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Review

Exploitation of Microalgae Species for Nutraceutical Purposes: Cultivation Aspects

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Abstract: Cyanobacteria and microalgae have been cultivated only for a limited number of bioactive compounds or biotechnological applications such as for carotenoids; essential omega-3 fatty acids; phycobilipigments; live cells, unprocessed or minimally processed complete biomass as aqua feed, animal feed and human health supplements as rich sources of proteins, carbohydrates, pigments, vitamins and minerals. However, cyanobacteria and microalgae have been reported through several research investigations as a potential source for various bioactive molecules with marketable nutraceutical and pharmaceutical properties. Therefore, more cultivation of cyanobacteria and microalgae species are waiting for new biotechnological applications. At present, the global demand for microalgal applications is focused on biofuels including biodiesel and bioethanol apart from a handful (mentioned above) of bioactive compounds which are mostly used as nutraceuticals. Thus, microalgal biorefinery is growing rapidly for multiple commodities production from both conventional and photobioreactor-based cultivation for biomass feedstocks for various biotechnological applications. This review presents the cultivation aspects of selected cyanobacteria and microalgae for commercial purposes.

Keywords: biotechnological applications; commercial cultivation; cyanobacteria; microalgae; out-door cultivation; photoautotrophic; photobioreactor cultivation

1. Introduction

Microalgae including cyanobacteria (hereafter, microalgae for simplicity unless specific mention of cyanobacteria is necessary) are considered one of the most promising feedstocks as a sustainable source of various commodities for both food and non-food applications (Figure 1; [1,2]). This is because microalgae are photoautotrophic similar to higher plants; they have a much higher growth rate compared to other land plants; can sustainably be cultivated in non-arable land; and they only need meagre amounts of growth nutrients, freshwater, brackish or marine water, and available sunlight or alternatively low energy consuming LED lights. Under specific growth conditions, certain microalgae biomass may contain macromolecule carbohydrates (up to 60%, [3,4]); lipids (up to 70%, [5,6]); proteins (up to 60%, [7]); and other high-value-added biomolecules (HVABs) such as astaxanthin in *Haematococcus pluvialis* (up to 7%, [8]); β-carotene in *Dunaliella salina* (up to 12%, [9]), EPA in *Phaeodactylum tricornutum* (39% of total fatty acids, [10]), DHA in heterotrophic microalga *Crypthecodinium cohnii* (45% of oil, [11]) and in photoautotrophic microalga *Diacronema lutheri* (19.2% of total fatty acids, [12]), phycocyanin in *Arthrospira* (*Spirulina*) *platensis* (17.5%, [13]), etc. During the last decade, microalgae have been researched for commercially viable production of biofuels, for which, the cultivation, harvesting and biomass extraction technologies remained cost challenging. To meet the global demand for renewable fuels in a cost-effective manner, microalgal cultivation and extraction received a biorefinery approach. Cyanobacteria and microalgae have been reported as a 'treasure house' for a variety of biological activities including anti-oxidant, anti-inflammatory, anti-bacterial, anti-diabetic, anti-fungal, anti-viral, anti-parasitic, anti-proliferative, anti-elastase, anti-trypsin, anti-chymotrypsin, angiotensin I-converting enzyme inhibitory (ACE-inhibitory), myofibroblast differentiation-inducing and hepatic fibrosis inhibitory activities, and as a source for bioactive peptide molecules with marketable nutraceutical and potential pharmaceutical properties [2]. Therefore, microalgae biomass can contribute not only in the biofuels sector but also in the food, feed, chemical and pharmaceutical sectors because of their HVABs and sustainable cultivation methods for both indoor and outdoor cultivations (Table 1).

