021), Ruiz-Domínguez et al. (2019), Marino et al. (2020)
Coelastrella striolata var. *multistriata*	Antioxidants	Canthaxanthin	Abe et al. (2007)
Scytonema sp.	Antioxidants	Mycosporine-like amino acids (MAAs)	Vega et al. (2020)
Anabaena vaginicola	Antioxidant and anti-aging agent	Lycopene	Hashtroudi et al. (2013)
Scenedesmus obliquus, *Scenedesmus rubescens*	Anti-inflammatory and anti-aging	*Scenedesmus* extracts	Cengiz and Sevilay. (2019), Campiche et al. (2018)
Porphyridium sp.	Anti-inflammatory and antioxidant	Sulfated polysaccharides	Matsui et al. (2003)) Arad and Levy-Ontman (2010), Arad and van Moppes (2013), Tannin-Spitz et al. (2005)
Botryococcus braunii	Antioxidants	Violaxanthin, β-carotene, lutein, and zeaxanthin	Koller et al. (2014)

3.2.1 Polysaccharides

Polysaccharides are the most abundant biochemical molecule made up of thousands of monosaccharide units. These polymeric compounds can be categorized as homo-polysaccharides or hetero-polysaccharides based on the single or different monosaccharide unit that makes the compound. The glycosidic bonds that frequently occur in these compounds are β-1,4 or β-1,3 and α-1,4, α-1,2, or α-1,6 linkages. The α-glucans (starch and floridean starch) have been reported in green, glaucophyte, charophyte, cryptomonad, dinophyte, red microalgae, and cyanobacteria (Mobin and Chowdhury 2019). Similarly, the β-glucans (chrysolaminarin and paramylon) have been found in euglenophytes, haptophytes, *Chlorella* genus, *Skeletonema* diatom, and also *Porphyridium* and *Nostoc flagelliforme* (Mourelle et al. 2017). The cosmetic action provided by β-1,3-glucans includes blood cholesterol levels reduction, antioxidant activities, as well as immune stimulator (Koller et al. 2014). Xia et al. (2014) discovered a novel β-glucans, chrysolaminarin, named CL2 obtained from *Odontella aurita* that showed strong antioxidant activities. Until recently, at an industrial level, Algatech has licensed their rights to the production of β-1,3-glucans from fermented microalgae *Euglena gracilis*. High concentration and purity of the biochemical in *Euglena gracilis* compared to common sources in the market such as oats, mushrooms, and yeast make them potential and effective bioresource with powerful health benefits (for an example see https://www.algatech.com/algatech-product/bioglena-beta-glucan). In a cosmetic formulation, use of hydroxy acid (HA) is promoted to provide skin moisturization; however, since it can be only extracted from plants and animals, the cost is relatively high. Studies have shown the application of polysaccharides from marine algal species like *Saccharina japonica* and *Chondrus crispus* can provide a much more cost-effective alternative to hydroxy acid (Wang et al. 2015). As reported by several authors, water-soluble polysaccharides from *Spirulina platensis* are another potential raw material for the cosmeceutical industry with strong antioxidant activities (Chaiklahan et al. 2013; Kurd and Samavati 2015).

Microalgal exopolysaccharides are complex glycopolymers that protect microorganisms against extreme environmental stress conditions like dehydration as it allows them to retain cellular water. It is thus widely accepted that this property of the microalgal exopolysaccharide can be utilized for moisturization and hydration of the skin (Stoyneva-Gärtner et al. 2020). Although only a few studies have been conducted on these lines, extracellular sulfated polysaccharide from red microalgae *Porphyridium* sp. has drawn prominent interest based on their remarkable nutritional, therapeutic, and cosmetic activity. Estee and Companies (2003) reported the in vitro and in vivo anti-inflammatory activity of polysaccharide material from *Porphyridium* sp. The bioactive sulfated polysaccharide prevented the polymorphonuclear leukocyte (PMN) migration involved in skin inflammation. In another study, the antioxidant properties of sulfated polysaccharide extract from *Porphyridium* sp. have been reported. At a concentration of 2 mg ml^{-1} and 10 mg ml^{-1}, *Porphyridium* polysaccharide gave antioxidant activity of 45% and 90%, respectively (Tannin-Spitz et al. 2005). Furthermore, the sulfated polysaccharide from *Porphyridium* sp. is also believed to be an excellent alternative to hyaluronic acid as a bio-lubricant with antioxidant activities (Raposo et al. 2013a). Based on the significant cosmetic properties, sulfated polysaccharides thus make a suitable candidate as a cosmetic bioresource (Arad and van Moppes 2013). Notably in a study conducted on polysaccharide, extract of *Caulerpa microphysa* (Chlorophyta) suggested excellent anti-inflammatory and wound healing with good moisture-holding capacity. *Caulerpa microphysa* extract (CME) showed complete inhibition of β-hexosaminidase, a critical inflammatory mediator in allergic reactions thus suggesting the anti-allergic potential of CME (Lee et al. 2021). Table 3.2 presents a list of microalgal polysaccharides and their specific features and cosmetic action.

