Biotechnology Advances 37 (2019) 193–222

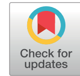
Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

New horizons in culture and valorization of red microalgae



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ARTICLE INFO

Keywords:
Red microalgae
Polysaccharides
Pigments
Porphyridium
Rhodella

ABSTRACT

Research on marine microalgae has been abundantly published and patented these last years leading to the production and/or the characterization of some biomolecules such as pigments, proteins, enzymes, biofuels, polyunsaturated fatty acids, enzymes and hydrocolloids. This literature focusing on metabolic pathways, structural characterization of biomolecules, taxonomy, optimization of culture conditions, biorefinery and downstream process is often optimistic considering the valorization of these biocompounds. However, the accumulation of knowledge associated with the development of processes and technologies for biomass production and its treatment has sometimes led to success in the commercial arena. In the history of the microalgae market, red marine microalgae are well positioned particularly for applications in the field of high value pigment and hydrocolloid productions. This review aims to establish the state of the art of the diversity of red marine microalgae, the advances in characterization of their metabolites and the developments of bioprocesses to produce this biomass.

1. Introduction

Microalgae constitute a diverse group of eukaryote photoautotrophic single cell-organisms present in many ecosystems including terrestrial, aquatic and airborne environments (Flechtner, 2007; Rajvanshi and Sharma, 2012; Tesson et al., 2016; William and Laurens, 2010). They are able to live in harsh conditions (Lewis and Flechtner, 2002; Pushkareva et al., 2016) and use solar energy, water, and inorganic nutrients to reduce CO_2 into complex organic compounds. Some of them have also the ability to use some organic carbon dividing their metabolism into three types: photoautotrophic, heterotrophic and mixotrophic even if they are mainly photoautotrophic organisms. Their systemic classification is based on their pigment composition leading to nine classes in which the main groups are Phaeophyceae, Chlorophyceae, Pyrrophyceae, Bacillariophyceae, Chrysophyceae and Rhodophyceae or red microalgae. It has been estimated that about 200,000–800,000 species exist (including Cyanobacteria) of which 50,000 species are described (Guiry, 2012; Richmond, 2004; Norton et al., 1996). The large diversity of microalgae species is correlated with a large variety of enzymes and metabolites including amino acids, peptides, proteins, carbohydrates (notably complex poly- and oligosaccharides), polyunsaturated fatty acids (PUFAs), carotenoids, sterols,

Abbreviations: AA, Arachidonic acid; ACP, Acyl-transporter-protein; APC, Allophycocyanin; ATP, Adenosine triphosphate; B-PE, B-Phycoerythrin; BPS, Bound polysaccharide; CA, Carbonic anhydrase; CCM, CO_2 concentrating mechanism; Chl *a*, Chlorophyll *a*; Chl *b*, Chlorophyll *b*; Chl *c*, Chlorophyll *c*; Chl *d*, Chlorophyll *d*; Ci, Inorganic carbon; C-PC, C-Phycocyanin; C-PE, C-Phycoerythrin; DHA, Docohexaenoic acid; DNA, Deoxyribonucleic acid; DW, Dry weight; EMP, Embden-Meyerhof pathway; EPA, Eicosapentaenoic acid; FACS, Fluorescence-activated cell sorting; FRET, Fluorescence resonance energy transfert; Gc, Compensation irradiance; GLA, γ-linolenic acid; GOGAT, Glutamate synthase; GS, Glutamine synthetase; Gs, Saturation point; IPS, Intracellular polysaccharide; Ma, Million years ago; MW, Molecular weight; MWCO, Molecular weight cut off; NADH, Nicotinamide adenine dinucleotide; NADPH, Nicotinamide adenine dinucleotide phosphate; Ni, Nitrite reductase; NR, Nitrate reductase; PAR, Photosynthetic active radiation; PBR, Photobioreactor; PC, Phycocyanin; PE, Phycoerythrin; PEC, Phycoerythrocyanin; PES, Polyethersulfone; PEG, poly(ethylene glycol); PFD, Photon flux density; PPP, Pentose phosphate pathway; PS, Polysaccharide; PUFA, Polyunsaturated fatty acid; PVC, Polyvinyl chloride; q0, incident light flux; RNA, Ribonucleic acid; R-PC, R-Phycocyanin; R-PE, R-Phycoerythrin; RPS, Released polysaccharide; RuBisCo, Ribulose-1,5-bisphosphate carboxylase/oxygenase; sPS, Sulphated polysaccharide; TAG, Triacylglyceride; TCA, Tricarboxilic acid; TUF, Tangential ultrafiltration; VLDL, Very low density lipoprotein