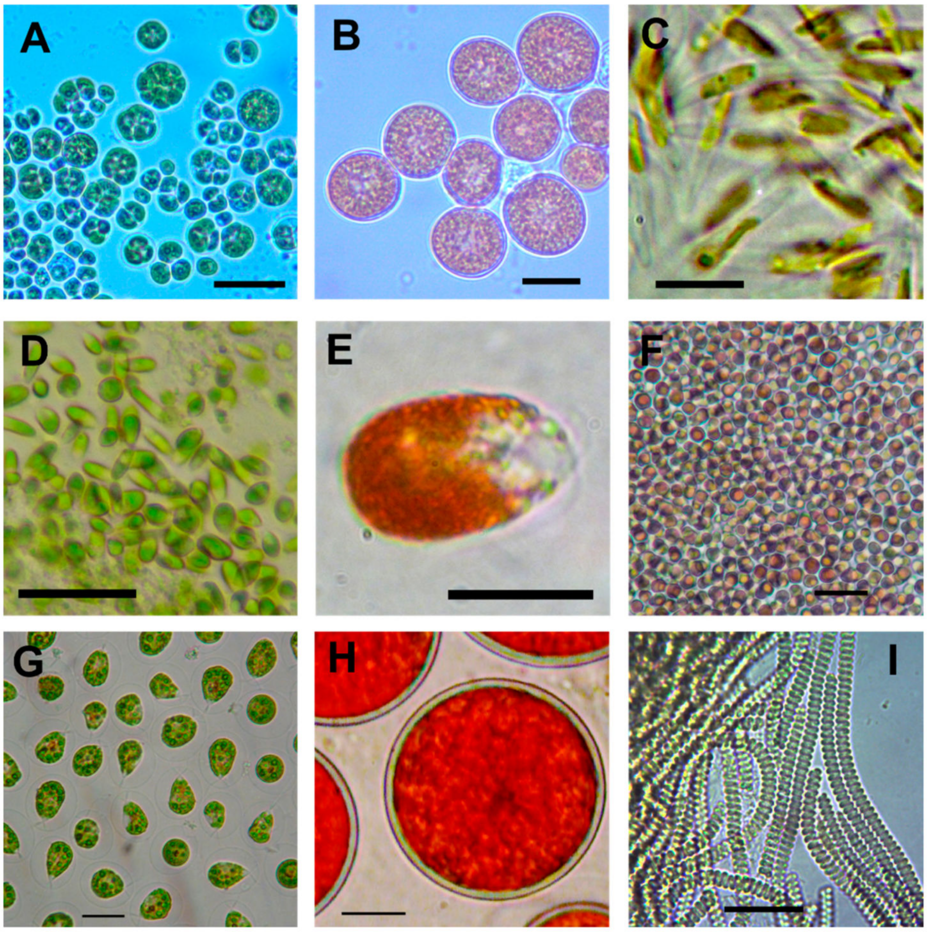

Figure 1. Light micrographs showing cellular colouration and morphological diversity of potential microalgae for commercial cultivation for various biotechnological applications. *Chlorococcum* sp. ((**A**,**B**); source for mixed carotenoids including β-carotene, astaxanthin, canthaxanthin, lutein), *Dunaliella salina* ((**D**,**E**); the source for β-carotene) and *Haematococcus pluvialis* ((**G**,**H**); the source for astaxanthin) are cultivated as two distinct growth phases: (1) green-phase ((A,D,G); for biomass generation) and (2) stress-phase ((B,E,H); for carotenoids and fatty acids accumulation). (**C**), *Phaeodactylum tricornutum* (the source for essential fatty acid EPA), (**F**), *Porphyridium cruentum* (source for natural pink colourant phycoerythrin and bioactive polysaccharides) and (**I**), *Arthrospira* (*Spirulina*) sp. (source for natural blue colourant phycocyanin and multiple health benefitting ingredients) are cultivated as single-phase actively growing biomass for the targeted biomolecules. All scale bars are 25 μm, except for (C,E), which are 10 μm.

Microalgae are a potential source for various bioactive molecules including pigments chlorophyll, β-carotene, astaxanthin, lutein, phycocyanin, allophycocyanin and phycoerythrin; polyphenols and α-tocopherols; polyunsaturated fatty acids (PUFAs); antioxidant molecules ascorbate, glutathione and ergothioneine; antioxidant enzymes superoxide dismutase, catalase, peroxidase, etc. Therefore, microalgae drew gradual attention for their use for health benefits. For example, several cyanobacteria and microalgae such as, *Arthrospira* (*Spirulina*) *maxima*, *Arthrospira* (*Spirulina*) *platensis*, *Aphanizomenon flos-aquae*, *Chlorella vulgaris*, *Dunaliella salina*, *Haematococcus pluvialis* and *Nannochloropsis oculata* have been commercially exploited for various available nutraceutical products in the market [2].

Table 1. Most popular microalgae and their mode of cultivation for various applications.

Microalgae Species	Cultivation History	Growth Type	Cultivation Conditions	Applications
Chlorella vulgaris	1951	Photoautotrophic	Open raceway pond, tubular PBR, flat-plate photobioreactor	Whole biomass for human nutrition as tablets, powders, nectar noodles; cosmetics; aquafeed
Crypthecodinium cohnii	1999	Heterotrophic	Large stainless steel Fermentor	DHASCO™ oil for the infant formula as DHA source
Dunaliella salina	1980	Photoautotrophic (two phase cultivation)	Unstirred open pond, lagoons, paddle wheel stirred raceway ponds, tubular photobioreactors	Carotenoid β-carotene for food and in cosmetics, human nutrition as powder, animal feed, source for proteins and glycerol
Haematococcus pluvialis	2000	Photoautotrophic (two phase cultivation), Mixotrophic	Open raceway pond, tubular enclosed outdoor PBR, bubble column and airlift photobioreactors, large plastic bags	Carotenoid astaxanthin, aquafeed, poultry feed, animal feed, human nutrition, cosmetics, pharmaceuticals, food-colourant, food-supplement
Nannochloropsis sp.	1997	Photoautotrophic, Mixotrophic	Raceway pond, Helical-tubular photobioreactor	EPA oil for human nutrition, aquaculture
Odontella aurita	1996	Photoautotrophic, heterotrophic or mixotrophic	outdoor open ponds, Pilot Tanks, cylindrical glass columns and flat-plate photobioreactors	Human nutrition, baby food as EPA and DHA source, cosmetics
Phaeodactylum tricornutum	1996	Photoautotrophic	Open pond, circular tanks, outdoor pilot-scale bubble column photobioreactor, large 400 L polyethylene bags supported by frames, air-lift photobioreactor	Aqaculture feed, EPA oil as health supplement
Porphyridium cruentum	1970	Photoautotrophic	Tubular PBR	Pink phycoerythrin pigment, sulfated polysaccharide, cosmetics
Schizochytrium sp.	1999	Heterotrophic	Large stainless steel Fermentor	Life's Omega™ oil as source for DHA and EPA
Arthrospira (Spirulina) platensis	1970	Photoautotrophic	Open raceway pond, tanks, earthen pots, basins, natural lakes	Whole biomass for human nutrition as tablets, capsules, powders; blue phycocyanin as colourant in food and in cosmetics; source for g-linolenic acid (GLA), vitamins and minerals

Carotenoids are accessory photosynthetic pigments of microalgae and certain species are known to accumulate high levels while esterified with specific essential fatty acids under stressful environments, such as, under nitrogen limitation and high light intensity as their survival strategies. Carotenoids are yellow, orange or red colour pigments that are very popular for various food including as supplements, functional ingredients and cosmetic products, especially astaxanthin and β-carotene from microalgae. Although certain cyanobacteria contain β-carotene (80% of total carotenoids) as the major carotenoids followed by zeaxanthin, the commercial source of β-carotene is only *Dunaliella salina* [14,15]. Likewise, *Haematococcus pluvialis* is a popular microalga as it is the richest natural source of commercial carotenoid astaxanthin, which has much higher antioxidant activities than β-carotene and Vitamin E [16].

Omega-3 fatty acids (popularly known as EPA, DHA, etc.) are essential fatty acids, which cannot be synthesized de novo by the human body and must be obtained in our diet. DHA was found as one of the major fatty acids of the brain and retina, and is considered responsible for visual and cognitive development [17]. EPA and DHA were shown to aid in the prevention of coronary heart disease, stroke, hypertension, dementia, Alzheimer's and depression [18,19]. The source of these fatty acids were wild harvest 'Fish oil' and 'Krill oil' until recently. However, nowadays, these fatty acids have been commercially produced from heterotrophic microalga *Crypthecodinium cohnii* and photoautotrophic microalgae *Phaeodactylum tricornutum* and *Nannochloropsis* sp. Therefore, an alternate "vegetarian source" for these fatty acids has been developed. There are several companies such as Aurora Algae (Hayward, CA, USA), Algae Biosciences (Holbrook, AZ, USA), Blue Biotech International GmBH (Kaltenkirchen, Germany), GCI Nutrients (Burlingame, CA, USA), Ingrepro BV (Borculo, The Netherlands), Live Fuels (San Carlos, CA, USA), Lonza (Basel, Switzerland), Martek Biosciences

(Columbia, MD, USA), Photonz (Auckland, New Zealand), Qualitas Health (Houston, TX, USA) and TerraVia (San Francisco, CA, USA) which are producing omega-3 polyunsaturated fatty acids for nutraceutical applications [2].