3.2.2 Fatty Acids

Traditionally, microalgae are considered a rich source of lipids, especially the long unsaturated fatty acids such as γ-linolenic acid, arachidonic acid, stearidonic acid, and ω-3 fatty acids: eicosapentaenoic acid (EPA) (ω-3 C 20:5) and docosahexaenoic acid (DHA) (ω-3 C 22:6), etc. (Mobin and Chowdhury 2019). The total lipid production in microalgae may reach up to 30–70% of their dry weight depending on the species type (Balasubramaniam et al. 2021). Microalgal species like *Chlorella emersonii*, *Dunaliella tertiolecta*, *Nannochloropsis* sp., *Porphyridium cruentum*, *Botryococcus braunii*, and *Neochloris oleoabundans* show high lipid content of more than 60% of their dry weight. Freshwater microalgal species like *Chlorella minutissima*, *Chlorella protothecoides*, *Chlorella vulgaris*, *Scenedesmus dimorphus*, and *Scenedesmus obliquus* and marine microalgal species like *Dunaliella salina*, *Nannochloris* sp., *Crypthecodinium cohnii*, *Isochrysis galbana*, *Nitzschia* sp., *Phaeodactylum tricornutum*, *Schizochytrium* sp., and *Skeletonema costatum* display moderate lipid content in the range of 40–60% of their dry weight. Microalgae that display lower lipid content, i.e., below 40% of their dry weight, include *Arthrospira maxima*, *Spirulina platensis*, *Dunaliella primolecta*, *Chlorella sorokiniana*, *Ankistrodesmus* sp., *Chaetoceros muelleri*, *Cylindrotheca* sp., *C. cohnii*, *Ellipsoidion* sp., *Euglena gracilis*, *Haematococcus pluvialis*, *Monodus subterraneus* UTEX 151, *Monallanthus salina*, *Oocystis pusilla*, *Pavlova salina*, *Thalassiosira pseudonana*, and *Tetraselmis suecica* (Mobin and Chowdhury 2019; Maity et al. 2014). Evidently, two genera of microalgae, viz., *Dunaliella* and *Chlorella*, show differential total lipid content at a species level: *Dunaliella tertiolecta* (more than 60%), *Dunaliella salina* (40–60%), and *Dunaliella primolecta* (less than 40%) and also *Chlorella emersonii* (more than 60%), *Chlorella vulgaris* (40–60%), and *Chlorella sorokiniana* (less than 40%). A greater emphasis must be thus given to the microalgal strains with high lipid content to empower cost-effectiveness for economic production.

The unique fatty acid profiles from microalgae can be broadly categorized into polar and neutral lipids. The polar lipids are mainly constituted of membrane lipids

Table 3.2 Microalgal polysaccharide features and their cosmetic activity

Microalgae species	Polysaccharide type	Major polysaccharide features	Cosmetic activity	References
Porphyridium sp. and *Porphyridium aerugineum*	Sulfated exopolysaccharide	MW 2000–7000 kDa; major sugars: xylose, glucose, and galactose	Antioxidants	Burg and Oshrat (2015)
Navicula sp.	Sulfated exopolysaccharide	MW 17 kDa; major sugars: Glucose, galactose, rhamnose, xylose, and mannose	Antioxidants	Fimbres-Olivarria et al. (2018)
Isochrysis galbana	Sulfated exopolysaccharide	MW 15.934 kDa; major sugars: Mannose, glucose, galactose, and rhamnose	Antioxidants	Sun et al. (2014a, b)
Cylindrotheca closterium	Sulfated exopolysaccharide	Major sugars: Glucose, galactose, xylose, mannose, and rhamnose	Not described	Staats et al. (1999)
Sarcinochrysis marina	Sulfated polysaccharide	MW 2595 kDa; major sugars: L-arabinose, D-fructose, and glucose	Antioxidants	Sun et al. (2014a, b)
Phaeodactylum tricornutum	Sulfated polysaccharide	MW 449 kDa; major sugars: Glucose, glucuronic acid, and mannose	Anti-inflammatory and immunomodulatory	Guzmán et al. (2003)
Pavlova viridis	Sulfated polysaccharide	MW 3645 kDa; major sugars: rhamnose, D-fructose, glucose, and mannose	Antioxidants	Sun et al. (2014a, b)
Pavlova viridis	Sulfated polysaccharide	MW 386.96 kDa; major sugars: D-fructose, glucose, and mannose MW 54.99 kDa; major sugars: D-fructose, glucose, mannose, and L-rhamnose	Immunomodulatory	Sun et al. (2016)
Chlorella stigmatophora	Sulfated polysaccharide	MW 22 kDa; major sugars: Glucose, glucuronic acid, xylose, and ribose/fucose	Anti-inflammatory and immunomodulatory	Guzmán et al. (2003)
Spirulina sp.	Sulfated polysaccharide	MW 200 × 103 g/Mol; major sugars: Acofriose and rhamnose	Anticancer	Senni et al. (2011)
Parachlorella kessleri	Non-sulfated polysaccharide	MW 3.05 × 105 and 9.84 × 104 g Mol − 1 with α-L-rhamnan and xylogalactofuranan in different ratios	Immunomodulatory	Sushytskyi et al. (2020)

