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**Published on:** 10 May 2013 - Journal of Industrial Microbiology & Biotechnology (Springer Berlin Heidelberg)

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### **To cite this version:**

Pignolet, Olivier  and Jubeau, Sébastien and Vaca-Garcia, Carlos  and Michaud, Philippe *Highly valuable microalgae: biochemical and topological aspects*. (2013) *Journal of Industrial Microbiology and Biotechnology*, 40 (8). 781-796. ISSN 1367-5435

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# Highly valuable microalgae: biochemical and topological aspects

Olivier Pignolet · Sébastien Jubeau ·  
Carlos Vaca-Garcia · Philippe Michaud

**Abstract** The past decade has seen a surge in the interest in microalgae culture for biodiesel production and other applications as renewable biofuels as an alternative to petroleum transport fuels. The development of new technologies for the culture of these photosynthetic microorganisms and improved knowledge of their biochemical composition has spurred innovation in the field of high-value biomolecules. These developments are only economically viable if all the microalgae fractions are valorized in a biorefinery strategy. Achieving this objective requires an understanding of microalgae content and the cellular localization of the main biomolecular families in order to develop efficient harvest and sequential recovery technologies. This review summarizes the state of the art in microalgae compositions and topologies using some examples of the main industrially farmed microalgae.

**Keywords** Biorefinery · Cyanobacteria · Eukaryotic microalgae · Biofuel · Lipid · Polysaccharide · Pigment · Protein

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## Introduction

Microalgae are unicellular and multicellular photosynthetic microorganisms from freshwater and marine systems that offer extremely interesting industrial potential. Definitions of microalgae include prokaryotic microalgae, i.e., cyanobacteria (Chloroxybacteria), and eukaryotic microalgae, i.e., diatoms (Bacillariophyta), green algae (Chlorophyta) and red algae (Rhodophyta). Eukaryotic microalgae are recognized as primitive plants and can be either autotrophic or heterotrophic. Autotrophic microalgae require only inorganic compounds such as CO<sub>2</sub>, N, S, P, and light as an energy source for their growth and development. They convert captured solar energy into biomass (photosynthesis) with efficiencies that generally exceed those of terrestrial plants (3 % reported for marine microalgae against 0.2–2 % for terrestrial plants) [108]. Some photosynthetic microalgae are mixotrophic, meaning they are able to perform photosynthesis and to catabolize exogenous organic nutrients. For autotrophic microalgae, photosynthesis is essential to convert solar radiation and CO<sub>2</sub> into reducing power, adenosine triphosphate (ATP), O<sub>2</sub>, and 3-phosphoglycerate, which are then used to support growth. With these simple growth requirements, microalgae can sustainably generate lipids, proteins, and carbohydrates at a large scale, offering promising environmentally friendly alternatives to the current consumer products. The most prominent examples are biofuels, human diet and animal feed supplements, and applications in the pharmaceuticals, nutraceuticals, and cosmetics industries. Some macroalgal compounds also show interesting functional properties (free radical scavengers) and could provide new treatments against diseases such as cancer or even AIDS [97]. However, these high-value compounds (primary products) are generally extracted individually through expensive processes that waste most of

the microalgal material (co-products). The microalgae industry's future hinges on enabling first-generation biorefineries to valorize the main primary products from a biomass and not only one of them. In a schematic way, first-generation biorefineries work to a three-step concept highlighted in a schematic flowsheet shown in Fig. 1. These steps are disintegration of cells, fractionation of main component families, and purification of valuable biomolecules. Nevertheless, microalgal biorefineries are hard to set up due to the broad chemical and morphological diversity of microalgae and the high-value fractions that need to be obtained for specific applications. Indeed, each unit operation has to preserve the specific properties of the obtained fractions and the stability of the target compounds in the remaining materials. Given that the main molecular content groups are often associated with specific intracellular compartments, unit operations could be performed on a deconstructed biomass in a conceptual biorefinery (Fig. 2). These main families are lipids, polysaccharides, proteins, and pigments. Even though pigments are biochemically varied, they can be considered a single biochemical family for the purposes of a valorization strategy. All these

compounds are qualitatively and quantitatively dependent on microalgal species used. However, some species have risen to prominence in the industry and are dedicated to specific applications due to their select biochemical composition. The aim of this review is to describe the biochemical composition and topology of selected microalgae and to highlight the compounds to be valorized in a first-generation biorefinery strategy.

## Lipids

Biological lipids are chemically diverse groups that nevertheless share the common defining feature of being insoluble in water. Their biological functions, like their chemistry, are equally diverse. Oil-rich algae (called oleaginous species) can be grown either autotrophically and heterotrophically. In a biorefinery concept, this class of compounds, and more especially reserve lipids, are at the origin of biofuel production. Microalgae for biofuel production can be grown in several places all year round with significant advantages such as good oil productivity exceeding the yields of the best oilseed crops, non-use of arable land, low water consumption compared to crop irrigation, and low environmental impact [78]. Microalgae growing under normal conditions actually produce significant amounts of lipids, but this lipid accumulation decreases when cultivation conditions are optimized to increase biomass yield [76, 109, 124]. Microalgae contain primarily polar lipids such as phospholipids and glycolipids together with neutral storage lipids such as monoglycerides (MAGs), diglycerides (DAGs), and triglycerides (TAGs), free fatty acids, hydrocarbons, and pigments [57]. Polar lipids are confined to cell organelle membranes such as the thylakoid membranes of the chloroplast [57]. TAGs are neutral lipids mainly stored in vacuoles within the cell [21, 57]. Most microalgae accumulate very few TAGs during the exponential growth phase but can produce and store substantial amounts of TAGs during the stationary phase or under adverse environmental conditions [13, 57, 114]. Fatty acid (FA) composition may differ between lipid classes. As a rule, saturated fatty acids (SAFA) and monounsaturated fatty acids (MUFA) comprise the storage lipid fraction while polyunsaturated fatty acids (PUFA) largely comprise the structural lipid fraction [57]. However, culture processes and species selection have a major influence on lipid composition, accumulation, and distribution. For many microalgae, growth stressors such as excessive solar energy captured by photosynthesis, nitrogen depletion, salinity, or high temperature lead to primarily lipid storage, with high TAGs levels [26, 31, 62, 108, 114] or to a shift in FA composition [38]. Some microalgae are well-known lipid producers extensively