Phycobilipigments are accessory light harvesting protein-pigments found predominantly in cyanobacteria and in a few red microalgae. These water-soluble phycobilipigments are mainly of four colour shades: (1) allophycocyanin (APC, bluish green); (2) phycocyanin (PC, blue); (3) phycoerythrin (PE, purple) and (4) phycoerythrocyanin (PEC, orange). PC and PE have established commercial applications in food and diagnostic purposes respectively. *Arthrospira* (*Spirulina*) *platensis* is commercially exploited for phycocyanin (a blue phycobilipigment) with reported antioxidant activities [20–22], while the red microalga *Porphyridium cruentum* is used for the production of phycoerythrin (a red phycobilipigment) that has antioxidant activities as well [23,24].

The traditional use of microalgae can be traced back several decades in indigenous populations around the world especially in China, Japan and Republic of Korea. Interestingly, the migration of these people around the world has disseminated the culture of algae consumption. The changes in the trend of microalgae use are also due to the recent enormous research on health and nutritional benefits of microalgae and our present day lifestyle. Microalgal biotechnology for nutraceutical applications are a fast growing segment of Health and Nutrition industry today and the benefits from upcoming microalgae are plenty, which are totally dependent on industrial involvements and technological developments.

2. Microalgae for Commercial Cultivation and Their Growth Conditions

2.1. *Haematococcus pluvialis*

This is one of the freshwater green microalgae, which has been identified as a significant natural source of carotenoid astaxanthin with high antioxidant activities and has long been used as a food colouring agent in aquaculture and poultry, and very recently in various human health products [25,26]. *H. pluvialis* grows as motile bi-flagellated green cells under optimum growth conditions, but due to changes in environmental conditions, such as increased light intensity, high temperature, nutrient limitation or high salt concentrations, the healthy green cells undergo morphological and biochemical changes as their survival strategies. They turn into red cysts by losing their motility, increasing cell size, thickening cell walls, inducing carotenogenesis, and particularly the accumulation of orange-red pigment astaxanthin esters that may constitute up to 95% of the total carotenoids and increased lipids biosynthesis with specific fatty acid production [27,28]. Therefore, *H. pluvialis* is generally cultivated as two-stage (green-phase and stress-phase (red)) under photoautotrophic conditions. Green-phase is maintained under low light as most of the strains are sensitive to light for induction of carotenogenesis, which will eventually cease the growth. Hence, green-phase is for generating enough actively growing green biomass, which then after gentle harvesting, is transferred to a stress medium and/or high light condition for cellular morphogenesis with appropriate biochemical changes, especially the accumulation of carotenoid astaxanthin and fatty acids [29]. Photoautotrophically, *H. pluvialis* is mainly cultivated in open raceway ponds or closed photobioreactors (tubular, bubble column and air lift). *H. pluvialis* can produce astaxanthin through single-stage cultivation, which is simple compared to a two-stage process, where the growth medium from first phase has to be removed before the progression of high-light and stress-medium-based cultivation for astaxanthin accumulation. The first method, however, yields significantly lower amounts of astaxanthin compared to the two-stage cultivation method. *H. pluvialis* have also been cultivated both in open raceway ponds or closed photobioreactors under heterotrophic and mixotrophic growth conditions. Most recently, a two-stage mixotrophic cultivation system for astaxanthin production in *H. pluvialis* was suggested [30]. Some studies considered *H. pluvialis* biomass for biodiesel production because of their suitable fatty acids profile [29,31]. It was proposed that *H. pluvialis* biomass after extraction of astaxanthin can be used as feedstock for bioethanol or biomethane production [32–34].

Algatechnologies Ltd. (*aka* Algatech) is the Israel-based company which cultivates *Haematococcus pluvialis* in a fully controlled closed system of glass photo-bioreactors energized by the sunlight of the Arava desert in Israel. They produce all-natural AstaPure® astaxanthin for application as a food ingredient (http://www.algaeindustrymagazine.com/algatech-expands-astaxanthin-line-brazil/). Cyanotech® Corporation, a Hawaii-based company produces BioAstin from *Haematococcus pluvialis*, that is cultivated in open ponds, and processed on the Kona coast of the Big Island of Hawaii (https://www.cyanotech.com/nutrex-hawaii/). Atacama Bio produces natural astaxanthin from *Haematococcus pluvialis* in their 250-acre facility in the Atacama desert in Chile by using both closed and open photo-bioreactors and in raceway ponds with paddle wheels (http://news.algaeworld.org/2018/02/the-natural-algae-astaxanthin-association-welcomes-atacama-bio-natural-products-s-a-as-a-new-executive-member/). Astareal corporate (Japan, USA, Sweden, Singapore, Australia and India) company together with their parent company Fuji Chemical Industries Co. Ltd., are commercially cultivating *H. pluvialis* in indoor tubular systems, outdoor raceway ponds and fermentation tanks for natural astaxanthin for various health products. Their cultivation process begins in the laboratory, where a seed culture is grown under sterile conditions with enriched nutrients and low light that allows for optimal green-phase growth with active biomass production. When the green-phase culture reaches its optimal density, the biomass is transferred to the stainless steel photobioreactors where the accumulation of astaxanthin (stress-phase) is initiated. Upon maturity of red cysts, biomass are harvested, crushed and dried as deep-red powder containing a high concentration of astaxanthin (http://www.astareal.com/about-astaxanthin/production). Valensa International producing astaxanthin-rich *Haematococcus pluvialis* biomass in an algal growing facility company, Alimtec S.A., located in Chile. They maintain primary cultures and inoculum under sterile conditions under laboratory environment and carry out initial cultivation in closed greenhouse systems throughout the green-phase growth. The resulting biomass is then transferred to open ponds for appropriate population density of green cells. Then stress-phase cultivation is induced in a series of cascaded open ponds for astaxanthin accumulation (http://valensa.com/vproducts/astaxanthin/). Algae Health Sciences produce astaxanthin from *Haematococcus pluvialis* by cultivating in their 80-acre farm with 100% glass-tube photobioreactors. Their cultivation strategies include keeping the microalga cells in a healthy state as green and motile cells by feeding them essential nutrients and protecting them from harsh sunlight including harmful UV rays. Then, during the final stage of growth they create stress to the microalga cells by starving for essential nutrients and exposing them to the intense sun in their pristine farm, which helps accumulating astaxanthin (https://www.algaehealthsciences.com/).

