Chapter

Biocompounds of Commercial Interest from Freshwater and Marine Phytoplankton

Irene Gallego

Abstract

Microalgae are photosynthetic microorganisms that produce a wide range of biocompounds, such as proteins, omega-3 fatty acids or pigments like astaxanthin; with various applications in the pharmaceutical, cosmetic, bioenergy and food sectors. This chapter provides an overview of the compounds and molecules synthesized by microalgae, ranging from polysaccharides to vitamins, minerals and other secondary metabolites. Additionally, the chapter reviews the key biological aspects that influence the production of such biocompounds, including strain selection, strain improvement and cultivation conditions, as well as the biotechnological factors necessary to optimize the production and processing of these compounds, such as cultivation system, extraction and purification. Lastly, the chapter presents the main applications of commercially relevant microalgae-derived compounds, emphasizing the most notable microalgae-based products currently being developed in the global market.

Keywords: microalgae, bioactive compounds, nutraceuticals, biofuels, animal feed, food supplements, cosmetics, biotechnology

1. Introduction

Biocompounds —chemical compounds derived from biological sources— are gaining attention in the global market because of their sustainable production and higher acceptance compared to synthetic compounds. In 2010, one third of the total β-carotene production had a 'natural' origin [1]. Today, the high demand for omega-3 fatty acids is searching for alternative, non-fish sources to reach the expected market size of 3.8 billion USD (US dollars) by 2026 [2].

Microalgae, a diverse group of photosynthetic microorganisms responsible for capturing 50% of the global atmospheric carbon [3], can also synthetize dozens of biocompounds with various commercial applications, including the pharmaceutical sector, food and feed industries, and the bioenergy sector, among others [4]. These cell microfactories use sunlight and carbon dioxide (CO_2) to sustainably produce high-value biocompounds, such as proteins, lipids, polysaccharides, pigments, vitamins, minerals and other secondary metabolites [5]. The commercial exploitation of some microalgae-derived biocompounds, such as β-carotene, astaxanthin, omega-3 fatty acids, pigments and extracts, is well stablished [1], with a global market size valued in 3.4 billion USD in 2020 [6]. Some microalgal biocompounds, with relatively low production costs, have been commercialized for decades. This is the case of β-carotene from *Dunaliella salina*, with a market price that may reach 300–1500 USD/kg, commercialized since the 1980s [1]. Other biocompounds, however, are still far from being economically viable and require further biotechnological advancements.

Microalgae are predominantly single-celled organisms widely distributed in aquatic ecosystems, —including freshwater, marine or brackish environments— as well as in terrestrial habitats, such as soils and sand [4]. Due to their fast life cycles and short generation times (around 24 h), microalgae can generate relatively high biomass without the need of fertile soils, as opposed to terrestrial crops [7].

According to Whittaker's classification system [8], microalgae can be divided into eukaryotic microalgae and prokaryotic cyanobacteria. While organisms belonging to the former group contain membrane-bound organelles, such as the nucleus or the chloroplast, prokaryotes lack these structures. Within the eukaryotic microalgae group, the most abundant groups belong to phylum Chlorophyta (green algae), phylum Ochrophyta (class Bacillariophyceae includes diatoms and class Chrysophyceae includes golden algae) and phylum Miozoa (class Dinophyceae includes dinoflagellates) [9]. Prokaryotic cyanobacteria (phylum Cyanobacteria) are commonly referred to as blue-green algae. Interestingly, taxonomists use the term 'microalgae' to refer exclusively to the eukaryotic microalgae. However, the physiology and biotechnological applications of both groups of microorganisms are similar, and cyanobacteria are colloquially considered as microalgae [7]. In this chapter, for the sake of simplicity, I will use the term 'microalgae' to indistinctively refer to cyanobacteria and/or eukaryotic microalgae.

Microalgae only require a few essential resources to grow and reproduce, namely light and nutrients. In most cases, a liquid culture medium with nitrogen, phosphorus and silica, in the case of diatoms will suffice to allow microalgal growth. However, to achieve competitive productivities and an economically viable production of microalgal biomass —and derived biocompounds—, some factors must be considered, such as the strain and optimal growth parameters, the type of cultivation system and the bioprocessing techniques to obtain the final product. Before targeting the production of one (or more) biocompounds of interest, it is recommended to follow a roadmap to clarify some questions, such as: (1) which strain(s) produce(s) larger amounts of biocompound(s); (2) how to optimize the strain cultivation conditions (nutrients, environmental variables, genetic techniques); (3) what is the preferred cultivation system (open systems *vs*. closed systems); and (4) what are the most common downstream processing techniques (harvesting and processing). In this chapter, I will focus on the most relevant biocompounds and metabolites that are produced by microalgae, how to increase their production yields, and what are the applications of these valuable compounds that are currently under development, or available in the market.

2. Compounds of interest

2.1 Main biocompounds of interest produced by microalgae

Microalgae are an excellent source of primary metabolites that are necessary for their own survival, e.g., proteins, essential amino acids, lipids, fatty acids, etc.; and secondary metabolites, which are not essential for microalgae survival, and include hydrocarbons, pigments and vitamins, among others (see **Figure 1**) [10].