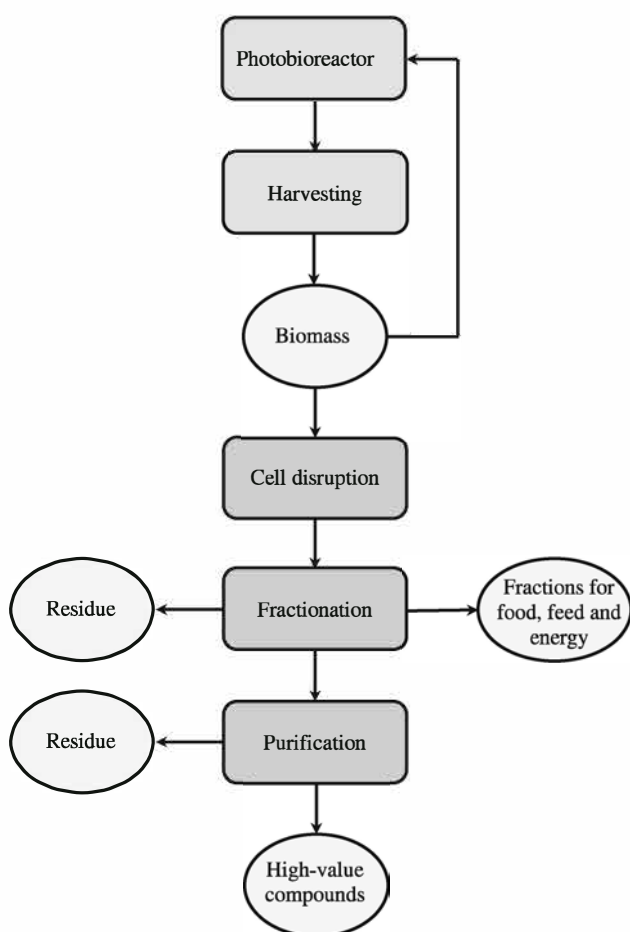
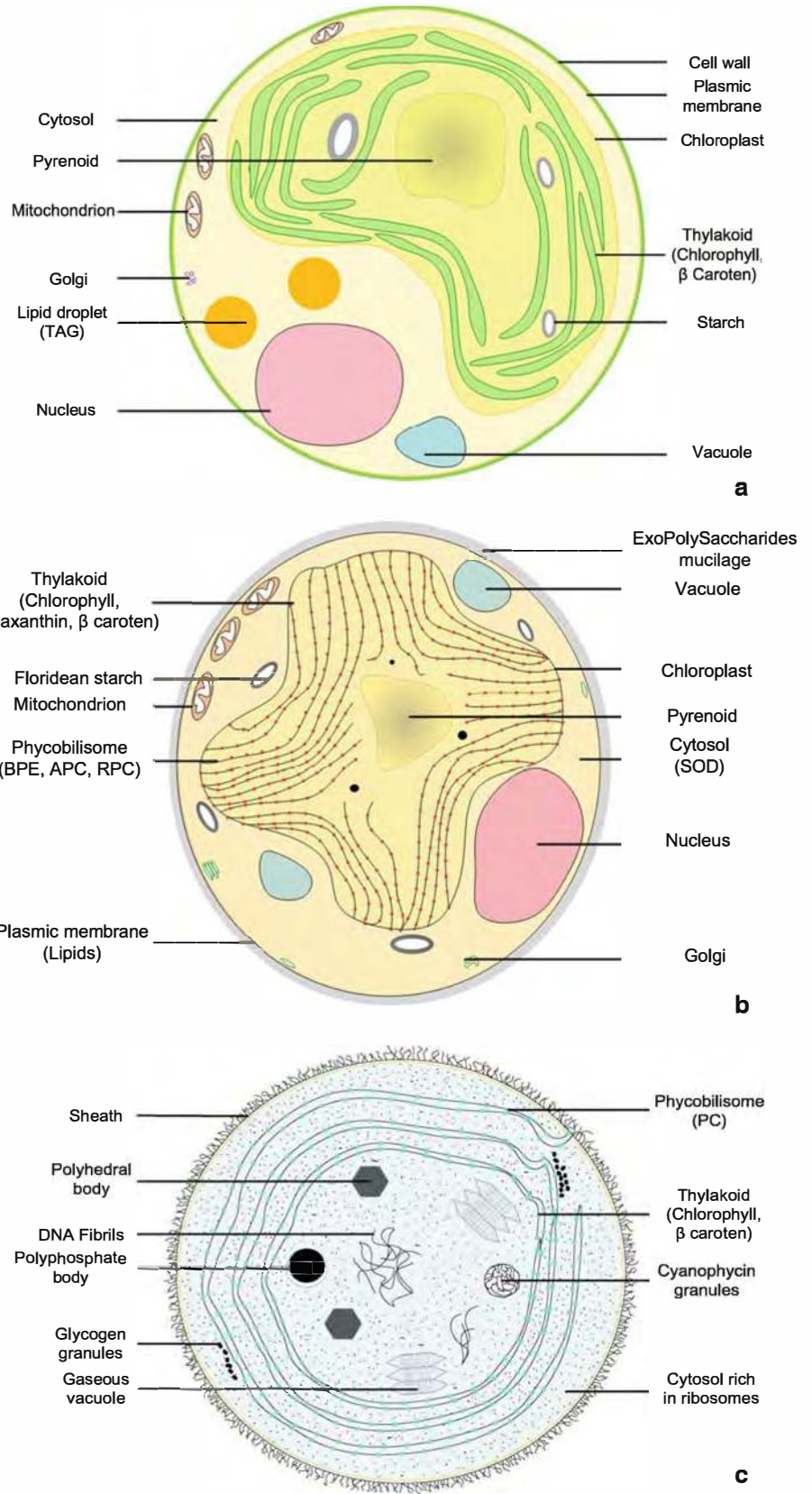


Fig. 1 A microalgae biorefinery

**Fig. 2** Ultrastructure of microalgae: **a** *Chlorophyceae*, **b** *Rhodophyceae*, and **c** *cyanobacteria* (based on Ref. [70])



studied for biofuel and more especially for biodiesel production. Biodiesel is defined as monoalkyl esters of vegetable oils or animal fats. It is produced by the transesterification of TAGs with methanol (or other alcohols) in the presence of an appropriate catalyst, with glycerol as by-product. Biodiesel standards around the world, including the USA (ASTM D 6751) and Europe (EN 14214), also address storage and handling issues—linoleic and linolenic acid esters, for example, are prone to oxidation and so need to be chemically reduced by a hydrogenation process [63]. Hydrogenation converts double bonds to single bonds by H addition. Other microalgae-producing lipids are exploited for food and feed applications, notably due to their unsaturated FA or pigment contents. A majority of the high-value lipid compounds extracted from these algae are long-chain PUFA and carotenoid pigments.

Although all microalgae are candidate lipid-producers, the “winners” for industry applications are the eukaryotic microalgae *Chlorella vulgaris* (up to 58 % dw) [2], *Nannochloropsis oculata* (up to 69 % dw) [19], *Botryococcus braunii* (up to 75 % dw) [27], and *Scenedesmus obliquus* (up to 50 % dw) [78]. Nonetheless, another eukaryotic microalgae such as *Porphyridium cruentum* may have futures for lipid-based industrial applications as they have original and/or highly specific lipid profiles, rich in long-chain PUFA. Indeed, *Porphyridium cruentum* is rich in high-value FA such as arachidonic acid (20:4 $\omega$ 6) and eicosapentaenoic acid (20:5 $\omega$ 3) [32] for a global lipid content of between 9 and 19 % [78].