Dictyosphaerium pulchellum and *Dictyosphaerium tetrachotomum*	Non-sulfated exopolysaccharide	Galactose, xylose, unidentified 2-OMe-hexose and fucose, and glucuronic acid	Immunomodulatory	Halaj et al. (2019)
Aphanothece halophytica	Non-sulfated exopolysaccharide	MW 2000 kDa; major sugars: glucose, fucose, mannose, arabinose, and glucuronic acid	Not described	Li et al. (2001)
Chlorella pyrenoidosa	Non-sulfated polysaccharide	MW 188 and 1020 kDa; major sugars: Arabinose and galactose	Immunomodulatory	Suárez et al. (2006)

such as phospholipids and glycolipids in a microalgal cell. On the contrary neutral lipids are localized in the cytoplasm as triacylglycerols (TAGs) (Yao et al. 2012). Long-chain polyunsaturated fatty acid (PUFA) usually belongs to polar lipids, whereas a neutral lipid consists of monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs) in the triacylglycerols (TAGs). Based on the percentages of characteristic fatty acids such as C 16:0/C16:1 and eicosapentaenoic acid (EPA) (ω-3 C 20:5) for neutral (TAGs) and polar fatty acids, respectively, fatty acid profiles of different microalgae can be compared. *Nannochloropsis oceanica* fatty acid percentage values in terms of r^2 of C16:0 and EPA were 0.94 and 0.97, respectively. Similarly, *Chlorella pyrenoidosa* r^2 values for C18:1/ C18:3 with TAG content were 0.91 and 0.99, respectively. This method of correlation thus allows researchers to precisely quantify TAGs in microalgae (Shen et al. 2016). Several species of microalgae have been explored for their rich lipids content and their subsequent application in the cosmetic industry. Gamma-linolenic acid from *Spirulina platensis* functions as the potential bioactive compound with anti-aging, anti-wrinkle, collagen synthesis, anti-inflammatory, and antioxidant activities (Hoseini et al. 2013). *Porphyridium cruentum* is an excellent source of polyunsaturated fatty acids (PUFAs) that provides photoprotection against the high intensity and UV light as well as prevents skin dehydration. ω-3 PUFAs, namely, eicosapentaenoic acid (EPA, 20:5 ω3) and docosahexaenoic acid (DHA, 22:6 ω3), from *Isochrysis galbana* show anti-inflammatory effect. Furthermore, several extracts of lipids, PUFAs, and pigments from *Isochrysis galbana* have been claimed to have skin and hair care properties (Abdoul-latif et al. 2021; Bonfanti et al. 2018). Microalgal squalenes have been reported to have moisturizing, antioxidant, and anti-aging properties. A large number of cosmetic products, viz., lipstick, lotions, eye pencil, eye shadows, eye makeup remover, and perfumes, utilize the squalene in their formulations. *Botryococcus braunii*, *Schizochytrium mangrovei*, and *Thraustochytrium* sp. are a few microalgae species that produce these thriving molecules. Microalgal galactolipids, i.e., monogalactosyl diacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), present another unique bioactive resource with cosmeceutical applications based on its anti-inflammatory activities. Bruno et al. compared dose-dependent anti-inflammatory activity of MGDG and DGDG and concluded that eicosapentaenoic acid (EPA) presence in MGDG further enhanced the activity (Bruno et al. 2005). Microalgal species such as *Chlorella minutissima*, *Chaetoceros*, *Cyclotella*, *Ellipsidion*, *Isochrysis*, *Monochrysis*, *Monoraphidium*, *Nannochloris*, *Nannochloropsis*, *Nitzschia*, *Phaeodactylum*, *Porphyridium*, *Skeletonema*, and *Thalassiosira* are the potential sources of the galactolipids (Asraful et al. 2020).

3.2.3 Pigments

Pigments are another widely explored bioactive resource from the microalgal biomass. Fat-soluble pigments such as chlorophyll and carotenoids including β-carotene, astaxanthin, and fucoxanthin as well as water-soluble phycobiliproteins such as allophycocyanin, phycocyanin, phycoerythrin, and phycoerythrocyanin are the most ubiquitous in the microalgal cell factory. *Agardhiella*, *Arthrospira*, *Chlorella*, *Dunaliella*, *Haematococcus*, *Muriellopsis*, *Nannochloropsis*, *Nostoc*, *Phaeodactylum*, *Porphyridium*, *Polysiphonia*, *Scenedesmus*, and *Spirulina* are major microalgal genera that produce these wide ranges of bioactive pigments. They are commonly present in cyanobacteria (blue-green algae), Rhodophyta (red algae), and unicellular eukaryotic algae (cryptomonads). Phycobiliprotein's properties as an antioxidant and free radical scavenging activities have drawn major attention in cosmetics as well as in food, pharmaceuticals, and biomedical applications. Furthermore, microalgal pigments also exhibit anti-inflammatory, antiangiogenic, antiviral, antiobesity, antidiabetic, anticancer, anti-osteoporotic, and neuro- and hepatic-protective activities (Paillriè-Jiménez et al. 2020). At present Dainippon Ink and Chemicals Inc. (Japan) utilizes *Arthrospira* spp. with a brand name of Linablue as a food colorant as well as cosmetic applications (Saini et al. 2021; Morocho-Jácome et al. 2020). Algenist (CA, USA) introduced a novel vitamin C from *Spirulina* which enhances skin tone, smoothens texture, and prevents photoaging (for an example see BLUE ALGAE VITAMIN C™ Skinclarity Brightening Serum (algenist.com)). Because of their pH and heat stability characteristics, phycobiliproteins are used as a natural colorant in eyeliners, lipsticks, and other cosmetic formulations (Balboa et al. 2015).