2.1.1 Proteins and derivatives

The two most common microalgae cultivated nowadays, *Chlorella* spp. (Chlorophyta) and spirulina—commercial name for *Arthrospira*/*Limnospira* spp. (phylum Cyanobacteria) [11]—have been historically exploited as an alternative source of proteins, as their average composition may contain up to 60–70% of proteins (dry weight, DW) [12]. The first documented records of the consumption of microalgae go back to the sixteenth century, when the Spanish chroniclers described that the Aztecs harvested spirulina from Mexican lagoons [13]. *Chlorella* spp. also has a relatively long tradition of culturing and consumption in Asia due to its high protein content, and commercial cultivation at large scale started to develop in Japan in the 1960s [13]. *Chlorella* spp. and spirulina are an excellent source of proteins in plant-based diets, generally with higher concentrations of essential amino acids (not synthetized) than other plant or animal protein sources [14]. Additionally, microalgae-derived peptides have demonstrated antioxidant, immune-protector, anticancer, hepatoprotective and anticoagulant properties, but the technology readiness level to produce microalgal peptides for biomedical applications is still in its early stage [15].

Microalgae also produce enzymes that can be directly used in the industry, and enzymes that are involved in the biosynthesis of microalgal biocompounds (carotenoids, peptides, etc.). For instance, L-asparaginase is an enzyme widely used in the food processing industry and is mainly produced not only by bacteria, but also by cyanobacteria, such as *Limnospira maxima* [16].

Mycosporine-like amino acids (MAAs) are a group of ~40 secondary metabolites that have recently gained commercial interest because of their photoprotective function against ultraviolet (UV) radiation [17, 18], being commonly referred to as

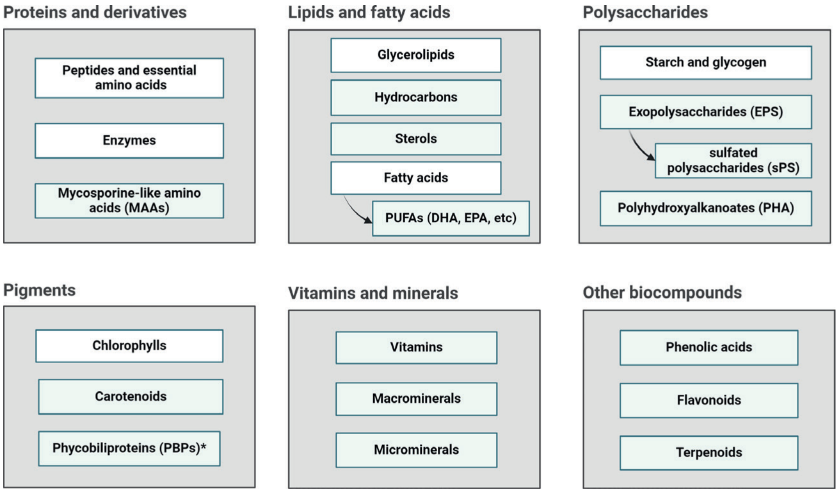

Figure 1.
*Compounds of commercial interest produced by microalgae, classified into six main groups. Primary metabolites are given in white boxes and secondary metabolites are given in green boxes. Arrows point to different subtypes of compounds. *Phycobiliproteins (PBPs), composed by proteins and phycobilins, are shown as pigments.*

'microbial sunscreens'. More than 150 species of marine microalgae are known to produce MAAs, including cyanobacteria, green microalgae, red microalgae, diatoms, cryptophyceans and dinophyceans [19].

Phycobiliproteins (PBPs) are proteins bound to microalgal photosynthetic pigments (phycobilins) that capture and transfer light inside the cell. Examples of PBPs are further detailed in the specific section on pigments (Section 2.1.4).

2.1.2 Lipids and fatty acids

Microalgae are renowned for their high ability to accumulate lipids, in comparison with plant oil crops [20]. The yield of microalgal oils is strain dependent, and several species have received high attention for commercial exploitation as potential biofuels and nutrition supplements. For example, the unicellular marine thraustochytrid *Aurantiochytrium* (formerly *Schizochytrium,* phylum Bigyra) may reach up to 77% of lipid content inside the cell, and the green microalgae *Botryococcus braunii* (Chlorophyta) can store up to 75% of lipids. Lipid accumulation is also dependent on the cultivation conditions, and nitrogen limitation has shown to be very effective to increase the lipid content [21].

Microalgae produce a large range of lipid-like compounds, such as glycerolipids, sterols and hydrocarbons (see also **Figure 1**) [22]. Fatty acids (FAs), the building blocks of lipids, can be categorized into two main groups: neutral, e.g., triacylglycerols (TAGs); or polar, with a more complex structure, e.g., long-chain polyunsaturated fatty acids (LC-PUFAs) [23].

Microalgal PUFAs have attracted commercial interest because of their multiple health benefits (antioxidant, anti-inflammatory, antibacterial activities and a protective effect against cardiovascular problems) [24]. The most extensively studied PUFAs are omega-3 docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA). Because mammals are unable to synthesize these molecules, they must be acquired through the diet. Traditionally, the primary source for PUFAs is fish oil, but its quality and availability are unfortunately declining due to pollution and unsustainable management practices. Microalgae are seen as a promising alternative and sustainable source of PUFAs, as they produce at least 70 different PUFAs [25]. *Aurantiochytrium* sp. and *Ulkenia* sp. (Bigyra) have the highest productivity of DHA [26]. The red microalga *Porphyridium purpureum* (formerly *Porphyridium cruentum*) is one of the richest natural sources of ARA, with 36% of the total FA composition [14]. *Phaeodactylum tricornutum* (Bacillariophyceae), *Pavlova* spp. (Pavlovophyceae), *Nannochloropsis oculata* (Eustigmatophyceae) and *Isochrysis galbana* (Coccolithophyceae) have also shown considerable levels of DHA and EPA [27, 28].