2.2. Arthrospira (Spirulina) spp.

Arthrospira (*Spirulina*) are spiral or coiled filamentous cyanobacteria with about 15 species and have long been used as human food in Chile, China, Mexico, Peru and the Philippines [2]. The Kanembu people of Africa used to harvest the microalgal blooms from Lake Chad and sun-dried them to obtain cakes called "Dihé", which was used as their healthy food. During the 1960s, this microalga was identified as *Arthrospira* (*Spirulina*), which nowadays become the "Super Natural Food". More than 250 tons of dry biomass is produced yearly by the women around Lake Chad, it is the highest volume of *Arthrospira* (*Spirulina*) with the cheapest production cost (Algae Industry Magazine report). *Arthrospira* (*Spirulina*) *platensis* were found to produce a novel sulphated polysaccharide named "Calcium-Spirulan" [containing calcium ions, sulphate, uronic acids, rhamnose, 3-*O*-methylrhamnose (acofriose), 2,3-di-*O*-methylrhamnose, 3-*O*-methylxylose, *O*-rhamnosyl-acofriose and *O*-hexuronosyl-rhamnose (aldobiuronic acid)] which has been reported to help prevent atherosclerosis because of its antithrombogenic, fibrinolytic, and anti-atherogenic properties [35]. Commercially, various species of *Spirulina* (aka *Arthrospira*) such as *A* (*S*). *platensis*, *A* (*S*). *subsalsa* and *A* (*S*). *maxima* have been cultivated for health and nutrition products including as a blue food colourant (e.g., LinaBlue by DIC LIFETEC CO, Tokyo, Japan). The colourant, which is derived from *Arthrospira* (*Spirulina*), is the preferred source of natural blue for the industry. The industrial biotech firm Scottish Bioenergy and scientists at the University of Edinburgh initiated a research partnership to develop a large-scale process to extract C-phycocyanin from the *Arthrospira* (*Spirulina*) because of the global demand for the natural blue colourant. The current market size of the food colourant has reached USD 1.88 billion in 2015, 10% up from last year and is expected to be around USD 2.5 billion by 2020. *Arthrospira* (*Spirulina*) spp. are cultivated photoautotrophically both in natural ponds, raceway ponds and/or precise controlled tubular photobioreactors by various cultivation companies in India, Japan, Czech Republic, Myanmar, USA and France. However, it was shown that the mixotrophic cultivation of this organism may increase productivity five-fold [36]. *Arthrospira* (*Spirulina*) is a photosynthetic microalga that is exposed to light and high oxygen concentrations (HOC), especially as high cell density cultures in closed photobioreactors. It was found that HOC can increase the antioxidative potential of *Arthrospira* (*Spirulina*) *platensis* by 2.3-fold [37]. To reduce the cost of production in photobioreactor cultivation of this cyanobacterium, LED light source has been tested and red LED was found to be important to achieve the highest specific growth rate and biomass production [38]. Although, the low-cost and easy in operation cultivation methods using open raceway ponds, tanks and basins were used for *Arthrospira* (*Spirulina*) spp. cultivation, these methods resulted in low biomass productivity [39,40]. The above cultivation systems are difficult in terms of maintaining optimal culture conditions due to high evaporation rates, temperature changes, etc. Importantly, *Arthrospira* (*Spirulina*) spp. is generally cultivated at alkaline pH (9.5 to 11.0 with an optimum at 10.5) [41] and is, therefore, less prone to other microalgal and cyanobacterial contaminations in the outdoor cultivation methods mentioned above. *Arthrospira* (*Spirulina*) *platensis* is one of the most promising sources of essential fatty acid γ-linolenic acid (GLA), which was found to be enhanced under optimum light/dark cycles both in laboratory and in outdoor cultivations [42].

2.3. Dunaliella spp.

A few species of *Dunaliella* are the most popular microalgae for β-carotene production. *Dunaliella* spp. are marine, unicellular, oxygen-evolving eukaryotic microalgae of Chlorophyceae. These microalgae grow as motile bi-flagellated green cells under optimum growth conditions and scavenge atmospheric CO_2 during photosynthesis. Some morphologically identical *Dunaliella* strains were found biochemically different especially with their carotenogenic and non-carotenogenic potential, with high protein content, glycerol producing capacity, etc., which has been exploited for commercial products development. Accumulation of glycerol with increasing salinity in *Dunaliella* sp. helps the organism in acting as osmoticum and regulating the osmotic pressure between cytoplasm and the environment. The glycerol concentration can be as high as 17% of the dry weight when the halotolerant *Dunaliella* sp. is cultivated with 25% NaCl based medium [43,44]. Under stressful environmental conditions, such as low temperature, high irradiance, nitrogen limitation or high-salt concentrations, *D. salina* cells accumulate carotenoids [45–48]. *Dunaliella salina* is the most halotolerant photosynthetic eukaryote known with a salinity tolerance range of 0.5 to 5 M salt concentration; however, it is mostly found in saline and hypersaline waters, which is one of the important factors essential for outdoor cultivation with minimum contamination issues [49]. Photoautotrophically, unstirred open pond, paddle wheel stirred raceway ponds, lagoons, and tubular photobioreactors have been used for the cultivation of *Dunaliella salina* and other best β-carotene producing *Dunaliella* species. For the production of β-carotene, *D. salina* have been mass cultivated in several countries including in Australia, Chile, China, India, Iran, Kuwait, Spain and the USA [50]. For the induction of carotenogenesis in *Dunaliella* a two-stage cultivation method has been adopted. However, there are at least two schools adopting this method as specific to their cultivation strains. One school cultivates *Dunaliella* green biomass in a nutrients-rich medium containing 18% NaCl in stage one, and in the second stage for induction of carotenogenesis, green biomass is transferred to the nutrients-depleted medium containing 27% NaCl [51]. The second school cultivates *Dunaliella* green biomass in small nursery ponds to attain optimal biomass in stage one. Then for the second stage of induction of carotenogenesis, the biomass are transferred to large production ponds with diluted nitrate concentration and altered salinity levels [52]. There is a growing demand for natural food colourant and β-carotene from *D. salina*; it has already been approved for various food applications, and consumers avoidance of synthetic version of colourants created a huge opportunity for increased production of natural β-carotene and other biotechnological ingredients from *Dunaliella* spp. Present commercial producers and new industries may adopt cutting-edge photobioreactors technology for the cultivation of *Dunaliella* under heterotrophic and mixotrophic conditions other than conventional autotrophic conditions for production of other novel molecules along with β-carotene production.