There is an increasing demand for carotenoids derived from microalgae considering their pharmaceutical and cosmetic role. *Chlorella*, *Dunaliella*, *Haematococcus*, and *Muriellopsis* are the routine sources for carotenoids. Astaxanthin extracted from *Haematococcus pluvialis* is reported to have antioxidant biological activity as well known to reduce skin pigmentation. A clinical study on two human subjects, 30 women aged 20–55 years old and 36 men aged 20–60 years old, concluded astaxanthin's role in improving elasticity, skin texture, moisture content of corneocyte layer, and corneocyte condition as well as reducing skin wrinkle and age spot size in both subjects (Goswami et al. 2021c; Tominaga et al. 2012). In another study, astaxanthin was found to significantly suppress transepidermal water loss and wrinkle formation thus preventing photoaging caused due to UV-A radiation (Komatsu et al. 2017). AstaPure is a commercial product of astaxanthin extracted from *Haematococcus pluvialis* utilized by Algatech (Israel) as topical creams and emulsions because of its cosmeceutical activity on skin health, eye health, and immunity ((Morocho-Jácome et al. 2020), also see AstaPure Natural Astaxanthin l astaxanthin manufacturer l bulk astaxanthin (algatech.com). Fucoxanthin mainly found in *Cercis siliquastrum*, *H. fusiformis*, *L. japonica*, *Undaria pinnatifida*, and *Sargassum fulvellum* also offers antioxidant properties. Fucoxanthin extracted from *Phaeodactylum tricornutum* showed protection against oxidative damage (Kawee-ai et al. 2013). A study reported the anti-pigmentary activity of fucoxanthin by inhibiting tyrosinase, a key enzyme in melanogenesis, and also preventing the UV-B radiation-induced skin pigmentation (Shimoda et al. 2010).

The most common carotene produced by the halotolerant microalga *Dunaliella salina* is β-carotene, i.e., up to 10% of its dry weight biomass. Apart from pro-vitamin A activity, β-carotene is also accepted as a cosmeceutical because of its properties like antioxidants, anticancer, anti-inflammatory, and immune modulators (Raposo et al. 2013b). Guruvayoorappan and Kuttan (2007) demonstrated the antiangiogenic activity of β-carotene on B16F-10 cell lines. The study showed that β-carotene prevented neovascularization, i.e., the formation of tumor-directed capillaries by tailoring the expression of proinflammatory cytokines such as matrix metalloproteinase 2 (MMP-2) and MMP-9 and also preventing nuclear translocation of the transcription factor. In another study, β-carotene exhibited antioxidant and anti-inflammatory activity on *H. pylori*-infected human gastric epithelial AGS cell lines. *H. pylori*-infected cell lines showed increased expression of matrix metalloproteinases (MMPs), key molecules in metastasis and cancer invasion. However, on treatment with β-carotene, the expression of MMPs was significantly controlled primarily by reducing the ROS levels in the cell line (Bae et al. 2021).

3.2.4 Proteins and Peptides

Mycosporine-like amino acids (MAAs) are water-soluble, low molecular weight (0.188 to 1.05 kDa) bioactive resources found in cyanobacteria, microalgae, macroalgae (chlorophyta and Rhodophyta), and several other marine organisms (Wada et al. 2015). Oligosaccharide-linked mycosporine-like amino acids were first reported in *Nostoc commune* which also showed significant photoprotection against UV-B radiation. These molecules have a characteristic UV-A and UV-B absorption, a critical feature for cosmeceutical application as sunscreens (Bohm et al. 1995). Schmid et al. (2006) characterized MAAs-based sunscreens (Helioguard 365) extracted from *Porphyra umbilicalis* in in vitro and in vivo conditions and demonstrated the anti-aging and antioxidant properties of the biochemical. Helioguard 365 containing porphyra-334 and shinorine showed protection from irradiation-induced DNA damage and prevented lipid peroxidation ultimately providing smoothness and firmness of the skin. In another study porphyra-334 extracted from *P. yezoensis* showed significant antioxidant properties with ROS production inhibition as well as induced type I collagen and elastin suggesting a role as an anti-wrinkle or anti-aging agent (Ryu et al. 2014). A commercial product named HELIONORI® with mycosporine-like amino acids (MAAs) as an important ingredient is derived from *Porphyra umbilicalis*. The cosmetic product protects against UV-A-induced skin damage and DNA damage and also prevents premature photo-aging ((Figueroa 2021), e.g., see HELIONORI® - GELYMA)). Until recently, MAAs has been reported in *Chlorella*, *Pseudochlorella*, *Stichococcus*, *Apatococcus*, *Bracteacoccus*, *Coccomyxa*, *Elliptochloris*, *Pabia*, *Prasiolopsis*, and *Pseudococcomyxa* (Stoyneva-Gärtner et al. 2020).