Glycerolipids (or glycolipids) are lipids with a glycerol backbone and FAs and mono- or oligosaccharide attached, and they can have either a structural function (in cellular membranes) or storage lipids (TAGs). Green microalgae (*Tetraselmis* sp.*, Scenedesmus* sp., *Tetradesmus* spp.) and the diatom *Phaeodactylum tricornutum* are known to produce different glycolipids with anticancer and anti-inflammatory activities [29].

Phytosterols are steroids widely used as additives in many food products. They have received great attention because they can reduce blood cholesterol and prevent cardiovascular disorders [29]. *Diacronema lutheri* (formerly *Pavlova lutheri*) (Pavlovophyceae) is the most promising strain for microalgal phytosterols, with a content over 5% DW [30].

Hydrocarbons derived from fatty acids, i.e., alkanes and alkenes, are ubiquitous not only in plants, in insects' external tissues, but also in cyanobacteria. Alka(e)nes of different chain lengths are important targets for biotechnology because they are major components of gasoline, jet fuels and diesel fuels. For instance, *Chlamydomonas reinhardtii, Chlorella variabilis* (Chlorophyta) and *Microchloropsis gaditana* (formerly *Nannochloropsis gaditana*) (Eustigmatophyceae) produce relatively large amounts of heptadecene [31]. Other relevant hydrocarbons for commercial use include carotenes, a subtype of carotenoid that will be described in Section 2.1.4.

2.1.3 Polysaccharides

Microalgae polysaccharides (and oligosaccharides) are considered as byproducts of pigments and/or lipids production [32], but there are some polysaccharides with industrial uses, e.g., moisturizing and aggregating agents in cosmetics, or substrates for bioethanol production [33, 34].

The most relevant polysaccharides for industrial exploitation include exopolysaccharides (EPS), i.e., saccharides excreted outside the cells as mucilage or in the culture media. The advantage of EPS is that the downstream processing (extraction and purification steps) can be simpler than for intracellular compounds [32]. Other polysaccharides of commercial interest are starch and glycogen, which can be used as feedstock and chemical products (**Figure 1**).

The composition of EPS may include sugars (glucose, galactose, fucose, xylose, arabinose, rhamnose, mannose, fructose, etc.) and other non-sugar substituents, such as proteins, or sulfated groups. Both freshwater and marine microalgae (mainly green microalgae, diatoms) segregate EPS with a content of protein and sulfate that can reach up to 26% of the total EPS composition (up 20% sulfate groups and 9% proteins). For more details, see [35, 36] and references therein.

Sulfated polysaccharides (sPS) have drawn attention because of their pharmaceutical and biomedical application; with antiviral, anti-inflammatory, antioxidant, hypoglycemic and anticoagulant properties [35]. For instance, the segregated sPS from *Porphyridium purpureum* (red microalga) show a strong inhibitory effect on *Herpes simplex* virus [37]. Spirulan, another sPS extracted from spirulina, presents antibiotic bioactivity [38]. Additionally, sPS can be used for other industrial applications, as drag reducers and biolubricants (see review in Ref. [33]).

Eukaryotic microalgae and cyanobacteria use the polysaccharides such as starch and glycogen, respectively, as energy storage. Microalgal starch is a potential candidate to replace synthetic polymers, particularly those used in packaging. Some examples of microalgae that produce a high content of intracellular starch are *Chlorella vulgaris*, *Tetraselmis subcordiformis*, *Chromochloris zofingiensis* and *Parachlorella kessleri* (Chlorophyta) [39]. Moreover, glycogen derived from cyanobacteria has demonstrated potential as an alternative fermentation feedstock to produce liquid fuels and chemicals. Heterotrophic organisms, e.g., yeasts, can use glycogen from cyanobacteria as substrate to produce biofuel [40]. The glycogen content in cyanobacteria is dependent on the strain and the growth conditions. For instance, while *Synechococcus* sp. PCC 6803 has shown a content of glycogen equivalent to 12% (DW) [41], spirulina may contain 13.7–63% of glycogen (DW), depending on the cultivation conditions [42].

Cyanobacteria also have the ability to autotrophically produce polyhydroxyalkanoates (PHAs) as storage compounds. PHAs are biopolymers (polyesters) produced by bacteria and cyanobacteria, and serve as a promising alternative to petroleum-based plastics. A recent review on cyanobacterial PHA production identified only six strains producing five types of PHAs relevant for the chemical industry, concluding that PHA productivity is still far from being economically viable and that research should expand towards more screening studies and genetic-engineered strains [43].

2.1.4 Pigments

Microalgae may contain three different pigments: chlorophylls, carotenoids and phycobiliproteins (**Figure 1**).