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https://doi.org/10.1016/j.biotechadv.2018.11.014
Received 18 May 2018; Received in revised form 26 November 2018; Accepted 26 November 2018
Available online 28 November 2018
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saturated lipids and others. The industrial exploitation of these metabolites for the sustainable production of biomolecules, biofuel, food or feed is at its early stages (Pignolet et al., 2013). Indeed, the maximum theoric photosynthetic efficiency of microalgae in terms of conversion of light energy into biomass energy is between 3 and 10% against 0.2–6% for C3 and C4 plants (Barsanti and Gualtieri, 2018). However, < 100 genera of microalgae have been cultivated at the laboratory scale and no > 10 were produced in an industrial context. They include *Chlorella*, *Nannochloropsis*, *Isochrysis*, *Muriellopsis*, *Odontella*, *Dunaliella*, *Haematococcus*, *Porphyridium* or *Rhodella* genera which are exploited for food/feed production (nutraceutical industries, poultry aquaculture and nutrients), pharmaceutical and ecological applications (bioenergy, water treatment, CO_2 emissions remediation, chemicals, materials, pharmaceuticals care) and also in the cosmetic industry (personal care) (Barsanti and Gualtieri, 2018; Liu et al., 2016; Vigani et al., 2015). The producers clearly focus on niche markets for high value microalgal products capable of counterbalancing the production costs as well as biomass treatments. Large markets like those related to the production of biofuels are not currently economically viable and the new challenge is to propose an innovative framework for microalgal biorefinery. So, special attention has been paid to obtaining biofuel and high value biomolecules *via* systems integration and engineering (Zhu, 2015).

Among the microalgae exploited at the industrial scale, red microalgae (Rhodophyta) such as those belonging to the genera *Porphyridium* and *Rhodella* are of increasing interest as a source of valuable compounds such as extracellular polysaccharides, phycobiliproteins and long chain PUFAs (Guihéneuf and Stengel, 2015; Pignolet et al., 2013). Among the phycobiliproteins which constitute the major accessory light-harvesting pigments, the phycoerythrin (PE, 540–570 nm) gives a pink/red color to the microalgae. Phycocyanin (PC, 610–620 nm) and allophycocyanin (APC, 650–655 nm) are two other phycobiliproteins of the red microalgae. Some of them such as PE are commercially exploited from red microalgae belonging to the genera *Rhodella* and *Porphyridium*. These pigments find applications in several markets such as foods, feeds, pharmaceuticals (*e.g.* immunolabeling factors) and cosmetics. Exopolysaccharides (EPS) from some red microalgae and notably *Porphyridium* species are the second family of high value compounds produced by these strains. They are secreted in the culture medium but can also remain associated to cell surface as a mucilage. They are exploited as sulphated polysaccharides (sPS) by several companies mainly in the cosmetic field as bioactive agents (Arad and Levy-Ontman, 2010; Delattre et al., 2016).

As for other microalgae, the exploitation of red microalgae can be divided into three steps: (i) the production of biomass in open-air systems or in closed photobioreactors (PBR), (ii) its recovery by harvesting and (iii) its refining including stabilization of the microalgae for further processing or direct use after mechanical or chemical disruptions. The fractionation of biomass should be ideally carried out using a scheme of processing routes that selectively extract targeted compounds without altering each other and leading to a final product with low value or for energy production. Therefore, the potential of red microalgae as producer of cosmetic, nutraceutic, therapeutic agents and other value compounds is discussed in this review.

2. What is a red microalgae?

2.1. Taxonomy

The accurate identification of microalgal species remains difficult and is based on estimation, literature data and Algae database resources (*i.e.*www.algaebase.org). Their systemic classification depends on their pigment composition, but other parameters such as life cycle, basic cellular structure, storage material and molecular data are also considered (Heimann and Huerlimann, 2015; Kim et al., 2014). Taxonomically, microalgae comprise both prokaryotic Cyanobacteria (also called blue-green algae) and eukaryotic microorganisms. The eukaryotic microalgae emerged phylogenetically later than Cyanobacteria and resulted from succession of two independent endosymbiosis steps, the first giving rise to the red (Rhodophyceae) and green algae (Chlorophyceae) and the second leading to the Dinoflagellates, Cryptophytes, Euglenida and Heterokont groups, consisting of brown algae (Phaeophyceae), yellow-green algae (Xanthophyceae), golden algae (Chrysophyceae) and diatoms (Baciliarophyceae).