Biopeptides are low molecular weight (3 kDa) short peptides derived from plants, animals, and microalgal sources. *Chlorella* and *Spirulina* are a few microalgal genera that are currently explored for biopeptides with cosmeceutical properties. Chlorella-derived peptides showed inhibitory activity towards UV-B-induced matrix metalloproteinase-1 (MMP-1) involved in the photoaging process and also restored collagen and TbRII (transforming growth factor, TGF-β, receptor) preventing further damage to the skin (Chen et al. 2011). In another study, *Chlorella*-derived peptide (CDP) prevented UV-C-induced cytotoxicity and DNA damage through inhibition of caspase-3 activity and Fas-associated death domain (FADD) expression (Shih and Cherng 2012). Sadeghi et al. (2018) reported spirulina-based peptides with antimicrobial and anticancer properties. In this study spirulina-based peptides (<3 kDa peptide fraction) showed prominent dose-dependent inhibition of SW480 cells (human colon adenocarcinoma cell), and the same fraction also demonstrated significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Furthermore a phycobiliprotein-derived bioactive peptide, SpirPep1, attained significant (angiotensin-converting enzyme) ACE inhibitory activity thus demonstrating anti-tumor and antihypertensive activity (Anekthanakul et al. 2019).

3.3 Microalgae for Biofuel Production

Due to environmental concerns including increasing greenhouse emissions and the world energy crisis, a need for an alternative source of energy with CO_2 mitigation potential has arisen. Microalgae provide a unique feedstock for biofuels production that can outcompete the traditional fossil fuels and crop-based biofuels providing a sustainable approach to the energy crisis as well as increasing the efficiency of biofuel production. In developing countries like India with 120 million tonnes of petroleum consumption per year, at least 21% of the agricultural land for biofuels production is necessary (Mondal et al. 2017). However, with microalgal bioresource, less than 2–3% of the agricultural land would be required considering the high oil yield productivity in terms of area utilized. Compared to the oil yield of crops such as caster, maize, oil palm, and physic nut which range from 172 to 5366 oil liters/hectare/year, microalgae with high oil yield can produce up to 136,900 oil liters/hectare/year (Medipally et al. 2015). Significantly microalgal land use efficiency for biofuel production was 338 times greater than corn biodiesel (Lum et al. 2013). Furthermore the CO_2 capture efficiency of microalgae is estimated to be 10–50 times higher than that of terrestrial plants (Li et al. 2008). Studies report CO_2 capture from many microalgal species such as *Chlorella kessleri*, *Chlorella emersonii*, *Chlorococcum littorale*, *Galdieria partita*, and *Synechococcus* PCC7942. The photoautotrophic potential of microalgae thus provides an added advantage to the utilization of greenhouse gas like CO_2 in the upstream application for biofuels like bioethanol, biodiesel, biogas, biohydrogen, bio-oil, and syngas.

3.3.1 Biodiesel

Major biodiesel standards specification like US specification (ASTM D6751), European biodiesel specification (EN 14214), and Indian biodiesel specification define biodiesel as a mixture of monoalkyl esters of long-chain fatty acids (Mondal et al. 2017). Transesterification is a primary process for the conversion of triglycerides (oils) generated from microalgae to biodiesel using different catalysts such as alcohols, acids, and enzymes. Tang et al. (2011) reported *Dunaliella tertiolecta* as a potential feedstock for biodiesel production extracted using methanol and chloroform. Fatty acid methyl ester (FAME) profile showed the significant percentage of methyl linolenate (C18:3), methyl palmitate (C16:0), methyl oleate (C18:1), and methyl linoleate (C18:2) with the highest cell density at 2–6% of CO_2 concentration. A study conducted on biodiesel production from *Spirulina platensis* using acid and methanol reported a viscosity value of 4.8 in accordance with the EN 14214 standard thus affirming its quality as a biodiesel substitute to fossil-derived fuels (El-Shimi et al. 2013). Investigation on the choice of the solvent for lipids extraction in *Nannochloropsis* sp. showed chloroform/methanol (1:2 v/v) with the highest percentage yield of 60.37% (Rahmanpour and Shariati 2015). Extraction process is one of the major factors affecting biodiesel yield apart from the choice of solvents. A study on microalgae *Schizochytrium limacinum* validated that direct transesterification of microalgal biomass using methanol and sulfuric acid along with chloroform, hexane, and petroleum ether produced higher biodiesel yield (10–20%) compared to indirect extraction-transesterification method using chloroform and methanol as extracting agent and additional sulfuric acid as transesterification agent (Johnson and Wen 2009). In another study using *Scenedesmus* sp. for in situ or direct transesterification process, a higher biodiesel production was recorded for alkaline catalyst (55.07 ± 2.18%) than acidic catalyst (48.41 ± 0.21%) (Kim et al. 2014). Nutrient limitation is another factor that can impact the efficiency of biodiesel production in microalgae by lipid accumulation, i.e., converting excess carbon into storage lipids. In one study nitrogen limitation increased the biodiesel yield in *Chlorella* sp. and *Desmodesmus quadricaudatus*. For *Chlorella* the unsaturated fatty acids increased from 37.92% to 41.6% in nitrogen-free medium, and similarly for *Desmodesmus quadricaudatus*, saturated fatty acids increased from 51.62% to 66.92% in nitrogen-free medium (Shafik et al. 2015).