2.4. *Chlorococcum sp.*

Chlorococcum is one of the unicellular microalgae of Chlorophyceae, which is cosmopolitan in distribution including in freshwater and marine habitats. The microalga cells are spherical or slightly oblong with varied size. The cells may be solitary or in irregular clumps, mucilage is thin and inconspicuous. Each cell has a single cup-shaped, parietal chloroplast with a single pyrenoid. *Chlorococcum* spp. has not been thoroughly researched for commercial biotechnological benefits except for few studies related to its potential for carotenoid and lipids production. This microalga was reported to produce carotenoid astaxanthin (free and esters), adonixanthin (free and esters), canthaxanthin, b-carotene, lutein, and some cis-isomers of ketocarotenoids [53]. Hence this microalga can be a balanced source for "multiple carotenoids" for various health benefits. A study suggested that the microalga *Chlorococcum* is another potential source for commercial ketocarotenoids because of its fast growth rate, it is relatively easy for outdoor tubular photobioreactor cultivation, and its tolerance to extreme pH and high temperature [54]. This microalga is generally cultivated photoautotrophically, but can grow heterotrophically on glucose supplementation with reduced carotenoids content. Upon glucose supplementation to the basal medium, the content of adonixanthin was higher than that of astaxanthin, but under photoautotrophic condition the difference in contents of astaxanthin and adonixanthin were meagre [53]. Other studies explored *Chlorococcum* for biodiesel production and especially a freshwater *Chlorococcum* sp. RAP13 was found well adapted to the sea water and accumulated up to 38% lipids (of DW biomass) under heterotrophic conditions. Both biomass and lipid yield were significantly higher under marine heterotrophic conditions than fresh water. Also the composition varied between phototrophic and heterotrophic growth conditions. Photoautotrophically, *Chlorococcum* sp. RAP13 produced a relatively higher amount of polyunsaturated fatty acids, while under heterotrophic conditions the microalga produced more of medium-chain saturated fatty acids and monounsaturated fatty acids suitable for biodiesel application [55]. In our laboratory, we have isolated a marine *Chlorococcum* and optimised its carotenogenesis conditions through a two-stage cultivation method which outperforms photoautotrophically under freshwater conditions.

2.5. *Porphyridium spp.*

The genus *Porphyridium* of Rhodophyceae has at least two of the most researched microalgae species *Porphyridium cruentum* and *P. purpureum*, which are of marine origin. The cellular content of this red microalga are encapsulated by a cell-wall polysaccharide complex, which are both free and bound forms, respectively called "soluble polysaccharide" and "bound polysaccharide". The polysaccharide content can reach 50% of the biomass. The "soluble polysaccharide" is also called exopolysaccharide (EPS) that is highly sulfated acidic heteropolymers consisting mainly of xylose, galactose and glucose. *Porphyridium purpureum* can be a commercial source of total proteins (28–39%), polysaccharides (40–57%), lipids (9–14%), and coloured phycobilipigments (Phycoerythrin 12.17%, R-phycocyanin 10.2% and allophycocyanin 2.86%) [56]. So far, this microalga has received commercial importance for phycoerythrin for diagnostics, sulphated polysaccharides for pharmaceuticals, whole biomass as "nutrients package" for health supplements and recently for the production of essential omega-3 fatty acid (EPA, eicosapentaenoic acid) [57–59]). Photoautotrophically, *Porphyridium cruentum* responds to the external irradiance and diurnal light availability in terms of growth rates and various biochemical compositions when cultivated outdoors in a pilot plant in a tubular photobioreactor. For example, fatty acids content decreased during the daylight and the total fatty acids profile was a function of growth rate. Likewise, quick response to solar irradiance with increased exopolysaccharides release as a protection against adverse light conditions [60], which is a phenomenon explored for EPS production in outdoor cultivation systems. However, Irradiance is a determining factor for bioproduction of phycobiliproteins [56] and therefore the use of outdoor photobioreactor systems for the production of phycoerythrin in *Porphyridum* spp. needs irradiance minimisation set-up. A cylindrical PBR-based study on *Porphyridium cruentum* found that temperature, illumination, nitrogen level, and CO_2 levels in the growth media significantly impact the cell growth, lipid content, and fatty acid compositions. Although, 5% CO_2 in air aided in higher lipid productivity and lipid contents but resulted in lower biomass [59]. Another study found that photoautotrophically, *Porphyridium cruentum* can grow faster under 18/6 h light/dark cycles but the highest lipid (19.3%, *w*/*w*) accumulation was achieved at 12/12 h light/dark cycles. Accumulation of lipids under heterotrophic conditions using glucose or glycerol was negatively impacted in *Porphyridium cruentum* [61]. Nutritional composition of the *Porphyridium cruentum* biomass may be influenced by residence time in the bioreactor and the amount of irradiance [57]. Presently, the source of commercial phycoerythrin is the red microalga *Porphyridium cruentum* and is mostly produced for diagnostic purposes and in diverse research applications. However, there is a demand for food-grade natural pink colourant and the pink phycoerythrin from this microalga is a major challenge because of the fact that this microalga produces huge amount of EPS, which increases down-stream processing costs. Therefore, production of food-grade natural pink colourant phycoerythrin from this microalga is waiting for new improved strain with less exopolysaccharides and/or a simple downstream process that is cost effective. Alternatively, certain cyanobacteria strains can be optimised for large-scale cultivation and enhanced production of phycoerythrin. This is for the fact that many cyanobacteria were reported to inherently produce phycoerythrin and relatively less amounts of polysaccharides, and their sustainable mass cultivation methods are very robust.