Chlorophylls are green pigments present in photosynthetic organisms (from microalgae and cyanobacteria to higher plants), involved in light energy absorption during photosynthesis, and the synthesis of other pigments [44]. In microalgae, the estimated amount of chlorophyll is around 1% DW [45]. Chlorophylls are commercially exploited as food colorants, cosmetic ingredients, and in biopharma due to their coloring properties, stimulating effects and antioxidant action [44]. There are five major groups of chlorophylls based on their colors and light absorption characteristics. The most abundant types of chlorophyll are chlorophyll *a* (dark green), particularly abundant in cyanobacteria and red microalgae —but present in all microalgae— and chlorophyll *b* (brilliant green), present in green microalgae. Spirulina is a good source of chlorophyll *a*, while *Chlorella* spp. contains high concentrations of both chlorophylls *a* and *b* [46]. Chlorophylls *c*, *d* and *f* are less abundant and are only present in certain microalgal groups [47]. Extraction of chlorophylls can be challenging, since the pigments can become unstable under oxygen, light, temperature and pH variations [45, 48].

Carotenoids are colored, liposoluble pigments that are responsible for the yellow, orange, red or purple color of some microalgae, as well as plants (fruits, vegetables and flowers), to protect them against photodamage. At least 1204 natural carotenoids have been described from 722 source organisms, 297 of which are algae (macro- and microalgae) [49]. Carotenoids can be classified into two groups: Carotenes, which are hydrocarbons, and xanthophylls, which are oxygenated derivatives of carotenes. Generally, the most commonly exploited carotene worldwide is β-carotene, while the most commonly used xanthophylls are astaxanthin, lutein, fucoxanthin and zeaxanthin. Carotenoids account for an average of 0.1% of the DW of algae, but some microalgae may reach 14% under certain growth conditions, such as the halophytic *Dunaliella salina* (Chlorophyta) [50]. Due to their photoprotective properties, carotenoids are widely used as antioxidants for human health and well-being.

Astaxanthin is the most stable carotenoid and has the most potent antioxidant action [51]. *Haematococcus lacustris* (formerly *Haematococcus pluvialis*) (Chlorophyta) can naturally accumulate up to 5% astaxanthin (DW), but achieving economically viable yields of astaxanthin is challenging and requires careful consideration of numerous factors [52]. *Chromochloris zofingiensis* can achieve productivity of astaxanthin close to 2.0 mg/L/d [53].

β-Carotene is a red-orange carotenoid, precursor of vitamin A (retinol), and together with lutein, it is one of the most pigments with the highest market value. The economic demand for β-carotene is expected to increase 3.8% yearly (from 2018 to 2026), with an expected global market size of 620 million USD [54]. *Dunaliella salina* (*Dunaliella bardawil*) are the most important species that naturally accumulate β-carotene (14%, DW) [55], with a usual range of β-carotene concentrations between 0.1 and 1 mg/L, in large production systems [56].

Lutein is also a potent antioxidant and has drawn much interest in its health-promoting functions. Dosages of 6 mg day^{-1} have been proven to be beneficial for human health, including eye health [57]. The lutein market is also important and may reach 357.7 million USD by the end of 2024 [58]. Several microalgae, including species of the genera *Chlorella*, *Chlamydomonas*, *Desmodesmus*, *Dunaliella* and *Scenedesmus*, naturally accumulate lutein in a range of 4.5–7.05 mg/g (DW) [59, 60], with dozens of reported cultivation strategies (even under different cultivation modes) to increase the content of lutein, particularly for *Chlorella sorokiniana* and *Auxenochlorella protothecoides* (formerly C*hlorella protothecoides*) (Chlorophyta) (see review in [61]).

Fucoxanthin, a brown pigment mostly present in marine microalgae, accounts for more than 10% of the total natural carotenoids [46]. Although the main natural sources of fucoxanthin are brown macroalgae, many microalgae groups (e.g., diatoms, haptophytes, chrysophytes, etc.) are potential candidates for fucoxanthin production. In addition to the antioxidant effect, fucoxanthin has also reported anticancer properties (antiproliferation of cancer cells and cytotoxicity) [62]. *Isochrysis galbana*, *Isochrysis zhanjiangensis* and *Tisochrysis lutea* (Coccolithophyceae) may contain up to 23 mg/g fucoxanthin (DW) after optimization [57]. The diatoms *Phaeodactylum tricornutum*, *Cylindrotheca* sp., *Odontella aurita*, *Chaetoceros muelleri*, *Amphora* sp. and *Navicula* sp. also show considerable fucoxanthin production capacity (see review in Ref. [63]).

Zeaxanthin and canthaxanthin are the orange-color xanthophylls, both precursors of astaxanthin. Both pigments have antioxidant properties and are used as food colorants [64]. Red microalgae (*P. purpureum* and *Rhodosorus* sp.), green microalgae *Chloroidium ellipsoideum* (formerly *Chlorella ellipsoidea*) and cyanobacteria (*Arthrospira* spp., *Synechococcus* sp.) are the main sources of microalgal zeaxanthin [57, 65, 66], while canthaxanthin is naturally occurring in *Chlorella vulgaris* and *Coelastrella striolata* (Chlorophyta) [65]. The production of these pigments requires complex downstream processing (extraction and separation), which increases costs [57].