The red algae (macro- and microalgae) are one of the most distinct eukaryotic groups whose origin resulted from the acquisition of a plastid from a cyanobacterium through primary endosymbiosis approximately 1600 Ma. Most of the Rhodophyta live in marine environments and are macroscopic (macroalgae). Their diversity is estimated around 700 genera and > 10,000 species (Kraft and Woelkerling, 1990; Silva and Moe, 1997). They constitute a monophyletic eukaryotic group whose member's affiliation is based on phylogenetic analysis of nuclear, plastid and mitochondrial genes (Burger et al., 1999; Freshwater et al., 1994; Ragan et al., 1994). In the 1970s and 1980s, morphology and molecular markers (16S, 18S, SSU and rbcl) based on taxonomy led to classification of Rhodophyceae into subclasses including the Bangiophycidae and the Florideophycidae (Dixon, 1973; Gabrielson et al., 1985; Garbary and Gabrielson, 1990).

More recently, the classification of red algae was revised and a new taxonomic model was proposed by Saunders and Hommersand (2004), and Yoon et al. (2006). In their classification scheme (Table 1), Saunders and Hommersand proposed, next to the phylum Rhodophyta, a new phylum called Cyanidiophyta, including a single class named Cyanidiophyceae. The phylum Rhodophyta was for its part divided in three subphyla (Rhodellophytina, Metarhodophytina and Eurhodophytina) where four classes (Rhodellophyceae, Compsopogonophyceae, Bangiophyceae and Florideophyceae) were identified. In support of large taxon sampling coupled to multigene studies, Yoon et al. (2006) (Table 1) established a more simplified classification of Rhodophyta with two new subphyla. The first one named Cyanidiophytina has a single class (Cyanidiophyceae) whereas the second one, the Rhodophytina was composed of six classes (Bangiophyceae, Compsopogonophyceae, Florideophyceae, Porphyridiophyceae, Rhodellophyceae and Stylonematophyceae).

Red microalgae include both unicellular and undifferentiated multicellular microscopic organisms, which were long considered as belonging to the order of Porphyridiales (Bangiophyceae class) (Van den Hoek et al., 1995). Based on Yoon et al. (2006) classification, red microalgae are present in the two subphyla, Cyanidiophytina and Rhodophytina. Cyanidiophyceae are unicellular red microalgae living in extremophylic conditions (acidic environment, high temperature and others) comprising two families in the order of Cyanidiales: the Cyanidiaceae and the Galdieriaceae (Table 2). Four genera, among which the more commonly known *Cyanidioschyzon* and *Cyanidium*, have been described in Cyanidiaceae. In Galdieriaceae family, five species belonging to the single genus *Galdieria* have been identified (Table 2). In the Rhodophytina, six classes have been established, three of which comprise microalgae: the Porhyridiophyceae, the Rhodellophyceae and the Stylonematophyceae. The Porphyridiaceae family (Porphyridiophyceae class, Porphyridiales order) record five unicellular genera (ten species) including *Erythrobolus*, *Flintiella*, *Porphyridium*, *Rhodoplax* and *Timspurckia*. *Porphyridium* genus remains the more commonly studied for biotechnological applications, notably *P. purpureum* (also considered by the World Register of Marine Species, as a synonym to *P. cruentum* and *P. marinum*) (Kim et al., 2017; Sato et al., 2017). Rhodellophyceae are divided in two orders, the Dixoniellales and the Glaucosphaerales respectively associated, according to Yoon et al. (2006) classification, to Dixonelliaceae and Glaucosphaeraceae families. The Dixoniellaceae include 2 genera and species, *Dixoniella grisea* and *Bulboplastis apyrenoidosa*, while the Glaucosphaeraceae contain four unicellular genera *Glaucosphaera*, *Rhodella*, *Neorhodella* and *Corynoplastis* (Scott et al., 2008; Yokoyama et al., 2009). In support of

Table 1
Classifications of red algae (including microalgae) according to Saunders and Hommersand (2004) and Yoon et al. (2006).

Saunders and Hommersand (2004)	Yoon et al. (2006)	Orders
• Subkingdom Rhodoplantae		Cyanidiales
◆ Phylum Cyanidiophyta	◆ Phylum Rhodophyta	
	- Subphylum Cyanidiophytina	
* Class Cyanidiophyceae	* Class Cyanidiophyceae	
◆ Phylum Rhodophyta		Rhodellales
- Subphylum Rhodellophytina	- Subphylum Rhodophytina	
* Class Rhodellophyceae	* Class Rhodellophyceae	
- Subphylum Metarhodophytina		Compsogonales
* Class Compsopogonophyceae	* Class Compsopogonophyceae	Erythropeltidales
		Rhodochaetales
	* Class Stylonematophyceae	Rufusiales
		Stylonematales
- Subphylum Eurhodophytina		Bangiales
* Class Bangiophyceae	* Class Bangiophyceae	
* Class Florideophyceae	* Class Florideophyceae	Hildenbrandiales
Subclass Hildenbrandiophycidae		
Subclass Nemaliophicidae		Bratachospermales
		Balliales, Nemaliales
		…
Subclass Ahnfeltiophycidae		Ahnfeltiales
		Pihiellales
Subclass Rhodymeniophycidae		Rhodymeniales
		Halymeniales

ultrastructural and molecular data established by Yokoyama et al. (2009), the Rhodellophyceae class was reconsidered by the authors and two orders were proposed, the Dixoniellales including *Dixoniella*, *Neorhodella* and *Glaucosphaera*, and the Rhodellales comprising *Rhodella* and *Corynoplastis*.