2.6. *Phaeodactylum tricornutum*

This is a marine photoautotrophic microalga (diatom) of Bacillariophyceae, which has received commercial interests for biodiesel production, essential omega-3 fatty acid EPA and the carotenoid fucoxanthin [10,62–64]. This microalga may remain as fusiform, oval or triradiate shape depending on growth conditions and can accumulate EPA (5% of dry weight biomass, [65]), in its storage lipids during stationary growth. Its EPA content may be influenced by growth conditions such as increased nitrate, urea, CO_2 and vitamin B12 concentrations. EPA content in *P. tricornutum* increased with increasing concentrations of nitrate and urea when supplemented with 1% CO_2 in air under optimum culture temperature (21.5 to 23 °C) and initial pH (7.6) [10]. Photoautotrophically, this microalga has successfully been cultivated in open pond, circular tanks for aqua-feed, outdoor pilot-scale bubble column photobioreactor, 400 L polyethylene bags supported by frames, air-lift photobioreactor as well as long-term quasi steady-state outdoor continuous cultivation in large-diameter (>0.15 m) vertical column photobioreactors. In a recent study regarding the growth and lipid composition of *Phaeodactylum tricornutum* in an outdoor pilot-scale bubble column photobioreactor cultivation under natural conditions in Chile, the findings of the study suggested that *P. tricornutum* oil from phototrophic outdoor mass cultivation could be used as an alternative feedstock for biodiesel production [66].

2.7. *Crypthecodinium cohnii*

Crypthecodinium cohnii of Crypthecodiniaceae is one of the heterotrophic, marine dinoflagellate microalga that produces mostly DHA of its total lipid [11]. This microalga has successfully been used by DSM nutritional company to produce DHA-enriched oil for the infant formula market through their patented fermentation technology. In general, the fermentation processes are mainly achieved in two stages: First the biomass is generated through excess of nutrients supplemented growth and then in the second stage the combined nitrogen source is removed so that the nitrogen-deprived cells use the stored energy to produce fatty acids. Dissolved oxygen concentration, pH, carbon and nitrogen ratio, and the salinity and temperature are the important factors to be adjusted for optimum fermentation of DHA-rich oils [11].

3. Outdoor and Indoor Cultivation of Microalgae

Although most microalgae under commercial cultivation are photoautotrophic and require only minimum nutrient and basic abiotic conditions, their mass cultivation technologies are still developing and are microalgae species-specific. Continuous efforts have been made through research and development on cultivation systems in the recent past, especially when there has been a global demand for biofuel production from microalgae in a cost-effective manner. Since the commercial cultivation of *Chlorella* sp. only started in the early 1960s, the present publicity plus significant research on improving microalgal cultivation through Photobioreactor technology sounds like "re-inventing the wheel". However, it is a fact that tremendous improvement through various modern cultivation technologies has been achieved, especially due to the demand for renewal biofuels production. Challenges still need to be overcome to reduce the cost of production of biofuels through an integrated biorefinery approach where the biomass is produced optimally for first-priority HVABs and then the spent-biomass is sequentially extracted/utilised for other commodities production, including animal feed, fertilizer, biodiesel, bioethanol, biogas, etc.

The cultivation conditions of microalgae and the nutrients composition in their growth medium significantly influence various ingredients of nutraceutical importance such as carotenoids, omega-3 fatty acids as well as biodiesel lipids in microalgae. The microalgal cultivation conditions can broadly be classified in to four major types:

(1) Photoautotrophic cultivation: This is the most commonly used microalgae cultivation condition that uses lights, such as sunlight or artificial lights that supply photosynthetically active radiation (PAR, 400–700 nm) as an energy source, and inorganic carbon (mostly as CO_2 gas in air and certain instances chemical CO_2 as sodium bicarbonate) as the carbon source to carry out the photosynthesis for the first product glucose. Nowadays, the use of LED (light emitting diodes) lights [29,38] as source of energy for photoautotrophic cultivation is developing because of the fact of low energy consumption as well as the supplying of narrow range lights (e.g., red LED, 624–634 nm; green LED, 515–525 nm; blue LED; 460–465 nm) for the enhancement of specific biomolecule production. The biomass yield and especially lipid productivity were reported to increase by using 2% CO_2 in air [67]. However, to reduce the cost and also to recycle industrial CO_2, the microalgae cultivation facility should not be far away from the CO_2 source.

(2) Heterotrophic cultivation: The organisms capable of heterotrophic cultivation lack the photosynthetic machinery and hence cannot generate energy through inorganic compounds oxidation [68]. Heterotrophic cultivation requires organic carbon (glucose, fructose, sucrose, lactose, galactose, mannose, acetate, glycerol, etc.) as both the energy and carbon source. However, most microalgae prefer glucose as it can easily be assimilated and produce energy-rich compounds such as neutral lipids. Glucose-grown microalgae showed higher growth rates compared to those grown on acetate and fructose [68]. The yield of lutein was found to be increased with the increase in glucose concentration in *C. protothecoides* [69]. However, under heterotrophic cultivation *H. pluvialis* grew very slowly and accumulated only 0.5% astaxanthin of dry weight biomass [70]. Certain microalgae are not obligate photoautotrophs and in fact prefer using organic carbon under dark cycle of growth, which is considered as heterotrophic microalgae. Heterotrophic cultivation was reported to be associated with higher biomass production and lipid productivity in *Chlorella protothecoides* [71]. However, cultivating microalgae under this condition may be challenging as this suffers from contamination problems and hence, maintenance of sterile seed-culture is very important. The merit for this type of microalgae cultivation includes the avoidance of lights limitation as faced in the high-density microalgae cultures in large-scale photobioreactors [72].

(3) Mixotrophic cultivation: This is an interesting capacity of certain microalgae that can perform photosynthesis using both organic carbon compounds and inorganic carbon (CO_2) as a carbon source for their growth. These microalgae are facultative photoautotrophic or heterotrophic, or even both. Compared to photoautotrophic and heterotrophic cultivation, mixotrophic cultivation of microalgae for nutraceutical applications is rare except reports for astaxanthin production in *H. pluvialis* [30] and increased biomass in *Arthrospira* (*Spirulina*) [36]. Under mixotrophic conditions, both growth and astaxanthin production in *H. pluvialis* were found to be increased [73]. Therefore, mixotrophic cultivation is more prefered for enhanced production of biomass, lipid and carotenoids yield in microalgal species.

(4) Photoheterotrophic cultivation: This is a typical type of cultivation where microalgae require light as an energy source while utilising organic compounds as the carbon source [74]. It appears that both mixotrophic and photoheterotrophic cultivations are the same but the subtle difference is that the mixotrophic cultures can use organic compounds as an energy source and photoheterotrophic cultures require light as the energy source.