#### *Chlorella vulgaris*

Lipid accumulation as chloroplastic and cytoplasmic droplets by the green microalga *C. vulgaris* in freshwater (Fig. 2) requires a pre-extraction cell lysis step. Growth on nitrogen-limiting media resulted in higher lipid accumulation (20–53 %) but lower growth rate, whereas mixotrophic cultivation yielded higher lipid content (40–53 %) and higher lipid productivity (67–144 mg/l/day) in all culture media [124]. The FA composition of microalgal lipids comprises over 60–68 % SAFA [124]. More specifically, the FA most accumulated are described as C18, C18:1, C18:2, and C18:3 (3.4, 16.3, and 79.4 % of total FA [68]. Choi et al. [30] compared the lipid production of mixotrophic and autotrophic cultures of *Chlorella* sp. Cultures were carried out in photobioreactors using various light periods and various CO<sub>2</sub> supplementations [30]. Methanol (1 % v/v) was used as sole organic carbon source in the mixotrophic conditions. The highest lipid accumulation (18.6 % dw) with high proportions of C16:0, C18:0, and C18:1 was obtained with 5 % CO<sub>2</sub> and 450  $\mu$ E/m<sup>2</sup>/s of 12:12 (h) irradiation cycles in mixotrophic mode. It is

interesting to note that in this study, mixotrophy gave better results than autotrophy. Previous data [30, 68, 124] suggest the lipid composition of *C. vulgaris* is suitable for biodiesel production. *C. vulgaris* was also grown in a column aeration photobioreactor filled with artificial wastewater in semicontinuous mode [41]. The highest lipid production and content were 147 mg/l/day and 42 % dw, respectively, in the optimal growth conditions, and the system was characterized as highly efficiency at removing nutrients from wastewater [41].

#### *Nannochloropsis oculata*

*Nannochloropsis oculata* is well known in marine biotechnology for its potential interest in biodiesel production. *N. oculata* NCTU-3 was grown in a semicontinuous system aerated with 2 % CO<sub>2</sub> and operated by one-day replacement with the objective of optimizing culture for long-term biomass and lipid productions [28]. A two-stage cultivation strategy has also been proposed to enhance the lipid production of *Nannochloropsis oculata* [109]. Biomass growth and lipid production were experimented in two separate stages. Final lipid yield was 2.82-fold higher in these conditions than in traditional single-stage batch cultivation systems [109]. Microalgae lipid content was strongly influenced by the physical–chemical environment and composition of the culture media. For example, a temperature increase from 20 to 25 °C doubled the lipid content of *N. oculata* (from 7.90 to 14.92 % dw), while a 75 % decrease in nitrogen concentration in the medium increased *N. oculata* lipid content from 7.90 to 15.31 % dw without impact on growth [33]. The authors identified high amounts of palmitic acid (16:0), which constituted about 60 % (mol/mol) of the overall lipid fraction, along with C18:1, C18:2, and C18:3. This result confirmed the finding that storage lipids in *Nannochloropsis* sp. consist largely of the SAFA palmitic acid (C16:0) and the MUFA palmitoleic acid (C16:1) [110]. However, Tonon et al. [114] found that 68 % of TAGs in *Nannochloropsis oculata* consisted of the PUFA eicosapentaenoic acid (20:5  $\omega$ 3, EPA) at the end of stationary phase compared to just 8 % in the exponential phase. Similarly, under nitrogen deprivation, the average biomass and lipid productivities of *Nannochloropsis* sp. reached 9.9 and 6.5 g/m<sup>2</sup>/day, respectively. Lipid content was 68.5 % in starved biomass compared to 39.1 % in non-starved biomass, and the two cultivation conditions led to significant differences in lipid classes and FA composition [19]. Effectively, under nitrogen deprivation, polar lipid content decreased whereas neutral lipid content increased. The major lipids represented under nitrogen starvation were TAGs, polar lipids, and hydrocarbons (79, 9, and 2.5 % of total lipids, respectively) [19]. The FA composition of the different lipid classes (neutral, polar, and

phospholipids) includes mainly C16:0, C16:1, and C18:1. Note that polar lipids and phospholipids have a significant ratio of C20:5 $\omega$ 3 (eicosapentaenoic acid) in their structure. Lipid content, FA profiles, MAGs, DAGs, and TAGs were analyzed for 2 years in *Nannochloropsis oculata* biomasses grown outdoors in closed vertical flat-panel photobioreactors [88]. Results showed that the highest lipid content was recorded during autumn, signaling an optimal non-linear response to light and temperature. The authors hypothesized that enhanced thylakoid stacking under reduced light conditions resulted in more structural lipids concomitantly with the increase in glycerides due to released photo-oxidative stress. The relative increase in MUFA during autumn suggested a synthesis either of structural FA as MUFA or a relative increase of C16:1 incorporated into TAGs and DAGs.

### *Botryococcus braunii*

*B. braunii* is a colonial hydrocarbon-rich green microalga [6] widespread in freshwater and brackish lakes at temperate, tropical, and Arctic latitudes. *B. braunii* has tremendous potential as a renewable biomass feedstock and high potential for the production of renewable liquid hydrocarbons. The lipids of *B. braunii* can be repeatedly extracted from wet biomass without the usual harvesting and dewatering steps [18]. By exposing *B. braunii* to biocompatible organic solvent, a substantial fraction of hydrocarbons was obtained without impairing cell viability [42], as the majority of biosynthesized lipids in *B. braunii* are located in the outer cell wall of the algal material [18]. Note that a high percentage of hydrocarbons in some petroleum fuels were probably derived exclusively from this specie [122]. Depending on the type of hydrocarbons synthesized, *B. braunii* is classified into A, B, and L strains but some strains of this species are not classified [61]. For example, strain *B. braunii* AP103 grown on open raceway pond produced a biomass concentration of  $1.8 \pm 0.13$  g/l. Its biochemical analysis revealed 19 % lipids, 33 % carbohydrates, 18 % proteins, and 11 % hydrocarbons [4]. The hydrocarbon profile showed the presence of heptadecane (34 %) and hexadecane (12.5 %). The major FA detected in the lipids extracted were oleic (25.7 %), linolenic (34.26 %), and palmitic (9.42 %) acid [4]. *B. braunii* race A is a ubiquitous colonial green microalga that is an important synthesizer of lipids in fresh and brackish water ecosystems [82]. Race A produces C23–C33 odd-numbered *n*-alkadienes, mono-, tri-, tetra-, and pentanes from FA. Linear olefins can account for up to 61 % of the dry matter of green active-state colonies. Note that the growth of *B. braunii* (race A) and its production of hydrocarbons and FA were influenced by environmental salinity [95]. The L race produces a single tetraterpene hydrocarbon

called lycopadiene (C<sub>40</sub>H<sub>78</sub>) and it accounts for up to 2–8 % of dry biomass. The B race produces polyunsaturated and branched C30–C37 terpenoid hydrocarbons called polymethylated botryococenes that are promising renewable energy sources as they accumulate at very high levels of 26–86 % dry weight in the algae [82]. Photosynthesis, growth, and metabolic productivity of *B. braunii* BOT-22 cells (race B) were saturated at approximately 1,000, 100, and 200  $\mu\text{mol}/\text{m}^2/\text{s}$ , respectively, under monochromatic red light. Lipid and hydrocarbon production was not directly supported by an increase in photosynthetic activity [102].