Phycobiliproteins are water-soluble fluorescent pigments, commonly present in cyanobacteria, red microalgae and some cryptophytes. Attending the pigment colors and light absorption characteristics, there are four groups of PBPs, namely, phycocyanin (PC), allophycocyanin (APC), phycoerythrin (PE) and phycoerythrocyanin (PEC). PC is a blue pigment abundant in spirulina, *Aphanizomenon* and other cyanobacteria; APC is also a blue pigment present in extremophilic red microalga *Galdieria sulphuraria* (Rhodophyta, Cyanidiophytina)*;* PE is a red pigment from *Porphyridium* spp. and *Rhodomonas salina* (Cryptophyceae), and PEC is a magenta pigment found only in some cyanobacteria [67]. PBPs are currently used as natural colorants in cosmetics and the food industry. Moreover, they show antioxidant and anti-inflammatory properties and can be used as fluorescent markers [68–70].

2.1.5 Vitamins and minerals

Microalgae constitute an alternative source of vitamins and minerals that can fulfill human and animal nutritional requirements. Consumption of microalgae-derived vitamins may offer some advantages over other sources: renewable source, low-carbon footprint production, good absorption, vegan origin and no toxic byproducts, and higher vitamin content than some terrestrial plants [71, 72]. Besides the high concentration of β-carotene (precursor of vitamin A), microalgae also contain vitamins C, D, E and K, and the entire B group. In their seminal study, Fabrega and Herrero [73] demonstrated that four marine microalgae (*Tetraselmis suecica*, *Isochrysis galbana*, *Dunaliella tertiolecta*, *Chlorella stigmatophora*) contain higher concentrations of vitamins A, E, B_1 and B_9 compared to conventional food sources typically recognized for their vitamin content. Another study showed that *Diacronema lutheri* (formerly *Pavlova lutheri*; Pavlovophyceae), *Tetraselmis suecica* (Chlorophyta) and *Skeletonema costatum* (Mediophyceae) contain ~20-fold more vitamin D than cod liver oil [74]. Vitamin K_1, essential for prevention of chronic diseases, was found in the highest concentrations in *Anabaena cylindrica* (Cyanobacteria), with levels of 200 μg/g (DW) [75].

Minerals represent the inorganic portion of human and animal diets, and can be classified as macronutrients (e.g., calcium, phosphorus, potassium, sodium, magnesium, sulfur) or micronutrients (e.g., zinc, iron, copper, manganese, cobalt). The mineral composition of microalgae is not only heterogeneous and varies across species, but also indicates variation between marine and freshwater microalgae [76]. A study that evaluated the mineral content of 11 microalgal strains revealed that marine *Tetraselmis chui* (Chlorophyta) contained significantly higher levels of calcium, copper and zinc than other strains; *Phaeodactylum tricornutum* and *Porphyridium aerugineum* (also marine species) were richest in magnesium and iron, respectively; and freshwater *Botryococcus braunii* contained the highest levels of phosphorus and manganese [77].

2.1.6 Other secondary metabolites

Microalgae produce other valuable compounds to different industries and human health, such as phenolic acids and flavonoids. These compounds with antioxidant properties have gained attention recently, but their bioavailability and bioefficacy are limited [78]. Cyanobacteria (*Anabaena* spp., *Phormidium* sp., *Nostoc* sp.) and Chlorophyta (*Scenedesmus* spp., *Chlorella* sp., *Haematococcus* sp.) may produce up to 20 different phenolic acids and flavonoids [79].

Terpenes (squalene, pinene, limonene, bisabolene) are isoprenoids with aromatic properties, responsible for scents. Due to the low production, metabolic engineering is currently applied to increase terpenoids' yields [80].

2.2 Factors influencing the productivity of compounds of interest

There are four main factors that must be carefully considered to enhance microalgal biomass production or microalgal biocompounds of interest: the type of strain, cultivation parameters, cultivation mode and cultivation system.

2.2.1 Strain selection

The selection of the strain will mainly depend on the product of interest for commercial use. Strains can be purchased from microalgae culture collections, donated by laboratories or directly isolated from the natural environment. The advantages of microalgae culture collections (living libraries of biological resources) are various: the strains have been previously isolated and are ready to use, additional information is provided (genetics, morphological and physiological traits, culture conditions), and usually meet regulatory standards. Strain isolation is a time-consuming process that involves different techniques, including traditional techniques such as serial dilution and/or micro-pipetting, and/or automated techniques, such as flow cytometry with cell sorting [81].

Additionally, purification methods must be applied if the ultimate goal is to obtain axenic strains. In this case, techniques such as sonication, agar plating and the use of antibiotics, might be necessary to decontaminate the microalgal culture [14, 81].

Strain growth rate is in some way interconnected to cell/colony size [82]. Strains of larger sizes are expected to show lower growth rates than smaller size strains, but a likely trade-off might arise, i.e., bigger strains might contain higher concentrations of biocompounds, in comparison with smaller strains. Therefore, using only strain size as a parameter for strain selection would not be recommended, as it is not informative enough.

Strain selection can be challenging due to the unknown productivity of the target compound(s), or because our target strain might be (physiologically) similar to other strains. In this case, strain screening is essential prior to strain selection. Since microalgae screening studies can include from dozens to hundreds of strains, e.g., [83, 84], the use of bioinformatics and AI tools can facilitate the microalgal analysis by providing more accurate identification and quantification of the strains [85].

2.2.2 Strain genomic improvement

To increase production yields, microalgae can be genetically improved by selective breeding, either with random (UV-induced) or with targeted (gene editing) mutagenesis. Microalgal production systems can also be optimized using genetic and metabolic engineering, by adding (or removing) specific genes in the metabolic pathways involved in the production and accumulation of biocompounds [86].