It is well known that the cultivation conditions, especially irradiance and nutrients pool, are significantly responsible for the biomass yield and targeted biomolecules of interest. In general, there are four types of microalgae cultivation conditions, (1) photoautotrophic, (2) heterotrophic, (3) mixotrophic and (4) photoheterotrophic [74]. However, predominantly both indoor and outdoor cultivations in commercial practice are photoautotrophic except a few including *Crypthecodinium cohnii*. Although the fermentation technology has a long history, microalgae-based fermented food items have yet to be developed [75]. However, lactic acid fermentation was demonstrated in several microalgae such as, *Chrolella* sp., *Tetraselmis* sp., *Diacronema lutheri*, *Chaetoceros* sp., *Nannochloropsis* sp. [76]. It was found that the biomass pre-treatment with enzyme cellulase enhances lactic acid fermentation [75]. Allmicroalgae Natural Products S.A., a Portuguese microalgae cultivation company started producing *Chlorella vulgaris* and other microalgae via fermentation, which complements the company's existing photobioreactor technology. They use a fermented variant of *Chlorella vulgaris* and several other pipeline strains for producing omega-3 DHA/EPA (http://www.algaeindustrymagazine.com/allmicroalgae-adds-fermentation-system/). Fermentation technology for valorisation of microalgal biomass remains an open challenge for future benefits.

Due to human activities such as domestic, agricultural and industrial water activities, various organic and inorganic substances are released into our environment, which lead to organic and inorganic pollution. Interestingly, microalgae cultures offer solutions for wastewater treatments, as they provide a tertiary bioremediation along with the production of several potentially valuable biomass applications. Microalgae absorbs nitrogen and phosphorus from the wastewater and cleans it by separating the nutrients as biomass. There are specific microalgae cultivation as an approach for wastewater treatment including in highly efficient photobioreactors for sustainable wastewater treatment (https://www.schott.com/tubing/english/water-treatment.html) and open ponds. This cultivation allows cleaning municipal, agricultural or industrial wastewaters. There are several research findings that suggest certain microalgae can remove heavy metals, as well as some toxic organic compounds, bioaccumulation of selenium, rubidium and lithium [77]; therefore, it does not lead to secondary pollution. However, the biomass obtained from wastewater treatment may not be suitable for food applications but can be used for example as bioplastics, industrial chemicals, organic agriculture, and biofuels.

Microalgae for nutraceuticals have been cultivated in large-scale outdoor systems for purposes other than biofuel production, such as aquaculture, for decades. Irrespective of cultivating microalgae in outdoor ponds, raceways, or open or closed photobioreactors, their biomass productivity is dependent on cultivation parameters, which include, but are not limited to, light source and intensity, depth of culture, cells mixing rate, temperature, nutrients composition and CO_2 supply. Microalgae have been cultivated in a wide range of cultivation systems that can be operated outdoors or indoors, such as open shallow raceway ponds [simple (Figure 2) but in multiples or combined], vertical column photobioreactors (Figure 3), and closed tubular photobioreactors with a gas exchanging tank elsewhere (Figure 4). There may be several variations of the photoautotrophic cultivation system depending on the need and the species used; the facilities available are: unstirred open pond, open raceway pond, paddlewheel stirred raceway ponds, tubular closed photobioreactor, flat-plate photobioreactor, lagoons, bubble column and airlift photobioreactors, large plastic bags, helical-tubular photobioreactor, pilot tanks, cylindrical glass columns, circular plastic tanks, outdoor pilot-scale bubble column photobioreactor, 400 L capacity polyethylene bags supported by frames, vertical photobioreactors (bubble column or air-lift), earthen pots, basins, natural lakes and concrete tanks, etc. [78–80]. Due to gradual technological developments and precise control of microalgal growth conditions, closed photobioreactors are gaining popularity. The merits for increasing the use of PBRs are: (i) substantial savings in net water use, (ii) minimal or no contamination and (iii) increased biomass density. However, the main pitfalls of closed PBRs are the costs associated with high power consumption for operation and artificial light sources. To overcome these challenges, closed PBRs are partially exposed to sunlight for photosynthetic activities of cells that are re-circulated from the reservoir tank. To reduce the cost of artificial lights, a few approaches were adopted such as implementing LED lights, and particularly through increasing the lighting efficiency with submersible lights with specific wavelengths, fiber optic cables and light guides [81].

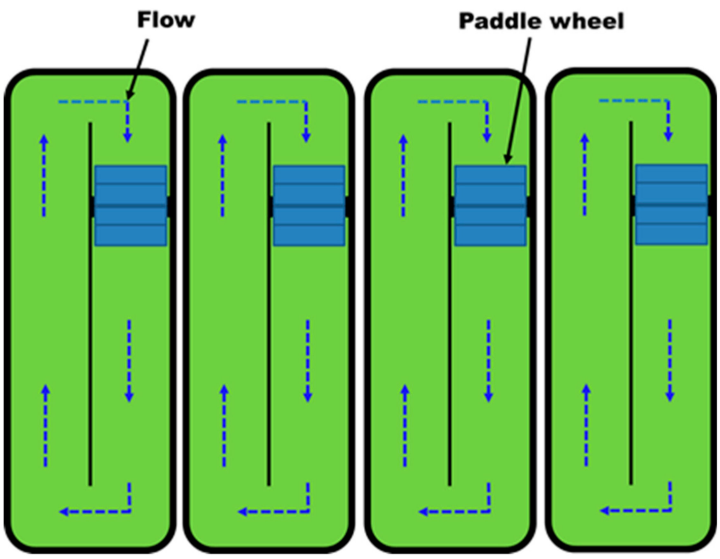

Figure 2. Schematic diagram showing simple open raceway pond with paddle wheel for gentle and uniform mixing of cells for outdoor cultivation.

Raceway ponds (Figure 2) are generally constructed in concrete or compacted earth, which is a specific form of open system built as a shallow, closed loop channel that helps circulating water for uniform mixing of cells. Raceway ponds are easy to construct with various shapes, but for a large area with multiple raceways, long stretched ponds possess 180° curves on either end. Raceway ponds are the most compact and efficient cultivation systems used for the commercial production of several micro-algae including *Haematococcus* and *Arthrospira* (*Spirulina*).