Red microalgae are also present in the class of Stylonematophyceae, divided in the orders Rufusiales and Stylonematales, themselves composed of eighteen genera according to Algae database resources (www.algaebase.org) (Table 2). Fourteen genera are microscopic organisms and most of the genera are filamentous (< 1 mm) (ie: *Rhodaphanes brevistipitata*, *Bangiopsis franklynottii*, *Chroodalycton ornatum*, *Stylonema alsidii*, …) and three are unicellular (*Rhodosorus*, *Rhodospora* and *Rufusia*) (Vis and Sheath, 1993; West et al., 2014).

In conclusion, red microalgae constitute a vast group, still remaining underappreciated and little exploited. Biotechnology applications are restricted to few species as mainly *Porphyridium* sp. (*i.e. cruentum*, *purpureum*) and *Rhodella* sp. (*reticulata* reclassified as *Dixoniella grisea*) (de Jesus Raposo et al., 2013).

2.2. Morphological and physiological characteristics of red microalgae

Microalgae are unicellular organisms whose size varies from one micron to one hundred microns depending on species and growth stage (Table 3). One of the main characteristic of these species is the lack of cell wall and the presence of a “polysaccharidic mucilage” of varying thickness surrounding the cell (Table 3, Fig. 1). Depending on culture conditions, a part of this polysaccharidic layer is solubilized in the culture medium significantly increasing its viscosity. In their cytoplasm, they contain many organelles necessary for their functioning and their metabolism.

Red microalgae are benthic marine organisms and do not possess flagella. Most of them are spherical or ovoid unicells (*Porphyridum* or *Rhodella* for examples) but some genera have been described as organized in pseudofilaments or branched pseudofilaments composed of spherical or elliptical cells (*Rufusia*, *Chroodactylon* and *Empseliums*) (Table 3). They usually contain a single parietal and multi-lobed chloroplast with various colors (blue, green, yellow or orange) even if some species such as those belonging to *Goniotrichiopsis* or *Neevea* genera possess several chloroplasts. Often, a single central, prominent, spherical to ellipsoidal or eccentric pyrenoid is present in their chloroplasts. Pyrenoids are sub-cellular micro-compartments in chloroplasts associated with the operation of CO_2-concentration mechanism (CCM). When present, they act as centres of CO_2 fixation, by generating and maintaining a CO_2 rich environment around the photosynthetic enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). Rarely, multiple pyrenoids may exist in the chloroplast as described with species belonging to the genus *Rhodella* or *Neorhodella* (Scott et al., 2011; Table 3). Indeed, Scott et al. (2011) described that the size of the pyrenoid is often larger than the *Rhodella*'s nucleus which is approximately 3 µm.

The morphology of *P. cruentum* is probably the most detailed in literature compared with other red microalgae. The outer layer of the *P. cruentum* nuclear membrane is covered by ribosomes. The nucleolus is located in the nucleus region adjacent to the chloroplast. It has a double plastid membrane and is star-shaped with lobes on the periphery of the cell. All red microalgae share the Golgi apparatus of eukaryotic cells. It is the place of synthesis of EPS for the *Porphyridium* genus. Starch granules (also called floridean starch or phytoglycogen) are often present in the cytoplasm and localized between the different cellular components. The mitochondria have flattened crests and are mainly located near the dictyosomes (stack of sacs belonging to the Golgi apparatus) at the periphery of the cell (Gantt and Conti, 1965; Scott et al., 2011).

The sole asexual reproduction done by cell division, autospores, biflagellate zoospores, endospores, monospores or archeospores has been described (Table 3).