2.2.3 Growth parameters

Choosing the most appropriate growth culture medium to ensure long-term cultivation is critical to avoid microalgae stress and to achieve a competitive productivity, at a later stage [81]. Furthermore, resource levels (nitrogen, phosphorus and light are essential non-substitutable resources for microalgae) and environmental conditions (pH, temperature, aeration, CO_2 levels) are highly connected to microalgal productivities and biomass [14, 87]. Optimization of growth conditions for each strain and product of interest remains crucial for enhancing productivity. A simple search of the terms 'microalga*' AND (('growth condition*' OR 'growth parameter*' OR 'optim*')) in the bibliographic database Web of Science shows more than 10,700 peer-reviewed scientific publications [88], which gives an estimation of the relevance of these factors to maximize the microalgae production. Since analyzing the output of Web of Science is beyond the scope of this chapter, I will only describe briefly the importance of light, temperature, pH, nutrient starvation, aeration and CO_2 levels.

It is well established that light intensity and the duration of the light:dark cycle (photoperiod) have a strong influence on microalgal growth, and that high light intensities, above the maximum threshold, will cause photoinhibition [14, 87]. Also, different light wavelengths can induce the production of certain compounds. For instance, it is known that microalgal lipid production is enhanced under relatively high light intensities (but the amount of PUFAs might decrease), and that the production of certain carotenoids is stimulated by blue light wavelengths [89, 90].

Temperature also plays a crucial role in cell growth and metabolism, and each strain is likely to have a different (negatively skewed hump-shaped) thermal performance curve, with optimal temperature levels relatively close to the threshold values, as it occurs in natural populations of phytoplankton [91]. The amplitude of the thermal gradient is also dependent on the strain, which is usually wider (and reach higher optimal temperatures) for cyanobacteria, and narrower (with lower optimal temperatures) for diatoms [92].

Nutrient starvation is recognized as one of the most common strategies to increase the production of storage compounds [14, 81]. In general, nitrogen limitation enhances the intracellular concentration of lipids or saccharides. A recent review on microalgal lipid production showed that 117 out of 189 studies induced nitrogen starvation [93]. Also, it is well known that the synthesis and accumulation of carotenoids, such as β-carotene and astaxanthin, can be stimulated under low nitrogen conditions [14]. In the case of diatoms (with silicon as a non-substitutable resource), low levels of silicon rather than low nitrogen, enhance lipid accumulation [14]. Increasing the salinity concentration in the medium is another strategy to induce the intracellular accumulation of valuable compounds. High salinity induces the accumulation of flavonoids, probably due to their antioxidant activity [79], and has also proven to be a highly effective method for enhancing lipid content [94].

Maintaining an equilibrium between air and dissolved CO_2 in the culture is crucial for the optimal strain growth. CO_2 removal rates increase with increasing biomass densities, and consequently, pH and dissolved CO_2 will fluctuate. Fluctuations in pH and dissolved CO_2 might change the availability of metals and minerals such as iron and calcium, and essential nutrients such as phosphorus [95]. These fluctuations can (and must) be minimized with CO_2 injections and/or continuous aeration. Also, pH is strain dependent, and strains from the same genus can show totally different values. For instance, *Chlorella vulgaris* optimal growth occurs at pH values between 7.5 and 8.0 [96], but *Chlorella sorokiniana* grows better at pH 6 [97].

The key variable(s) for enhancing the production of high-value biocompounds will depend on both the strain and type of biocompound. A recent review (>200 studies) on growth variables from 95 marine and freshwater phytoplankton species revealed that temperature, light-dark cycle and irradiance levels were the most frequently manipulated parameters to enhance the production of lipids, concluding that understudied factors, such as pH, phosphorus limitation or metals, might lead to higher lipid yields [93].

2.2.4 Cultivation mode

Three cultivation modes can be used for large-scale microalgae production: photoautotrophic (using CO_2 and light to generate biomass), mixotrophic (using an organic carbon source in the presence of light) and heterotrophic (using an organic carbon source in the absence of light) conditions. Mixotrophic cultivation allows shifting from photoautotrophy (in the presence of light and CO_2) to heterotrophy under dark conditions, while heterotrophic cultivation cannot use CO_2 as a carbon source, since the accumulation of the gas will decrease the pH of the medium. Mixotrophic cultures have demonstrated higher biomass productivity than photoautotrophic cultures, and more cost-efficient system than heterotrophic cultivation modes. The use of byproducts as organic substrates (e.g., acetate) enhances biomass productivity and reduces cost and environmental impacts [98].

2.2.5 Cultivation system

Microalgae can be cultivated in open or closed systems. Open ponds (OPs) are shallow ponds or tanks that usually allow mixing the culture, have low energy requirements and construction costs, and are easy to scale up. Photobioreactors (PBRs) are closed systems (transparent culture vessels) designed to enhance light penetration and photosynthesis. Fermenters are also closed systems used to cultivate mixotrophic and heterotrophic microalgae. The advantage of closed systems is that growth parameters can be easily controlled and monitored, there is low risk of contamination and the scale-up process is accelerated. PBRs are the predominant cultivation systems within the European Union (EU) [99]. However, the global use of fermenters has increased over the last decade, particularly for food production [100].