### *Scenedesmus obliquus*

The green microalgae *Scenedesmus obliquus* is an oleaginous alga well known for its ability to accumulate lipids, notably in fresh wastewater [77]. The lipids of *S. obliquus* grown under controlled conditions have been characterized as 7.24 % neutral lipid, 2.45 % glycolipid and 1.48 % phospholipid on a dry weight basis. The major neutral lipids were di- and triacylglycerol, free and esterified sterols, and hydrocarbons. The glycolipids were monogalactosyl diglyceride, digalactosyl diglyceride, free and esterified sterol glycoside. The phospholipids included phosphatidyl ethanolamine, phosphatidyl glycerol and phosphatidyl choline. The 14 FA identified in the lipid fractions were led by C18:2, C16:0, C18:3(alpha), C18:1, C16:3, C16:1, and C16:4. Unsaturated and essential FA were quantified at 80 and 38 % of total algal lipids, respectively [29]. The C16/C18 FA groups of *S. obliquus* lipids obtained from cultivation under nitrogen-starved conditions could reach up to 92.4 % of total FA [56]. Oleic acid, an important indicator of biodiesel quality, accounted for 35.10 % of total FA. The main FA of *S. obliquus* cultivated in nutrient-rich media with 10 % CO<sub>2</sub> were C16:0 (15–16 %), C16:1 (2–3 %), C18:0 (17–18 %), C18:1 (15–16 %), C18:2 (13–14 %), C18:3 (3–4 %), and several minor component FA with a carbon number lower than C16 and higher than C18 [56, 76]. These data were confirmed by [119]. Note also that under P-deficiency and thiosulphate supplementation, the lipid content of *S. obliquus* increased up to 30 % [76].

### Polysaccharides

Polysaccharides are defined as polymers of monosaccharides linked by glycosidic bonds. As for other organisms, microalgae polysaccharides demonstrate strong structural variability and biological functions [97]. Based on their role in the microalgae physiology, they can be split in three classes: structural polysaccharides generally associated to

cell walls, energy polysaccharides like starch, and polysaccharides implicated in cellular communication and recognition sites. Structural investigations into polysaccharides are complicated by the very broad diversity of their constitutive monosaccharides (pentoses and hexoses), non-sugar substituents (e.g., sulfate, acetate, or pyruvate) and glycosidic linkages. If we exclude starch, exopolysaccharides (also known as exopolymeric substances or exopolymers when associated to proteins) have gained significant attention in the published literature. This popularity is explained by their extractability, as these biopolymers are produced by microalgae in the extracellular medium without covalent linkages with cell walls. In some cases, it is difficult to distinguish these exopolysaccharides from the cell wall in which they are embedded (e.g., exopolysaccharides of *Porphyridium* sp.) [3]. Their physiological role is unclear, but some authors have hypothesized that they prevent desiccation [94] or offer a mechanical protection enabling the cells to develop in a range of environments [3]. Microalgal production of polysaccharide and especially exopolysaccharide (EPS) could be also one way to control photosynthetic activity. Algae colony formation is linked to polysaccharide production [113], which is catalyzed by various factors including C/N ratio. Reports suggest glyoxylate, a stimulator of carbon metabolism, is able to inhibit photorespiration and increase photosynthesis in higher plants [87] and some cyanobacteria [14]. Adding glyoxylate to algae like *Anabaena cylindrica* [15] and *Cyanospira capsulate* [91] triggers an excess of carbon flux resulting in intracellular polysaccharide accumulation and soluble extracellular polysaccharide release. A culture study by Liu et al. [73] found that *Scenedesmus obliquus* supplemented with glyoxylate increased exopolysaccharide production.

In recent years, there has been a surge of interest in polysaccharide-producing bacteria, cyanobacteria, and microalgae for industrial applications. Effectively, EPSs are regarded as an abundant source of structurally diverse polysaccharides and some may possess unique properties for special applications. Excluding the EPSs from *Porphyridium* sp. [46] or *Arthrospira platensis* [116], which have been partially characterized, and starch (known as floridean starch) located in cytoplasm and chloroplasts, structural characterization data on microalga-produced polysaccharides remains scarce, even on cell-wall polysaccharides. Vilchez et al. [120] tried to document a global composition of microalgae and described mono- and oligosaccharides as mainly represented by trehalose, glucose, sucrose, and mannose but also polyols. Polysaccharides were more or less ignored, and the authors only gave a global composition for monosaccharides (glucose, galactose, xylose, methylxylose, and glucuronic acid).

### *Chlorella vulgaris*

The most important substance in *C. vulgaris* for human health is a cell-wall  $\beta$ -(1,3)-glucan that has potential as an antitumor agent [85]. This class of polysaccharide is well known as an active immunostimulator, free radical scavenger, and reducer of blood lipids [66]. To our knowledge, the exopolysaccharides or other cell wall polymers from this microalgae have not been described. The only exception is an acidic polysaccharide containing high contents of glucuronic acid and rhamnose identified as a cell wall component [86].

### *Arthrospira platensis*

*Arthrospira platensis*, (Spirulina) is a Gram-negative cyanobacteria species. *A. platensis* grows in tropical and subtropical waters with pH between 8 and 11 and high carbonate and bicarbonate concentrations. The EPS of *A. platensis* is generally considered as a polyanionic sulfated heteropolymer containing proteins, carbohydrates, and sulfated groups. Contents were estimated at 55, 13, and 0.5 %, respectively in *A. platensis* Compère 1968/3786 strain grown in photoautotrophic-mode for 25 days. Carbohydrates are mainly composed of seven neutral sugars [galactose (14.9 %), xylose (14.3 %), glucose (13.2 %), fructose (13.2 %), rhamnose (3.7 %), arabinose (1 %), mannose (0.3 %)], and two uronic acids [galacturonic and glucuronic acids; 13.5 % together] [116]. Trabelsi et al. [115] evaluated EPS production in relation to culture mode: high EPS levels were obtained in culture medium using mixotrophy with a short cultivation period whereas photoautotrophy and heterotrophy cultures produced low EPS levels at longer cultivation periods (290; 219 and 30 mg/l, respectively; 1.5 g/l glucose and/or 100  $\mu$ mol photon  $m^{-2}s^{-1}$ ). Optimizing light and glucose concentrations led to a peak production level of 369 mg EPS/l in mixotrophic mode.

EPS from *A. platensis* exhibits interesting functional properties. It has value to industry due to a non-Newtonian pseudoplastic behavior, but the main applications remain pharmaceutical in nature due to its wide range of biological properties. The sulfated EPS from *A. platensis* acts as anticoagulant, anti-thrombogenic and anti-atherogenic [97]. In addition, calcium and sodium spirulans isolated from hot water-extracted EPS interfere with the adsorption and penetration of viruses like herpes or HIV [55]. Chalouf et al. [25] ran cytotoxicity and antioxidant assays on solvent-isolated EPS and concluded that its biological activity is closely tied to the molecular weight and conformation of EPS in terms of solvent-polymer interactions and the presence of sulfated moieties.