Rhodophytes are classified among the protist group with primary plastids due to the presence of two outer membranes (cell and polysaccharide membranes) and phycobiliproteins. These latters show an evolution with respect to Cyanobacteria and Glaucophytes by the presence of an extra pigment group, *i.e.* gamma phycobiliproteins (Larkum, 2016). Therefore, red microalgae hold their physiological characteristics of the very photosensitive supernumerary pigments (phycobiliproteins named also phycobilins). In fact, the phycobilins of Rhodophyceae are composed of 4 proteins: PE, PC, phycoerythrocyanins (PEC) and APC absorbing light at a wavelength between 490 and 650 nm. At these wavelengths, chlorophyll *a* (Chl *a*) and carotenoids abound little (Larkum, 2016). Thanks to their photosynthetic equipment, red microalgae can live at different depths up to 250 m. These phycobilins have been linked together by alpha, beta, and sometimes gamma polypeptides and form a complex called phycobilisome which have a hemi-discoidal shape. The complex consists of a tri-cylindrical

Table 2
Situation and taxonomic affiliation of red microalgae in support of algae database (www.algaebase.org, consulted on 2017, august the 22nd) and the World Register of Marine Species (WoRMS).

Class	Order	Familly	Genus	Number of species	Examples of species	Type of algae
Cyanidiophyceae	Cyanidiales	Cyanidiaceae	*Cyanidioschyzon*	1	*Merolae*	Microalgae-unicellular
			Cyanidium	1	*Caldarium*	Microalgae-unicellular
			Pluto	–	–	Microalgae-unicellular
			Rhodococcus	–	–	Microalgae-unicellular
		Galdieriaceae	*Galdieria*	5	*Daedala*	Microalgae-unicellular
					Maxima	Microalgae-unicellular
					Partita	Microalgae-unicellular
					Phlegrea	Microalgae-unicellular
					Sulphuraria	Microalgae-unicellular
Porphyridiophyceae	Porphyridiales	Phragmonemataceae	*Glauconema*	3	*Ramosum*	Macroalgae
		Porphyridiaceae	*Chaos*	–	–	–
			Erythrolobus	3	*Australicus*	Microalgae-unicellular
					Coxiae	Microalgae-unicellular
					Madagascarensis	Microalgae-unicellular
			Flintiella	1	*Sanguinaria*	Microalgae-unicellular
			Porphyridium	5	*Aerugineum*	Microalgae-unicellular
					Purpureum	Microalgae-unicellular
					Sordidum	Microalgae-unicellular
			Rhodoplax	1	*Schinzii*	Microalgae-unicellular
			Sarcoderma	–	–	–
			Timspurckia	1	*Oligopyrenoides*	Microalgae-unicellular
Rhodellophyceae	Dixoniellales	Dixoniellaceae	*Bulboplastis*	1	*Apyrenoidosa*	Microalgae-unicellular
			Dixoniella	1	*Grisea*	Microalgae-unicellular
	Glaucosphaerales	Glaucosphaeraceae	*Glaucosphaera*	1	*Vacuolata*	Microalgae-unicellular
			Neorhodella	1	*Cyanea*	Microalgae-unicellular
			Rhodella	2	*Violacea*	Microalgae-unicellular
					Maculate	Microalgae-unicellular
			Corynoplastis	1	*Japonica*	Microalgae-unicellular
Stylonematophyceae	Rufusiales	Rufusiaceae	*Rufusia*	1	*Pilicola*	Microalgae-unicellular
	Stylonematales	Stylonamataceae	*Bangiopsis*	3	*Franklynottii*	Microalgae-filamentous
			Chroodactylon	2	*Ornatum*	Microalgae-filamentous
			Chroothece	8	*Richteriana*	Unicellular-colonies
			Colacodyction	–	–	macroalgae
			Empselium	1	*Rubrum*	–
			Goniotrichiopsis	–	–	Macroalgae
			Kyliniela	–	–	Macroalgae
			Neevea	1	*Repens*	Microtahllus > 2 mm
			Petrovanella	–	–	–
			Purpureofilum	1		Macroalgae-filamentous
			Rhodaphanes	2	*Brevistipitata*	Microalgae-filamentous
			Rhodosorus	2	*Marinum*	Microalgae-unicellular
			Rhodospora	1	*Sordida*	Microalgae-unicellular
			Stylonema	13	*Alsidii*	Microalgae/macroalgae-filamentous
			Tsunamia	1	–	–
			Vanhoeffenia	–	–	–
			Zachariasia	–	–	Unicellular-colonies

center (APC) from which 6 arms consisting of PE and PC are developed (Fig. 2). Phycobilisome is present on the surface of thylacoid membranes allowing the transfer of energy (Kathiresan et al., 2007). The first absorbing the solar energy is PE transferring this energy towards PC in turn. APC then constitutes the final acceptor. This energy will eventually be transmitted to the chlorophyll of the reaction center to release electron from the photosynthetic system II (Roy et al., 2011).