A recent meta-analysis on cultivation modes (OP *vs* PBRs) elucidated that the environmental performance of microalgae cultivation not only depends on the cultivation system, but also on the location and the species considered, concluding that no cultivation system is favorable in terms of productivity [101]. OP systems require the least amount of land because these systems are usually placed in locations with high temperatures and irradiances, which increase the productivity [101].

2.2.6 Downstream processing

Extraction and purification of microalgal compounds is challenging because conventional techniques are highly energy-dependent. Common harvesting techniques include mechanical pressing, milling and solvent extraction, which are time- and energy-costly [102]. Indeed, ionic liquids may be toxic and pose environmental risks if they are not treated properly before discharge [103]. The use of genetic techniques to produce cell-wall deficient microalgal strains can be advantageous to optimize the production system, but in general, these techniques have a focus on improving a microalgal trait with a clear commercial application rather than improving the production system [104]. Sometimes, strains that were improved with random-mutagenesis techniques are used instead. This is the case of the model organism *Chlamydomonas reinhardtii* and its recombinant cell-wall deficient UVM4 strain.

2.3 Commercial applications of microalgae

2.3.1 Dietary supplements and functional foods

The global market of dietary supplements and functional foods has exponentially increased in the last years, both in terms of sales and variety of products available [105]. Microalgae play an important role in both sectors due to the variety of valuable compounds including pigments with antioxidant properties, PUFAs and vitamins, and the high protein content and balanced amino acid profiles, with numerous health benefits [15, 106]. A bioinformatics-based review on microalgae genomes and metagenomes shows that microalgae contain compounds with antiviral, antibacterial, anti-inflammatory, anticancerogenic, antioxidant, immune-protective and prebiotic activity [107]. Microalgal extracts rich in astaxanthin, β-carotene, lutein or chlorophylls, soft gels containing microalgal oils rich in omega-3 PUFAs, lyophilized capsules containing microalgal biomass rich in vitamins and proteins, are some examples of what is currently available in the market [108].

Also, feed enriched with microalgal biomass improves animal immune response, disease resistance and gut function of the animal. Camacho et al. reviewed the health benefits of microalgae-derived biocompounds on animals, and they proposed potential industrial applications of microalgae to increase the quality of cattle, poultry, piglets, lamb, fish and crustaceans' meat, as well as the production (and quality) of eggs [109]. For example, astaxanthin is utilized in aquaculture as feed additive, not only to enhance the color of farmed fish and shrimp, but also to improve the quality of seafood for human consumption [110].

Incorporating microalgae into foods can also lead to potential benefits for human and animal health due to the presence of several biocompounds. Nowadays, it is not uncommon to find in our supermarkets and stores a variety of products with microalgal extracts as ingredients: biscuits, breads, snacks, oils, drinks, pasta, condiments, emulsions and dairy products [15, 106, 111]. A higher consumer demand of non-animal protein sources and more sustainable production might explain this trend.

Additionally, most microalgal pigments (chlorophylls, β-carotene, astaxanthin, lutein, phycocyanin, phycoerythrin) are used in the food and beverage industry as natural food/beverage colorants, to replace synthetic colorants and ensure food safety. For example, phycocyanin from spirulina is a blue natural colorant commonly used in beverages and some foods.

Spirulina and chlorella, colloquially referred to as 'superfoods', dominate the microalgal market due to their nutrient-rich profile: high protein content, PUFAs, pigments, vitamins (including the B group) and minerals [12].

2.3.2 Fertilizers and biostimulants

The new paradigm of circular bioeconomy, with valorization of the microalgal residual biomass after extraction of high-value compounds, has potential applications in agriculture. Crop production and quality can be improved with the use of microalgae-derived biostimulants rich in minerals and micronutrients. Some benefits of using microalgae include the mineralization with simpler molecules for direct uptake by plants, plant protection against pathogens, pH buffering, higher resilience against stressors (droughts or salt stress) and stimulation of plant growth [112]. Osorio-Reyes et al. compiled some recent applications of foliar and soil application of microalgae and their effects on plant crops, and spirulina and/or *Chlorella* spp. were the most common biostimulants [113].

2.3.3 Cosmetics and personal care products

Different biocompounds are used in the cosmetics industry. Astaxanthin and lutein are usually included in cosmetics formulations due to their powerful antioxidants and antiaging activity [52]. MAAs are well-stablished photoprotective agents, used in sunscreens, anti-photoaging agents and wound-healing agents [18]. EPS is endowed with moisturizing and hydrating properties [32].

2.3.4 Biofuels

Despite the considerable amount of research on biofuels over the past decades, large-scale production of third- and fourth-generation biofuels (with microalgae as feedstock) remains challenging, but the microalgal biofuel industry (particularly in China) is expected to experience rapid development [114]. Alternative bioenergy sources include the production of biohydrogen, bioethanol or biobutanol, among others [115]. Genetically improved cyanobacteria can produce polysaccharides that will be used as a substrate for the production of bioethanol (by anaerobic fermentation) or produce bioethanol directly [86].

2.3.5 Pharmaceuticals and biomedicine

Microalgae can also be genetically transformed to generate cell factories with applications in biomedicine, such as the production of recombinant proteins. A recent review on microalgal recombinant vaccines has compiled 18 vaccines against various animal and human infectious diseases, with *Chlamydomonas reinhardtii* as the preferred cell factory [116].