## *Botryococcus braunii*

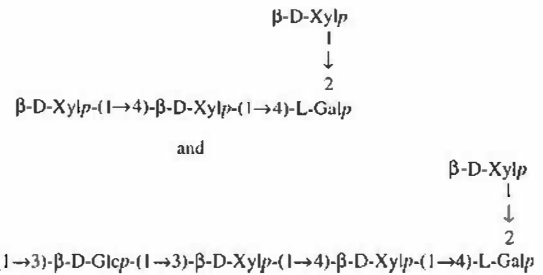
Race-A and race-B strains produce EPS at up to 250 g/m<sup>3</sup> whereas L-race strains produce EPS at up to 1 kg/m<sup>3</sup> [6]. However, EPS production level varies with strain and culture conditions such as culture media, illumination, agitation, and above all salinity [35, 36, 95]. *B. braunii* appears to produce carbohydrates as an osmoprotectant. The authors failed to describe the structures of these exopolymers and only quantified them after ethanol precipitation of culture medium supernatant and/or phenol-sulfuric assay [35, 36, 95].

## *Dunaliella salina*

*Dunaliella salina* is a type of halophile pink microalgae especially found in sea salt fields. EPS production by this strain increases with salt concentration in the culture medium, and peak production (944 mg/l) was obtained at 5 M NaCl [83]. Isolated EPS showing emulsifying activity slightly reduces with salinity. <sup>1</sup>H NMR, FT-IR, and HPLC have given structural clues on the presence of uronic acids, halides, sulfides, alkyl amine, and/or cyclic amine with polysaccharides. Galactose, glucose, xylose, and fructose were detected as constitutive monosaccharides [83]. Fine-grained monosaccharide composition analysis in five fractions of a polysaccharidic crude extract fractionated by DEAE chromatography using reverse-phase HPLC showed that PD1 and PD4a fractions were acidic heteropolysaccharides mainly containing glucose and galactose, respectively, and that PD4a contained sulfated groups. PD2 and PD3 were identified as a glucan. PD4b was a complex of polysaccharide covalently linked with nucleic acids. Compositional analysis of PD4a confirmed the deduction made by Fabregas et al. [39] that water extracts with antiviral activity from *D. salina* contained sulfated polysaccharides with a molecular weight of a hundred-thousand Da [34]. These EPS proved thermostable up to 270 °C and their dynamic viscosity was significantly high at pH 3.0 but decreased with shear rate, confirming pseudoplastic rheological properties [83].

## *Porphyridium* sp.

*Porphyridium* sp. is a red marine microalga (Rhodophyta) with spherical cells whose cell walls lack cellulose microfibrillar components. These microalgae are encapsulated within a sulfated polysaccharide gel (red microalgae cell wall). During growth, the outer gel dissolves in the growth medium while 50–70 % of the polysaccharide stays attached to the cell [106], forming an outer layer that shields the microalgae against extreme environment conditions (e.g., desiccation or salinity). The polysaccharides



**Fig. 3** Structures of oligosaccharides isolated from the exopolymeric fraction of *Porphyridium* sp. [46]

have a molecular weight in the range  $2\text{--}7 \times 10^6$  Da and are negatively charged due to the presence of glucuronic acid and sulfate groups. They are composed of up to ten monosaccharides, the most abundant being xylose, glucose, and galactose. Note that proteins represent 5.5 % of polymer dw. The structure of EPS from *Porphyridium* sp. was partially characterized by Gloagen et al. [46] with oligosaccharides obtained after polysaccharide degradation by lithium (Fig. 3). This polysaccharide has various concrete applications in the cosmetics, drugs, crop growth promoters, and health food markets. A feature of these EPSs is their fluid-dynamic behavior that gives highly viscous solutions at relatively low polymer concentrations in a wide range of pH and temperature values, yielding comparable rheological properties to industrial polysaccharides [3, 89]. Sun et al. [111] tested the antioxidant activity of degraded (microwave) polysaccharides from *P. cruentum* and found that smaller-sized polysaccharides have a better antioxidant effect. It was also shown that these polysaccharides exhibit antiviral (anti-herpes) and antitumor activities [106, 112].

## Pigments

Pigments are complex molecules or macromolecules that have the ability to change the color of reflected or transmitted light as a result of wavelength-selective absorption. Chlorophylls are green pigments with polycyclic planar structures esterified by a long phytol side-chain where  $\text{Mg}^{2+}$  occupies the central position. The chlorophylls differ by the substituents on their ring. The chlorophylls identified in microalgae are a, b, and c and are located in the thylakoids of chloroplasts [69]. Depending on microalgae species, some chlorophylls are not always present in photosynthetic structures. Cyanobacteria and red algae employ phycobilins such as phycoerythrobilins and phycocyanobilins [9]. These open-chain tetrapyrroles have the polyene system of chlorophylls but not their cyclic structure or central  $\text{Mg}^{2+}$ . They are covalently linked to specific proteins, forming phycobiliproteins. Phycobiliproteins are

associated in order complexes called phycobilisomes. Phycobilisomes are the primary light-harvesting structures of these microorganisms and are attached in regular arrays to the outer surface of the thylakoids [8]. Phycobilisomes absorb light over a wide range of wavelengths and transfer the energy to the reaction centers in the photosynthetic membranes for conversion into chemical energy [16]. In addition to chlorophylls, thylakoid membranes contain accessory photosynthetic pigments called carotenoids. Carotenoids are a class of hydrocarbons (carotenes) and their oxygenated derivatives (xanthophylls) consist of eight isoprenoid units (Fig. 4). Carotenoids may be purple, yellow, or red. The accumulation of secondary carotenoids is generally thought to be a survival strategy employed by the algae under photo-oxidative stress or other adverse environmental conditions [58]. The most important are red-orange  $\beta$ -carotene, an isoprenoid, and the yellow lutein. This section focuses on xanthophylls and phycobiliproteins, which are high-value compounds for industrial applications. Among the microorganisms detailed for these molecular families in the literature, the following eukaryotic microalgae emerge as the most significant: *Chlorella vulgaris*, *Dunaliella salina*, *Haematococcus pluvialis*,

*Porphyridium cruentum*, *Arthrospira platensis*, and *Haslea ostrearia*.

#### *Chlorella vulgaris*

Like other species of microalgae, *C. vulgaris* is able to activate metabolic pathways and accumulate carotenoids in response to high irradiance stresses, nitrogen availability, and other changes in environmental conditions. Gouveia et al. [50] studied carotenoid biosynthesis by *C. vulgaris* grown in a reactor for 22 days under a light intensity of  $38/\mu\text{E/s/m}^2$ , saline stress (30 g/l NaCl), and nitrogen starvation. They observed that  $\beta$ -carotene and lutein were first biosynthesized along with chlorophyll-*a* during the exponential phase. Carotenogenesis was activated just before the stationary phase, and  $\beta$ -carotene and lutein concentrations decreased while canthaxanthin, astaxanthin, and astaxanthin esters were biosynthesized in stressed cells. It was suggested that the accumulation of canthaxanthin resulted from the oxidative transformation of  $\beta$ -carotene and that hydroxylated/oxidized astaxanthin was biosynthesized from zeaxanthin/lutein precursors towards hydroxylative pathways. Lutein and  $\beta$ -carotene content in