2.3. Genomic data and metabolism

Knowledge of genomic data contributes to the acquisition of valuable information for the estimation of functionally features such as metabolism pathways, physiology, environmental adaptation and evolution of organisms. In the last decades, several microalgae sequencing projects led to the access to the complete sequences of nuclear, plastid and mitochondrial genomes of about 30 microalgae, mainly belonging to Chlorophyta taxon (https://jgi.doe.gov) (Lû et al., 2011; Sasso et al., 2011) and including only 3 red microalgae, *Cyanidioschyzon merolae* (Matsuzaki et al., 2004), *Galdieria sulphuraria* (Schönknecht et al., 2013) and *Porhyridium purpureum* (Bhattacharya et al., 2013). The analysis of nuclear genomes of *C. merolae*, *G. sulphuraria* and *P. purpureum* showed structural differences on the sizes of genomes (between 13 Mbp and 20 Mbp) as well as on a number of chromosomes (between 2 and 20) (Barbier et al., 2005; Bhattacharya et al., 2013; Matsuzaki et al., 2004; Muravenko et al., 2001) (Table 4). In addition, these genomic data highlighted singular features of red microalgae such as (i) the relatively small genome size, notably for *C. merolae* in comparison to other eukaryotes, (ii) a nearly complete absence of introns for *P. purpureum* and *C. merolae*, (iii) the low degree of genetic redundancy and high portion of coding sequences in *C. merolae* (Barbier et al., 2005; Misumi et al., 2008). Due to its genomic structural simplicity, *C. merolae* has been considered as one of the most primitive photosynthetic eukaryotes (Cunningham et al., 2007).

The genomic data are a valuable resource for the estimation of metabolic pathways. So, the identification of 121 genes involved in the metabolism of lipids in *C. merolae* genome contributed to design the complete diagram of lipids metabolism in Cyanidiales (Mori et al., 2016; Sato and Moriyama, 2007). The analysis of carbohydrates metabolism genes in *P. purpureum* and the identification of 116 CAZymes (http://www.cazy.org/), including a 33% greater number of families compared to *C. merolae* was in accordance with the complexity of *P. purpureum* cell-wall (Bhattacharya et al., 2013). Genomics studies also

Table 3
Characteristics of red microalgae depending on Algaebase database (www.algaebase.org).

Genus	Nature of matrix	Form	Size (μm)	Chloroplast	Mitochondrion	Vacuole	Detected Pigments	Storage products	Sexual/asexual reproduction
Cyanidioschyzon	Mucilage	Oval	1–4	Blue-green parietal, polymorphic chloroplast (no pyrenoid)	1	ND	Chl *a*, C-PC	Phytoglycogen	NR
Cyanidium	Mucilage	Spherical	1.5–6	Blue-green, parietal, plate-like chloroplast (no pyrenoid)	1	Described in older cells	PC	Starch	NR
Galdieria	Mucilage	Spherical	3–11	Blue-green parietal, multi-lobed chloroplast (no pyrenoid)	Several	Vacuolar system	Chl *a*, C-PC	Floridean starch	NR
Erythrolobus	ND	Spherical/Oval	5–12	Bright red, parietal, multi-lobed chloroplast (central pyrenoid)	1	Small vacuoles	B-PE, R-PC, APC	Floridean starch	Asexual (fission)
Flintiella	Gelatinous	Spherical	9–20	Massive, parietal, reddish chloroplast (no pyrenoid)	ND	ND	PE, R-PC, Chl a, zeaxanthin, ß-carotene	Floridean starch	Asexual (cell division)
Porphyridium	Mucilage	Spherical/obovoid	5–16	Stellate chloroplast (prominent central pyrenoid)	ND	ND	ND	ND	ND
Rhodoplax	Gelatinous	Spherical/ellipsoidal	10–18	Single, parietal chloroplast (pyrenoid)	ND	ND	ND	ND	Asexual (autospores and zoospores)
Bulboplastis	Mucilage	Spherical	6.5–23	Grayish green, multi-lobed chloroplast (no pyrenoid)	ND	ND	ND	ND	Asexual (fission)
Dixoniella	Mucilage	Spherical	8.5–17	Olive green to grayish green, multi-lobed chloroplast (pyrenoid)	ND	ND	No phycobilisomes	ND	Asexual (fission)
Neorhodella	ND	Spherical	22–40	Large, peripheral, blue-green, multi-lobed chloroplast (pyrenoids)	ND	ND	Sparse opposed phycobilisomes	ND	ND
Rhodella	Mucilage	Spherical	ND	Large, lobed, parietal chloroplast (pyrenoid)	ND	ND	B-Type II PE	ND	Asexual (fission)
Corynoplastis	Mucilage	Spherical	18–33	Single or multiple multi-lobed chloroplast(s), (pyrenoid)	ND	ND	ND	ND	ND
Rufusia	ND	Pseudofilaments with spherical/ elliptical cells	(5.5–15) × (3.5–10)	Reddish-violet, parietal, discoid to band-shaped chloroplasts (no pyrenoid)	ND	ND	ND	ND	Asexual (endospores)
Chroodactylon	Gelatinous	Pseudofilaments with globose/ elliptical cells	(3–16) × (6–20)	Blue-green stellate, axial chloroplast (pyrenoid)	ND	ND	ND	ND	Asexual (monospores and fragmentation)
Chroothece	Gelatinous	Ellipsoidal/cylindrical	(20–30) × (30–45)	Axial, stellate, blue-green to yellow-brown or orange chloroplast (pyrenoid)	ND	ND	ND	ND	Asexual (cell division)
Stylonema	Mucilage	Pseudofilaments	ND	Single stellate chloroplast (pyrenoid)	ND	ND	B-PE	ND	Asexual (archeospores)
Empselium	Mucilage	Pseudofilaments with uniseriately and/or multiseriately arranged cells	ND	Single, band-shaped, parietal chloroplast (pyrenoid)	ND	ND	ND	ND	unknown
Goniotrichiopsis	Mucilage	Pseudofilaments	ND	Several parietal, disk-shaped chloroplasts (no pyrenoid)	ND	ND	B-PE	ND	Asexual (archeospores)
Neevea	Mucilage	Pseudofilaments	ND	Several parietal, disk-shaped or irregular chloroplasts (no pyrenoid)	ND	ND	ND	ND	Asexual (archeospores)