Vertical column photobioreactors (VCPs) (Figure 3) are a type of tubular photobioreactor made of a split-cylinder (acrylic or glass) internal-loop suitable for outdoor and indoor cultivations and has an air-sparger system for keeping cells suspended during illumination either from sun or artificial lights. Generally, the air-sparger device is installed at the bottom of the cylinder; which allows the inlet of air-CO_2 mixture to release as tiny bubbles, generating a driving force for homogenous suspension of cells, mass transfer of CO_2, and removal of O_2, the by-product of photosynthesis. VCPs can be classified as bubble column or air-lift photobioreactors depending on the pattern of liquid flow inside the cylinder. The height of the cylinders of the bubble column photobioreactors are greater than their diameter, with high surface-area-to-volume ratio, satisfactory heat and mass transfer, homogenous cultures, and the efficient release of O_2 and residual gas mixture. The design of sparger is critical for the performance of any bubble column photobioreactor as air bubbling provides the required mixing and gas transfer within the cylinder. Therefore, in tall bubble column photobioreactors, perforated plates can be adopted to break up and redistribute coalesced bubbles for scale-up cultivation. Air-lift photobioreactors are made of a cylinder with two interconnecting zones: (i) Tubular zone for a gas riser where the gas mixture flows upward to the surface from the sparger and (ii) the other zone within the cylinder called the downcomer, which does not receive the air-gas mixture, however, it helps the growth medium flow down to the bottom and circulate within the riser and the downcomer. The source of illumination for VCPs is generally from the outside of the column as sunlight or artificial lights (fluorescent tubes, white LEDs, or other PAR illumination). Nowadays, an inner-illumination as a light cradle of LED lights is becoming popular due to efficient light penetration within the dense cultures and corresponding biomass productivity. This is the low cost photobioreactor most suitable for seed culture generation as well as biomass production in a manageable scale. VCPs are further developed as hybrid photobioreactors (HPBRs) by implementing a porous membrane in the bubble-column photobioreactors for supporting the growth of biofilm-forming microalgae. HPBRs have a high surface area for uniform lighting, reduced dead zones and improved mixing of nutrients [81].

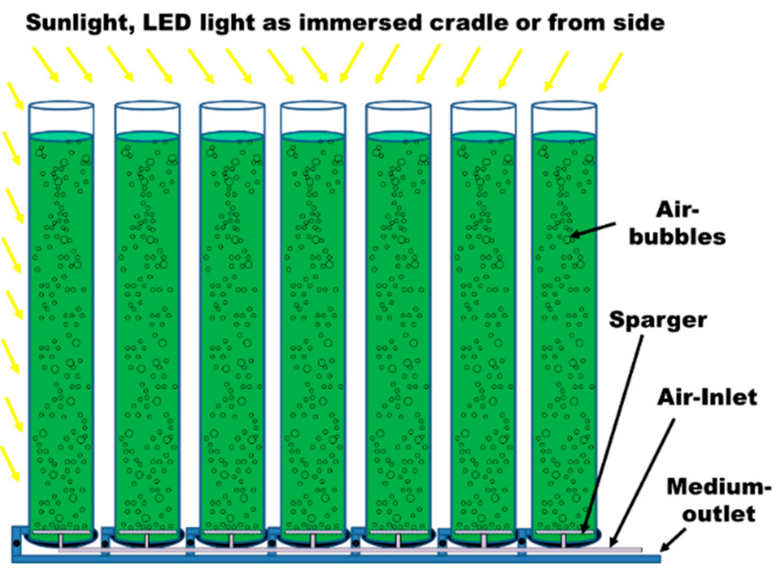

Figure 3. Schematic diagram showing vertical column photobioreactors suitable for outdoor and indoor cultivations.

Closed tubular photobioreactors (Figure 4) are one of the large variety of closed systems used for microalgae production. These closed systems are very efficient in terms of contamination-free cultivation of high-density biomass but it needs huge capital investment and maintenance costs. Tubular systems can be operated either vertically or horizontally both outdoors and indoors but in some cases they are operated inside greenhouses with precise control for HVABs production, for example astaxanthin.

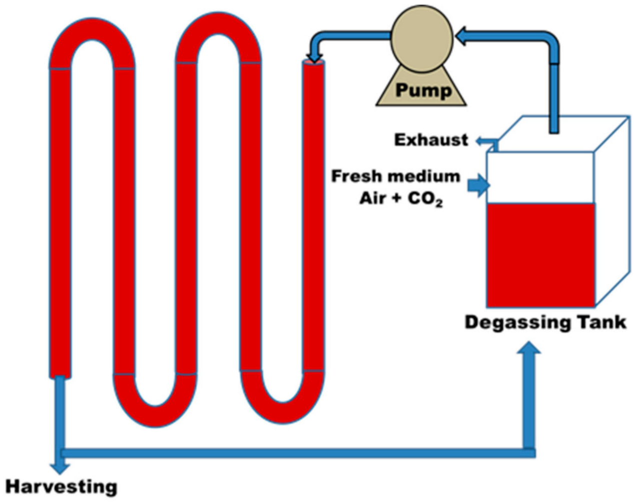

Figure 4. Schematic diagram showing simple tubular photobioreactor that can be operated both vertically or horizontally with continuous supply of nutrients and mixing of cells through pump, with CO_2 supply and exhaustion of produced gases.

4. Discussion and Conclusions

Microalgae are not only considered as a promising feedstock for next generation biofuel production but also for several biotechnological commodities useful in food as functional ingredients, natural colourants as well as health supplements [1,2]. Based on the evidences from several research findings, the World Health Organization approved the use of *Arthrospira* (*Spirulina*) as one of the greatest superfood on this planet [82]. There are numerous microalgae identified as potentials for commercial applications but only a few selected ones are commercially cultivated through conventional open and raceway ponds as well as modern tubular photobioreactor technologies [72]. In the recent past, microalgal biotechnology has developed a lot due to the improvements in industrial photobioreactors and the technical know-how around contamination prevention, light distribution within the dense cultures and control on appropriate cell mixing. Although the majority of microalgal production still occurs in outdoor cultivation photoautotrophically, within the enclosed glass and/or tubular photobioreactors such as for *Haematococcus* and *Chlorella* cultivation. With the evolution of microalgal cultivation technologies and biorefinery concepts, the diversity of microalgal products are increasing. However, more biomass production capacity from already optimised microalgae and also from new potential microalgae would be required to meet the demand for biofuel, HVABs and other natural ingredients such as natural colourants and essential omega fatty acids.

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