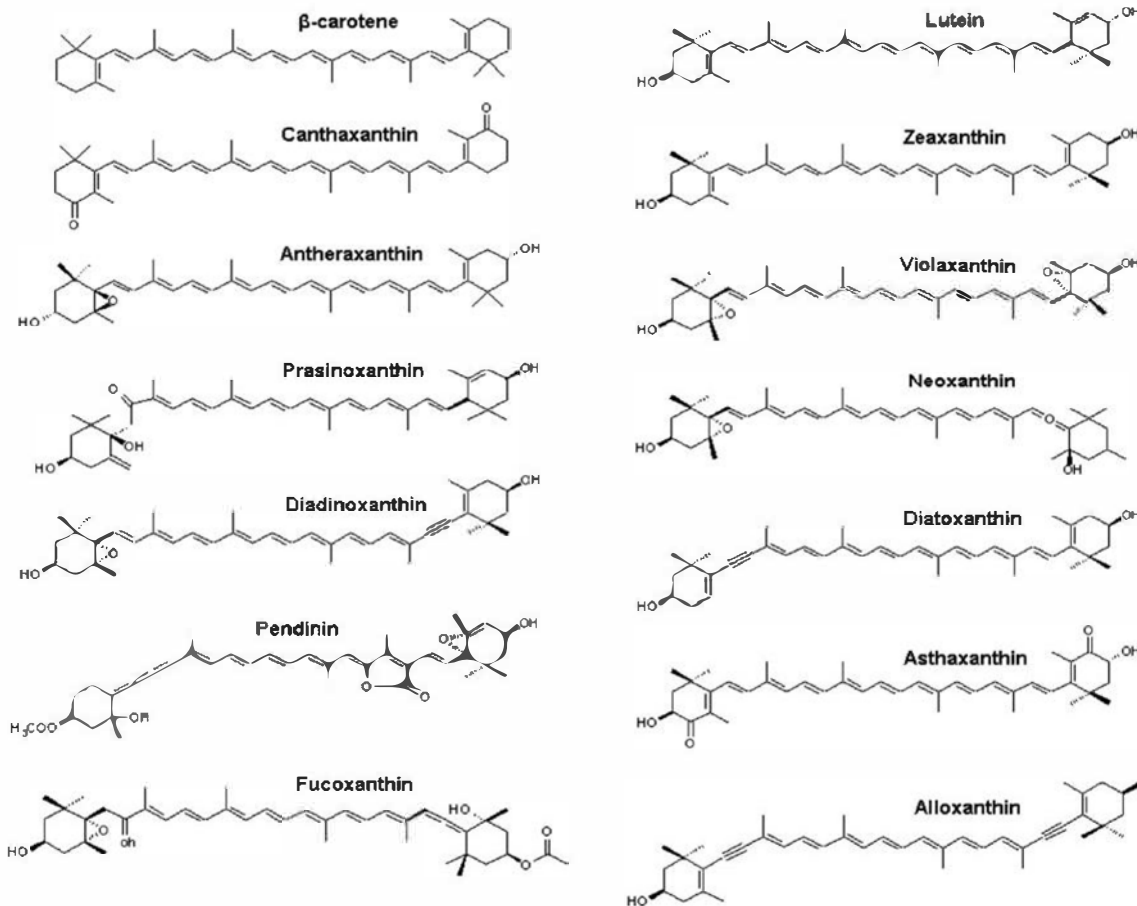


Fig. 4 Structure of the main microalgal carotenoids [43]

“green” *C. vulgaris* grown under normal conditions is about 8 and 5 mg/100 g, respectively [24, 100]. Nevertheless, Mendes et al. [79] extracted pigments of “orange” *C. vulgaris* obtained from a carotenogenesis-optimized culture. Supercritical CO<sub>2</sub> extraction was estimated to be efficient at removing carotenoids from well-crushed biomass (content: 547 mg/100 g dw) compared to solvent (acetone) extraction (content: 133 mg/100 g dw). Astaxanthin and canthaxanthin composed 2/3 of the extracted carotenoids [80]. However, carotenoid content is strain-dependent. Ruen-ngam et al. [100] estimated the lutein content from spray-dried high lutein content *C. vulgaris* to be 7.9 mg/g dw. Carotenoids from *C. vulgaris* have important pharmaceutical and food-industry applications. Indeed, semi-purified carotenoid (mainly lutein) extracts showed antiproliferative activity in an MTT assay on HCT116 cells with interesting IC<sub>50</sub> values [24]. Carotenoids from *C. vulgaris* have also been tested for their coloring and antioxidant properties in egg yolk, fish, food emulsions, and soybean oil [49, 50].

#### *Arthrospira platensis*

C-phycoyanin is the major phycobiliprotein of the cyanobacterium *A. platensis* (also known as *Spirulina platensis*). This bright-blue pigment is part of the phycobilisome and is used for photosynthetic light collection and transfer of chemical energy to the photosynthetic apparatus. C-phycoyanin has an estimated molecular weight of 100–200 kDa according to filtration techniques [60]. It is formed of two sub-units  $\alpha$  and  $\beta$  with molecular weights of 20.5 and 23.5 kDa, respectively [8]. *A. platensis* has a chemical composition that shows promising effects on human and animal health. Indeed, the whole alga is used as nutrient ingredients, and C-phycoyanin is used as natural dyes for food and cosmetics. C-phycoyanin also shows promise as a potential therapeutic agent for oxidative stress-induced diseases and as a fluorescent marker for biomedical research. C-phycoyanin content could reach 20 % [103]. However, *A. platensis* does contain other phycobiliproteins: Patel et al. [90] determined C-phycoyanin, allophycoyanins, and phycoerythrin contents to be 17.5, 3.8, and 1.2 % dw, respectively. *A. platensis* is also rich in carotenoids, 80 % of which are  $\beta$ -carotenes, the rest being physoxanthin and cryptoxanthin. The  $\beta$ -carotene and cryptoxanthin contents are very low under normal growth conditions (just 0.2 and 0.01 % dw, respectively) but these two carotenoids warrant mention as they are both convertible to vitamin A by mammals [22]. Abd El-Baky et al. [1] studied the influence of nitrogen and NaCl concentrations on *A. platensis* growth in batch culture and found that cyanobacterium responded to nitrogen starvation and high NaCl levels by accumulating carotenoids. High carotenoid

contents were obtained when *A. platensis* was grown at a low nitrogen level (51 ppm N), with values ranging from 19.82 to 24.1 mg/g (dw).

#### *Haematococcus pluvialis* and *Haematococcus* sp.

*Haematococcus pluvialis* is a freshwater unicellular green eukaryotic microalgae belonging to the class Chlorophyceae. It can grow under autotrophic or heterotrophic conditions and is commercially well known for its ability to produce massive amounts of astaxanthin (3,3'-dihydroxy- $\beta$ , $\beta$ -carotene-4,4'-dione), a red ketocarotenoid accumulated at up to 2.0 % (dw). Astaxanthin is biosynthesized through the isoprenoid pathway [51]. It is a high-value product for pharmaceutical, nutraceutical and animal nutrition applications [74]. Astaxanthin from *Haematococcus* algae is under consideration for clearance by the US FDA and has already been cleared for sale in several European countries as a dietary supplement ingredient for human consumption. *H. pluvialis* changes from a motile, flagellated cell to a non-motile, thick-walled aplanospore during the growth cycle [20, 64]. The astaxanthin is contained in the aplanospore. Astaxanthin content in the aplanospores is about 1–2 % of dw, and the thick wall requires physical breakage before the astaxanthin can be either extracted or made available to organisms consuming the alga [81, 107]. Zhekisheva et al. [125] suggested that astaxanthin accumulation under a monoesterified form under high light or nitrogen starvations was linked with FA accumulation. In both cases, the newly formed FA mostly correspond to oleic acid. The production of oleic acid-rich triacylglycerols and the esterification of astaxanthin enable the oil globules to maintain a high content of astaxanthin esters. The highest rate of carotenoid biosynthesis was observed almost simultaneously with the highest rates of cell division and chlorophyll biosynthesis after exposure to limited nitrogen and high irradiance [54]. A photo-protective role for astaxanthin has been suggested based on comparisons of astaxanthin-rich cells with astaxanthin-free vegetative cells, along with lower lipid peroxidation [53], a higher Fv/Fm value [52], or a higher oxygen evolution rate [54, 121].