3.3.2 Bioethanol

On account of low ligno- and hemicellulose content in microalgae compared to lignocellulosic biomass observed in conventional crops, microalgae with high levels of carbohydrates provide an appropriate choice for bioethanol production. Bioethanol production from biomass requires processes such as pretreatment, saccharification or enzymatic hydrolysis, fermentation, and distillation. Mild pretreatment is required for microalgal biomass owing to the absence of lignocellulose in algal biomass. During the process of pretreatment and saccharification, the complex polysaccharide is broken down into fermentable monomeric sugars. Several methods of pretreatment and saccharification are employed, i.e. physical or mechanical (ultrasonication, high-pressure homogenization, autoclave, bead beating), chemical (acidic hydrolysis, alkaline hydrolysis, and supercritical CO_2), or enzymatic treatment (Agrawal et al. 2020; Kumar et al. 2020a; Phwan et al. 2018; Behera et al. 2015). Pretreatment studies using alkaline hydrolysis produced a maximum reducing sugar concentration of 88 mg/g dried biomass and 81 mg/g dried biomass in *Chlorella* sp. and *Tetraselmis suecica*, respectively (Behera et al. 2015). In another study, microalgae *Chlorococcum infusionum* was used for alkaline pretreatment which produced the highest glucose yield of 350 mg/g dried biomass with the highest bioethanol yield of 0.26 g/g dried biomass (Harun et al. 2011). Khan et al. (2017) reported that calcium oxide (CaO) treatment before acid and enzymatic hydrolysis significantly doubled the yield of monomeric sugars in *Microcystis aeruginosa*. Furthermore, a high temperature of 120 °C (autoclave disruption treatment) combined with acidic treatment showed the highest disruption and sugar extraction efficiency for microalgae *Scenedesmus obliquus* (Miranda et al. 2012). Jeon et al. (2013) investigated the ultrasonic pretreatment prior to microbial fermentation for bioethanol feedstock production in *Scenedesmus obliquus* YSW15. The results demonstrated that ultrasonic treatment at 15 min duration increased the dissolved carbohydrates concentration at 0.12 g/g dried biomass further increasing the bioethanol yield through microbial fermentation. Scanning electron microscope (SEM) images confirmed that rupture of microalgal cell wall allowed fermenting microbes to ingress the microalgal cellular system thus further enhancing the treatment efficiency. After pretreatment and saccharification, microbial fermentation process then converts the fermentable monosaccharides into bioethanol and other bio-products. Several fermenting microorganisms have been utilized for the process, viz., yeast and fungi such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces fragilis*, *Kluyveromyces marxianus*, etc. as well as bacteria such as *Escherichia coli*, *Klebsiella oxytoca*, and *Zymomonas mobilis.* A study on bioethanol production from red microalgae *Porphyridium cruemtum* depicted that simultaneous saccharification and fermentation (SSF) was a better method than separate hydrolysis and fermentation (SHF). A bioethanol yield of 65.4 and 70.3% using *Saccharomyces cerevisiae* KCTC 7906 was achieved for seawater *Porphyridium cruemtum* and freshwater *Porphyridium cruemtum*, respectively (Miyamoto et al. 1979). Using *Escherichia coli* as a microbial fermenting organism, three marine algae *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, and *Undaria pinnatifida* were explored for bioethanol production. The highest bioethanol yield of 0.4 g/g biomass was observed for acidic and enzymatic pretreated *Chlorella vulgaris* biomass (Lee et al. 2011). Many other fermenting microorganisms such as *Saccharomyces bayanus*, *Saccharomyces cerevisiae* S288C, *E. coli* KO11, and *Zymomonas mobilis* have been utilized for fermentation treatment for microalgae such as *Chlorococcum* sp., *Chlamydomonas reinhardtii* UTEX 90, *Chlorella variabilis*, and *Chlorella vulgaris* FSP-E, respectively, at different pretreatment procedures and fermentation conditions (Phwan et al. 2018).