(*continued on next page*)

Table 3 (*continued*)

Genus	Nature of matrix	Form	Size (μm)	Chloroplast	Mitochondrion	Vacuole	Detected Pigments	Storage products	Sexual/asexual reproduction
Purpureofilum	Clear matrix	Uniseriate and multiseriate filaments	10–25	Single parietal multilobed, liver- to purple-coloured chloroplast (no pyrenoid)	ND	ND	ND	ND	Asexual (monospores)
Rhodaphanes	ND	Uniseriate and/or multiseriate, clavate	ND	One greyish-ruby to cinnamon coloured multi-lobed chloroplast (pyrenoid)	ND	ND	ND	ND	Asexual (archeospores)
Rhodosorus	Clear matrix	ND	4–9	Multi-lobed parietal plastid (pyrenoid)	ND	ND	ND	Floridean starch	Asexual (cell division)
Rhodospora	Gelatinous	ND	18	Parietal, discoid, red-violet to olive-green/yellow chloroplasts	ND	ND	ND	Floridean starch	Asexual (cell division)
Tsunamia	ND	Filaments with apical cells	ND	Single, peripheral, purple to pink, multi-lobed chloroplast	ND	Central vacuole	ND	ND	ND

Abbreviations: ND-Not Described, NR-Not Reported

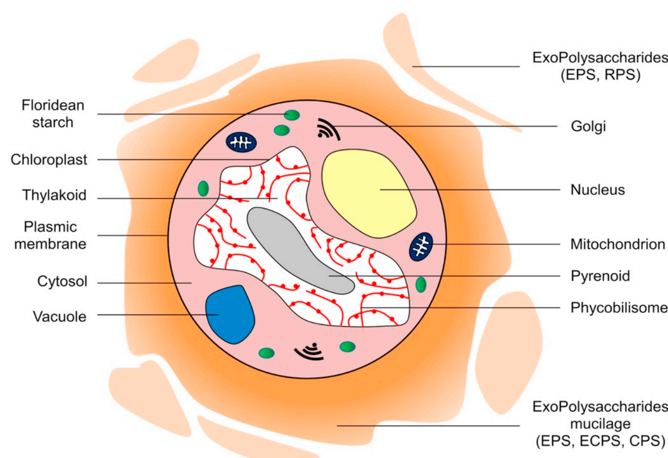

Fig. 1. A diagram of the ultrastructure of red microalgae.