2.3.6 Biopolymers

The production of microalgal biopolymers, such as PHAs, starch and cellulose, may contribute to a more sustainable economy and reduce the production of non-biodegradable plastics. Nonetheless, the industrial production of microalgal polymers is still in its infancy, but the number of scientific studies on microalgae-derived plastics is growing [117].

2.3.7 Bioremediation and wastewater treatment

Microalgae can remove nutrients, metals and organic contaminants, and have been proven to decontaminate industrial, urban and agricultural effluents. Wastewater treatment plants use microalgae in their tertiary treatment to remove pollutants and excess macronutrients -nitrogen (N), phosphorus (P)- from effluents. Green microalgae (*Tetradesmus obliquus, Chlorella* spp., *Chlamydomonas* spp., filamentous *Spirogyra* spp.) and cyanobacteria (*Limnospira maxima*) have a high nutrient and/or metal removal rate (see review in Ref. [118]). Microalgae can also improve air quality by removing toxic gases from the atmosphere and increasing oxygen concentrations, as shown in Ref. [119].

2.3.8 Carbon sequestration

Microalgae are known as the most efficient biological sequesters of CO_2, which are used to produce compounds of interest. The voluntary carbon markets are including microalgae production as a nature-based solution to reduce the emission of greenhouse gases, and the number of microalgal biotechnologies providing this service has recently multiplied [108].

2.4 Examples of microalgae-based products currently commercialized

Microalgae are commercially exploited worldwide. Here, I selected some examples of microalgal producers across the globe (**Table 1**). In the Pacific region, the Hawaiian biotech Cyanotech Corporation is one of the pioneers in microalgae nutritional supplements, and a world leader in astaxanthin production from *Haematococcus lacustris* [120], followed by the Swedish AstaReal. In Japan, with a long tradition of incorporating microalgae as food ingredients, the biotech corporation Euglena Co. has developed a full line of products from *Euglena gracilis* (Euglenophyta) as functional food and nutritional supplement. The omega-3 fatty acid DHA is produced at industrial scale in at least 20 countries, including France [121]. DHA from *Schizochytrium* sp. is generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA) and commonly added to infant formula products. In Inner Mongolia (China), the largest agglomeration of alkaline lakes allows the cultivation of spirulina under optimal growth conditions, with an annual production estimated in >3000 t [122].

Location	Main compound	Strain	Cultivation system	Product/service	Notes
Hawaii (USA)	Astaxanthin Spirulina biomass	*H. lacustris*	OP, PBR	BioAstin® nutritional supplement	Net sales (2024) 15.1 M USD
Nacka (Sweden)	Astaxanthin	*H. lacustris*	Indoor bioreactor	AstaReal®	Estimated revenue (2024) 16.9 M USD
Tokyo (Japan)	Biomass rich in amino acids and glutamic acid	*Euglena gracilis*		"Euglena for the Body" nutritional supplement and health foods	Net sales (2024) 23.649 M JPY (2024)
Libourne (France)	LC-PUFAs (DHA)	*Schizochytrium* sp.	Fermenter	DHA ORIGINS©	—
Inner Mongolia (China)	Spirulina biomass Phycocyanin	Spirulina	OP (enclosed)	Food supplements Food colorants (blue)	87 Ha cultivation area Annual production >3000 Ton
Malaysia (HQ in Japan)	Biomass	—	Flat-panel PBR	Carbon capture	Largest carbon capture farm (5 Ha, 100 Ha in 3 years)
Lyon (France)	Recombinant proteins	Genetically improved strains	PBR, fermenter	NINKARAK® and ALGAVAX® platforms	—

Abbreviations: HQ = headquarters, OP = open pond, PBR = photobioreactor, H. lacustris = Haematococcus lacustris.

Table 1.
List of seven examples of relevant microalgae-derived products currently commercialized or under development, at a global scale.

Innovative services include carbon sequestration using microalgae to boost the corporate climate finance (voluntary carbon markets). The largest microalga biomass production farm for carbon capture is located in Malaysia, with an estimated total surface of 100 Ha in 2027. In France, microalgal cell chassis are genetically improved to produce therapeutic recombinant proteins at large scale: Immunotoxins for immunotherapy, thermostable vaccines, etc.

3. Conclusions

Microalgae production is increasing worldwide due to the growing interest in biocompounds. These microorganisms are a potent source of proteins, lipids, pigments, vitamins and other valuable compounds with industrially relevant applications. Different economic sectors, including the food and feed industries, agriculture, cosmetics, pharmaceutical and biomedicine, bioenergy sector, or carbon capture markets, are incorporating microalgal compounds to fulfill the society demands and to achieve a more sustainable economy. Selection of the adequate strain and optimization of the growth conditions (e.g., light intensity and photoperiod, temperature, nutrient optimal and limiting levels, salinity, pH) are crucial steps to maximize the production of microalgae valuable compounds. Yet, productivities of some microalgal-derived compounds are not fully optimized, but new screening techniques, innovative cultivation and bioprocessing technologies, as well as strain improvement with genetic and metabolic engineering, may contribute to enhance the production of microalgal biocompound(s) and to move towards a circular bioeconomy.

Conflict of interest

The author declares no conflict of interest.

Author details

Irene Gallego
Research Division Agroecology and Environment, Agroscope, Zurich, Switzerland

*Address all correspondence to: irene.gallego@agroscope.admin.ch

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