3.3.3 Biogas

Owing to the presence of polysaccharides such as agar, alginate, carrageenan, laminarin, and mannitol and low cellulose content, microalgae present a superior bioresource for biogas extraction. Biogas production occurs through several anaerobic fermentation steps involving primarily conversion of insoluble high molecular weight organic molecules into soluble organic fraction. In the subsequent steps, volatile fatty acids and alcohols are released by acidogenesis; these volatile organic compounds are then converted into acetic acid and hydrogen and finally conversion of acetic acid and hydrogen into methane and carbon dioxide gas. The entire process of anaerobic fermentation requires microorganisms for biodegradation such as acidogenic bacteria, acetogenic bacteria, and methanogens (Behera et al. 2015). It is essential that the hydrolysis of the microalgal cells must be efficient enough to increase the biodegradation of microalgal biomass. A study on anaerobic biodegradation of *Scenedesmus obliquus* and *Phaeodactylum tricornutum* under similar experimental conditions showed that different microalgae have different biodegradation efficiencies owing to their unique physiology and thermal tolerance (Zamalloa et al. 2012). Several works have been reported for the methane and biogas yield from microalgae such as *Scenedesmus obliquus*, *Chlorella vulgaris*, *Nannochloropsis salina*, *Spirulina maxima*, *Phaeodactylum tricornutum*, *Isochrysis galbana*, etc. (Jankowska et al. 2017; Goswami et al. 2021d). A study on methane gas production from *Spirulina maxima* reported that mechanical and thermochemical treatments had a positive effect on acidogenic bacteria; however, it had no subsequent effect on the methanogens (Samson and Leduy 1983). Similarly, thermal pretreatment of *Scenedesmus* sp. showed a significant anaerobic biodegradability of 48% when the temperature was increased from 70 to 90° C thus establishing the effect of higher temperature on the biodegradation efficiency (González-Fernández et al. 2012). Another condition that increases the yield of biogas like methane from microalgae was a reduction of sodium concentration in the biodegradation mixture. Santos et al. (2014) described a 71.5% increase in methane yield by removing inhibitory sodium in the anaerobic digestion mixture in microalgae *Isochrysis galbana*. Another important variable that affects the yield of methane biogas includes the inoculum/extract (I/S) ratio. A consistent I/S ratio of 1 produced effective specific methane productivity of 0.304–0.557 L CH4/g volatile solids in five microalgae, viz., *Chlorella vulgaris* UTEX 395, *Phaeodactylum tricornutum* CCMP 632, *Nannochloropsis* sp., *Nannochloropsis salina*, and *Nanofrustulum* sp. (Zhao et al. 2014).

3.3.4 Biohydrogen

As an alternative energy and fuel source, hydrogen is advantageous as its combustion only releases water thus mitigating CO_2 levels and reducing greenhouse emissions (Nagarajan et al. 2021). Hydrogenases are the main enzymes involved in biohydrogen production in microalgae. These enzymes accept electrons through many sources like photosynthesis; an approach of direct photolysis is applied where the electrons released during photolysis of water are utilized by the enzymes for biohydrogen production. Also, other approaches include indirect photolysis; in this mechanism electrons released during the fermentative metabolism of stored carbon catalyze the biohydrogen production (Limongi et al. 2021; Goswami et al. 2021a; Nagarajan et al. 2021). A study on thermophilic algae *Mastigocladus laminosus* described the inhibition of hydrogen production yield due to hydrogen consumption by oxygen and carbon monoxide. Conclusively at the higher temperatures, the decrease in oxygen concentration enhanced the biohydrogen yield by 50% (Miyamoto et al. 1979). Kose and Oncel (2014) further enhanced the biohydrogen production in green microalgae *Chlamydomonas reinhardtii*. Using genetically engineered *Chlamydomonas reinhardtii* with mutations in D1 protein, a higher biohydrogen yield rate was generated 1.3 ± 0.5 mL/L.h compared to the wild strain with twofold lower yield rate of 0.57 ± 0.2 mL/L.h. The mutated D1 protein blocks PSII repair system preventing the generation of oxygen and subsequently increasing the activity of oxygen sensitive hydrogenases. Apart from *Chlamydomonas reinhardtii*, biohydrogen production is also reported in *Chlorella fusca*, *Chlamydomonas moewusii*, *Chlorococcum littorale*, *Lobochlamys culleus*, *Scenedesmus obliquus*, and *Tetraselmis subcordiformis* (Limongi et al. 2021).

3.3.5 Bio-Oil and Syngas

Bio-oil or biocrude oil is liquid fuel derived from organic biomass at anaerobic and high-temperature conditions (Behera et al. 2015). Pyrolysis is the main method for bio-oil production as it provides high-temperature conditions (400–600 °C) in the absence of oxygen. Microalgae *Chlorella protothecoides* has been investigated for biocrude oil production with highest yield of 52% produced at a temperature of 500 °C for a relatively short time of 5 min (Peng et al. 2000). Hydrothermal liquefaction is generally at a lower temperature (250–350 °C) under high-pressure conditions. In a study, a maximum biocrude yield of 65 wt% ash-free dry weight (AFDW) was produced at 350 °C in 5 min for halophytic microalga *Tetraselmis* sp. (Eboibi et al. 2014). Furthermore, in a study on high-protein high-ash microalgae, *Cyanobacteria* sp. and *Bacillariophyta* sp., a significant bio-oil yield of 21.1% and 18.21% per dry biomass weight was observed at 325 °C for 45–60 min (Huang et al. 2016). At a temperature of 350 °C, *Nannochloropsis* sp. generated the highest bio-yield of 43% weight at a holding time of 60 minutes (Brown et al. 2010).