#### *Dunaliella salina* and *Dunaliella* sp.

When the halophilic eukaryotic microalgae *D. salina* is under appropriate (carotenogenic) cultivation conditions, it produces more than 10 % (on a dw basis)  $\beta$ -carotene versus just 0.3 % for other microalgae and higher plants. This massive accumulation of  $\beta$ -carotene seems to be a protection mechanism to counteract the negative effects of solar radiations [44, 123]. High  $\beta$ -carotene content makes the cell display bright red coloration owing to the characteristic absorption spectrum of  $\beta$ -carotene. *Dunaliella*

species concentrate  $\beta$ -carotene in the interthylakoid spaces of the cell's single chloroplast in the form of lipid globules under carotenogenic conditions. Few or no globules exist when the cell is cultivated under non-induced conditions [12]. One analysis found that this membrane-free lipid globule was exclusively composed of  $\beta$ -carotene (more than half), neutral lipids (mainly with triacylglycerols) and a small amount of protein [12].

#### *Porphyridium* sp.

The main accessory light-harvesting complexes of *Porphyridium cruentum* are phycobilisomes. *P. cruentum* phycobilisomes are described as hemiellipsoidal, containing a tricylindrical core subassembly in the semi-spherical centre and several peripheral rods radiating out from the center to form the rounded surface of the semi-sphere. The core contains allophycocyanins, while the peripheral rods are mainly composed of phycocyanins and phycoerythrins. It has been shown that *P. purpureum* (*P. cruentum*) contains four phycobiliproteins: allophycocyanins (5%), R-phycocyanin (11%), b-phycoerythrin (42%), and B-phycoerythrin (42%) [17]. *Porphyridium* sp. is the potential organism for the production of phycoerythrins and especially B-phycoerythrin. B-phycoerythrin is the major light-harvesting pigment and the most valuable of the three main phycoerythrins classes due to its high fluorescence efficiency (intense unique pink color) and therapeutic value (immunomodulatory and anticarcinogenic activity). Reboloso-Fuentes et al. [98] studied the influence of dilution rates, residence time, and solar irradiation conditions on the biomass profiles of *P. cruentum* grown on outdoor photobioreactor systems. The authors reported peak values of 3.6% dw PB and 2.8% dw phycoerythrins, but allophycocyanins and phycocyanin were also detected at up to 0.36 and 0.43% dw, respectively. It has also been demonstrated in *P. purpureum* that phycoerythrin production is dependent on salt concentration in the culture medium. Sodium chloride seems to possess the maximum effect, and it was established that PB and phycoerythrins production can reach over 4.8 and 3.3% dw, respectively, under optimal cultivation conditions at lab scale. Further optimization of specific culture parameters (light and sodium bicarbonate concentration) of a culture of *P. purpureum* led to a pigment-rich content composed of B-phycoerythrin (12.17% dw), R-phycocyanin (10.2% dw), and allophycocyanins (2.0% dw) [118].

#### *Haslea ostrearia*

*Haslea ostrearia*, previously known as *Navicula ostrearia*, is a diatom involved in the greening of the French oysters called "*Fines de Claires*" [65]. This microalga synthesizes

a blue-green water-soluble pigment called marennine that is first stored at the apex of the cells then released into the surrounding environment [96]. Marennine is present under two different location-specific forms: extracellular marennine (EMn; molecular weight: 9,893 Da) and intracellular marennine (IMn; molecular weight: 10,751 Da) [92]. Although the structure of the molecule remains unknown, Pouvreau et al. [92] have shown that it is a polyphenolic compound close to the proanthocyanides. The specific absorption coefficients of purified EMn and IMn were 12.13 l/g/cm at 677 nm and 6.88 l/g/cm at 672 nm, respectively. This pigment is not involved in photosynthesis processes but instead acts as a photoprotector that is produced in response to different stresses such as high light intensity [84] or nitrogen and silica depletion [84, 99]. Marennine possesses antiproliferative, antiviral, and photoprotective activities [45, 93].

#### Proteins

Proteins constitute a large fraction of the actively growing eukaryotic microalgae and cyanobacteria. They are generally undervalued compared to minor products such as unsaturated FA or pigments for medical, pharmaceutical, and nutraceutical applications. Today, there are still no significant applications of purified proteins from microalgae, since the presence of non-protein components generally leads to undesired changes in color, taste, and protein structure [104]. Nevertheless, efforts are now turning to the impressive genetic potential of microalgae on one hand and the specific activities of microalgal proteins on the other. However, some algal biomasses are often used directly as a supplement in human diet, animal feed, or aquaculture, largely due to their high protein levels.

#### *Chlorella vulgaris*

*C. vulgaris* cultures grown in semicontinuous photobioreactor mode under constant irradiance contained up to 58% protein content [10]. Seyfabadi et al. [105] studied the effects of irradiance and photoperiod on the total FA, pigment and protein contents of *C. vulgaris*. Total protein was then estimated to be 46% at 100  $\mu\text{mol photons/m}^2/\text{s}$  and 16:8 h photoperiod. Mahboob et al. [75] determined the biochemical composition of a thermotolerant strain of *C. vulgaris* grown in mixotrophic mode with the aim of determining the biological value of the biomass for further aquaculture applications. Crude protein production was 2.26 g/l/d with urea as nitrogen source, and maximum protein content was 60% dw. The crude protein extracts contained 17 amino acids, including the essential amino acids (AA) isoleucine, leucine, lysine, methionine, phenylalanine, and valine.

Comparative analysis against FAO/WAO requirements for aquaculture development was performed based on AA and essential AA profile. It was confirmed that the thermotolerant *C. vulgaris* grown under mixotrophic mode is suitable for aquaculture applications. In addition, the high scores obtained for lysine and threonine suggested that this biomass may be utilized as a feed supplement in grain-based diets. Janczyk et al. [59] tested the nutritional quality of pretreated (cell lysis) *C. vulgaris* on growing Wistar rats. Note that the pretreatment was necessary to degrade the rigid cellulosic cell wall, which is not digestible for humans and other non-ruminants, and to make the intracellular compounds accessible. Animals were fed with the algal products as sole protein source (150 mg N per 100 g of bw), and digestion coefficient, net protein utilization, biological value, and protein efficiency ratio (PER) were recorded. Ultrasonication emerged as the best pretreatment, with digestion coefficient, biological value and protein efficiency ratio values of 64, 101, and 2.1, respectively. Protein digestibility was close to 70 %. Comparable results were obtained by Becker [10] on drum-dried *Chlorella* sp. with maximum values for digestibility, biological value, net protein utilization, and protein efficiency ratio of 89, 77, 68, and 2.00, respectively, highlighting that the pretreated biomass was of good nutritive quality.