A study investigating optimum thermochemical liquefaction operations in *Spirulina platensis* reported a remarkable biocrude quality at par with the petroleum crude. At 350–380 °C, the biocrude product has similar energy properties with density of 34.7–39.9 MJ/kg compared to that of petroleum (42.9 MJ/kg) (Jena et al. 2011). The thermochemical conversion of microalgal biomass can be directed to bio-oil as well as syngas production through hydrothermal liquefaction and hydrothermal gasification, respectively (Barreiro et al. 2013). Gasification is operated at high temperatures of 800–1000 °C which converts biomass into combustible gas mixtures like carbon dioxide, carbon monoxide, methane, hydrogen, and nitrogen. In a study, hydrothermal gasification of *Spirulina platensis* produced syngas mixtures of methane, carbon dioxide, carbon monoxide, and hydrogen. Using ruthenium catalyst in supercritical water, it was then possible to separate the methane from syngas producing a methane-rich biogas that can be further explored for biofuel properties (Stucki et al. 2009). Duman et al. (2014) reported catalytic steam gasification of *Nannochloropsis oculata* and seaweeds including *Fucus serratus* and *Laminaria digitata* which produced maximum hydrogen gas yields of 413 cc/g, 937 cc/g, and 1036 cc/g algal residue. The syngas produced hydrogen as the major component with carbon monoxide and methane in trace amounts. The method thus offers promising means to maximize hydrogen production from micro- and macroalgal biomass.

3.4 Future Perspectives

To attain the promising utilization of microalgal bioresource, persistent efforts are being carried out in the genetic modification of microalgae. Genetic engineering of microalgae aims to provide novel strain with exclusive features like high lipids yield, greater biomass accumulation, higher expression of bioactive compounds, superior CO_2 capture, and possible role in wastewater treatment. Several steps are required in the genetic manipulation of microalgae. The choice for genetic transformation techniques such as electroporation, particle bombardment, etc. is an important barrier to strain improvement. *Chlamydomonas reinhardtii* has been utilized in the past for genetic transformation through electroporation. Apart from transformation, efficient expression of our choice gene through codon optimization and promoter selection is also remarkably important (Chaturvedi et al. 2020; Barrera and Mayfield 2013). Barrera and Mayfield (2013) investigated overexpression of malic enzyme in *Phaeodactylum tricornutum* generating genetically improved transgenic cell with 2.5-fold higher lipid yield as well as higher biomass, thus suggesting novel prospects in biodiesel production. In another study enhanced lipid content of 46.4–52.9% was achieved in *Chlorella ellipsoidea* by overexpressing GmDof4 transcription factor from soybean (*Glycine max*) (Zhang et al. 2014). Couso et al. (2011) reported the stable increase in carotenoids production by overexpression of exogenous phytoene synthase gene in the *Chlamydomonas reinhardtii*. In recent times genetic improvement of the microalgal bioresource is currently pursued by the development of gene-editing tools like clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) (Kumar et al. 2020a, b). CRISPR/Cas9-based technology was utilized for the expression of omega-3 fatty acid desaturase (fad3) gene in *Chlorella sorokiniana* and *Chlorella vulgaris* FSP-E. Higher lipid content of 46% (w/w) in *C. vulgaris* FSP-E was achieved using the novel genetic tool (Couso et al. 2011). Success of genome editing has also been reported in *Nannochloropsis oceanica*, *Phaeodactylum tricornutum*, and *Chlamydomonas reinhardtii* (Vazquez-Villegas et al. 2018). Although genetic tools provide a propitious opportunity for better products development from microalgae, it must be also stringently regulated and monitored to prevent compromise on human health and safety issues. In silico simulation studies show that upon escape to the natural habitat, these genetically superior microalgae with excellent growth kinetics can cause the formation of harmful algal blooms, creating a nutritional challenge to zooplanktons and ultimately giving rise to ecological imbalance (Flynn et al. 2013). Thus as more industries are exploring genetically modified microalgae for commercial applications, a need to monitor appropriate regulation and risk assessment has arisen. A new greener technology with minimal environmental pollution as well as highly regulated road map for the microalgal industry is thus the need of the hour.

3.5 Conclusions

The enormous bioactive compounds in microalgae provide a plethora of opportunities for several applications including biomedical, cosmetics, nutraceuticals, and biofuels. Prominent among the bioactive components include polysaccharides, fatty acids, pigments, proteins, and peptides. Till date, several interesting cosmetic formulations have been developed utilizing the unique metabolites present in microalgae. A wide range of health benefits with antioxidants, immune-modulatory, and anticancer activities provides a much needed drive to the cosmeceutical industry. The prospect of next-generation fuels such as biodiesel, bioethanol, biogas, etc. has allowed industries to explore the microalgal diversity. Although numerous microalgal species have been utilized for several applications, many are yet to be characterized. Industries have utilized several approaches to amplify the yield such as nutrient limitation, photochemical parameters, and genetic modification. Favorably numerous microalgae have shown promising yield thus providing scope for future developments.

Competing Interests All the authors declare that they have no competing interests.

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