#### *Arthrospira platensis*

*A. platensis* is considered an alternative protein source and a very promising food supplement or additive, as its biomass contains up to 70 % dw of proteins [5]. The quality of powders from *A. platensis* (drum-dried and sun-dried biomasses) was assessed using parameters representative of nutritional quality. Maximum digestion coefficient, net protein utilization, biological value, and protein efficiency ratio values were 77.6, 83.9, 65.0, and 2.10, respectively. These values are relatively close to those of reference nutrition proteins such as casein or egg proteins, highlighting the quality of *A. platensis* as protein source for human nutrition or as a feed supplement [10]. Additional data indicate that proteins from *A. platensis* are up to 90 % digestible and contain about 60 % essential AA [37]. Depleted culture media have a significant impact on the protein content of *A. platensis*. In a study on batch cultures systems, the crude protein content of *S. platensis* harvested at the stationary phase was 67.4, 53.5, and 5.6 % dw for controls, 50 %-nitrogen-depleted media and 100 %-nitrogen-depleted media, respectively [117].

#### *Scenedesmus obliquus*

*S. obliquus* is commonly used for biofuel production but is also important as a source of proteins, with maximum

content reaching 56 % [10]. However, many authors have described *S. obliquus* as a good candidate for wastewater treatment and the conversion of nutrients or waste compounds to biomass. The protein content of *S. obliquus* grown in outdoor conditions in artificial wastewater during 3 days under seasonal irradiance levels was ranged from 33.5–38 % dw in winter to 51 % dw in summer [47]. Çelekli and Balci [23] evaluated the production of proteins from *Scenedesmus* sp. grown in Johnson's medium as a function of P and N concentration in the culture medium, and found that protein production was strongly related to nutrient concentration. Maximum protein yield was 32 mg/l for 0.3 mM P and 12 mM N. In another experiment, Ruiz-Martin et al. [101] showed that a semicontinuous culture of immobilized *S. obliquus* did not appear to allow sustainable culture for wastewater treatment compared to a free-cell culture. Protein content first increased but then tailed off toward the end of the treatment (250 h), meaning that the exponential phase was maintained for a few cycles before the culture collapsed. However, protein content hit a peak of 30 % dw compared to only 16 % dw in the free-cell culture. The immobilized system thus appears suitable for separating biomass from wastewater whereas the free-cell system appears to be the best option for producing biomasses with high nutritional potential. This nutritional quality of *S. obliquus* was confirmed by the maximum biological value, digestion coefficient, net protein utilization, and protein efficiency ratio values (75.0, 88.0, 67.3, and 1.99, respectively) and the essential AA content that ranged from 30 to 45 % of total AA. It was concluded that low concentrations of metallic pollutants stimulated the production of biomass and improved total protein content and AA biosynthesis whereas high concentrations were inhibitory [10].

#### *Dunaliella* sp.

The marine microalgae *D. salina* is mainly identified as a carotenoid producer, and is widely used in cosmetics. There has been little research on the nutritional, functional, and biological properties of the proteins but intensive research on their specific role in the mechanisms of salt tolerance. Certain specific proteins (e.g., two membrane-bound proteins: a 60-kDa carbonic anhydrase and a 150-kDa transferrin-like protein) and enzymes (e.g., dihydroxy acetone reductase reduction of dihydroxyacetone in glycerol) can be induced by different salinities in the osmotic response processes [72]. More, *Dunaliella* sp. is amenable for genetic manipulation and is a good candidate for the induction, accumulation and production of several novel bioactive compounds, including valuable enzymes. For instance, dihydroxy acetone reductase has been obtained from *D. salina* at the industrial scale and subsequently produced for commercialization [11].

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## *Porphyridium* sp.

*P. cruentum* is not necessarily known for its nutritional value despite its rich FA composition (eicosapentaenoic acid, docosahexaenoic acid, etc.) and significant protein content (up to 35 %) [48], which includes a significant fraction of phycobiliproteins, EPS-embedded proteins, biological membrane or cell wall proteins, and free proteins, but more crucially, superoxide dismutase. Superoxide dismutase is a free cytoplasmic enzyme that is synthesized in abundance and considered a high-value compound for biomedical applications such as the treatment of cancers or HIV involving free radicals and other active forms of oxygen [7]. Moreover, among the proteins of the EPS cell wall of *Porphyridium* sp., the most prominent is a 66-kDa glycoprotein (consisting of a 58-kDa polypeptide and 8-kDa sugar moieties) that is tightly bound but not covalently linked to EPSs. This glycoprotein merits attention due to its role in biorecognition and its potential biotechnological applications such as for understanding the *N*-glycosylation mechanisms linked to the production of therapeutic proteins, where the algae could be used as a “cell factory” [71].

## *Tetraselmis suecica*

*Tetraselmis suecica*, a marine green microalgae, is widely used as a source of nutrients for invertebrates in aquaculture due to its homogenous chemical composition and high protein content ranging from 26 to 69 % dw, depending on growth conditions [40]. Bondioli et al. [19] recorded a substantial decrease in protein content when *T. suecica* was grown in semicontinuous-mode in outdoor “green wall panels” under both N and P starvation. Indeed, the protein content of N-sufficient, N-starved, and N-and-P-starved *T. suecica* were 52, 8, and 9 % dw, respectively, in 14-day cultures. Nevertheless, Laws et al. [67], studying the relationship between growth rate and biochemical composition, concluded that the control of *T. suecica* growth rate in a continuous-mode system and phosphate-limited conditions is a convenient way to optimize the biochemical composition of *T. suecica* and its nutritional value to invertebrate consumers in aquaculture systems. Protein content under this protocol was estimated to range from a minimum of 7 % at zero growth rate to a maximum of 59 % at a relative growth rate of 1.0.

## Conclusions

The past two decades have seen huge strides forward in our understanding of the chemical composition, cellular

compartments, and culture conditions of eukaryotic microalgae and cyanobacteria, culminating in the development of new-generation photobioreactors enabling large-scale microalgae biomass farming. Moreover, the recent industry drive for microalgae applications in the near future, mainly for biodiesel production but also for more specific applications in the fields of food, feed, cosmetics, and therapeutics, has prompted a significant increase in funding for this field of science. However, the fractionation of all components of microalgae biomasses under a biorefinery framework is an emergent concept that starts to be translated into real-world industrial practice. The complexity of membrane bound organelles, granules, and droplets containing specific biomolecule classes combined with inadequate methods for separating them has meant that in many cases, only a single biomolecular fraction extracted from specific species actually gets used. A biorefinery approach generally entails upgrading spent biomass after lipid extraction at the expense of alternative bulk or fine chemical production streams. Some breakthroughs are still needed in the design and development of technologies that can reduce costs and increase yields, but even more important is the development of a methodology and technical processes for the global utilization of microalgae. The strategy can be considered as the eco-design of an integrated production scheme. Integrated studies will probably attain the goal of using lipid-producing strains under adapted growth conditions and optimized processes to successfully create an economically viable biorefinery of microalgae.

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