constitute a relevant approach to appreciate the divergence and flexibility of metabolism pathways in red microalgae, related to environmental conditions. The comparative analysis between the genome sequence of *C. merolae* and a large EST collection available for *G. sulphuraria* showed that > 30% of the *Galdieria* sequences are specific to this organism, without equivalence with related genes in *Cyanidioschyzon* species (Barbier et al., 2005). In addition, *G. sulphuraria* is able to grow photoautotrophycally, heterotrophycally and mixotrophically whereas *C. merolae* is restricted to photoautotrophycally growth. This metabolic versatility of *G. sulphuraria* seemed due to a limited set of genes encoding for appropriate carbohydrate transporters required for the internalization of extern carbon sources (Barbier et al., 2005; Lee et al., 2017). However, Moriyama et al. (2015) demonstrated the ability of *C. merolae* to grow heterotrophycally in glycerol-containing medium, without the requirement of aquaporin-type glycerol permease. Carbohydrate metabolism is divergent within red microalgae, as the storage glucans of *C. merolae* is semi-amylopectin-type starch while other species (e.g. *G. sulphuraria*, *Cyanidum caldarium*) accumulate glycogen-type glucans or both semi-amylopectin and amylose-type polysaccharides (Hirabaru et al., 2010). Candidate genes involved in glucan anabolism and catabolism, including genes encoding for UDP-Glc: glycogenin glucosyltransferase, glycogen synthase, α and β-amylases, glycogen phosphorylase and heteroglucan degradation enzymes (α-gucosidase, mannosiadase, galactosidase), were identified in the two cyanidales genomes (*C. merolae and G. sulphuraria*) (Barbier et al., 2005). Some of the genes, such as those encoding for amylopullulanase and glucan-(1,4)-α-glucosidase were only detected in *G. sulphuraria*, indicating a larger glucan metabolism in *Galdieria* species compared to *Cyanidioschyzon* ones. The relative simplicity, compared to green algae, of starch metabolism in *P. purpureum* appeared relatively singular, with an unusually enzyme network encoding by only 19 genes (Bhattacharya et al., 2013). In it, critical biochemical steps are represented by single enzymes, such as a starch synthase (GT5), displaying potentially several properties related to the primary polysaccharide synthesis and the elongation of different chains present on amylopectin or a unique isoamylase gene not shared with other red algae. In addition, the presence of amylose is a unique feature in Porphyridiales compared to other Rhodophyta and has been correlated with the identification of a α-glucans synthase in *P. purpureum* genome. The analysis of *C. merolae* genome revealed the sole detection of mannosyltransferases and glucosyltransferases genes putatively involved in the mucilage layer synthesis and then explained the absence of typical cell wall. Note that some strains such as *G. sulphuraria*, having a more complex mucilage layer, have some genes encoding for additional enzymes (e.g. fucosyltransferases, galactosyltransferases, xylanases) in their genomes (Barbier et al., 2005).

Floridoside, an osmolyte, is one of the major photosynthetic

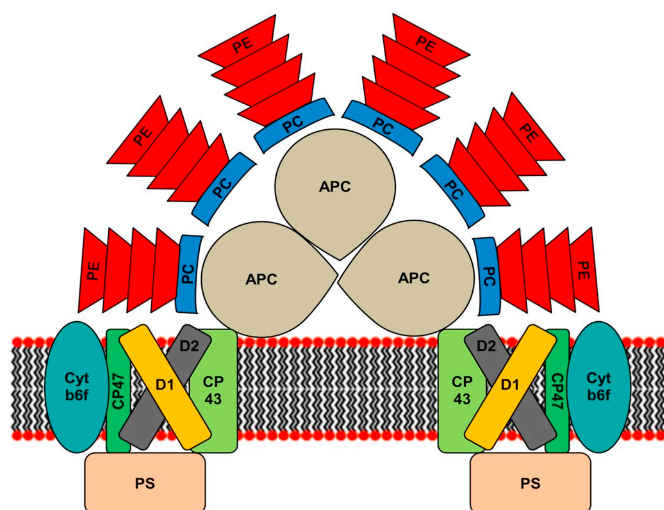

Fig. 2. Structural and energetic funnel-models of phycobilisome. PE: phycoerythrin; PC: phycocyanin; APC: allophycocyanin; D1 and D2: proteins related to chlorophyll; cyt: cytochrome (Wiethaus et al., 2010).

products of red microalgae (Bean and Hassid, 1955; Li et al., 2001). Although its occurrence in *Cyanidioschyzon* species was controversial (Nagashima and Fukuda, 1983; De Luca and Moretti, 1983), the comparative genomic highlighted several candidate genes, among which those encoding for Suc-P synthase and trehalose-P synthase could be implicated in floridoside biochemical pathways, both in *G. sulphuraria* and *C. merolae* (Barbier et al., 2005; Pade et al., 2015). The genomic comparative approach led also to identify potential genes involved in the metabolism of the trehalose playing a role in osmotic stress protection (Goddijn and van Dun, 1999). The genome of the Cyanidiales, *G. sulphuraria* and *C. merolae*, encode all enzymes required for trehalose synthesis (trehalose-P synthase, trehalose-P, trehalose synthase) and for trehalose breackdown (trehalase) (Barbier et al., 2005). Moreover, the absence of genes related to sucrose catabolism and the characterization of four genes encoding putative sucrose transporters in *P. purpureum* genome suggested its ability to exploit this disaccharide as an osmotically active solute (Bhattacharya et al., 2013).

Moreover, the inventory of genes encoding for subunits of photosystem I and phycobilisomes in *G. sulphuraria* and *P. purpureum* genomes has improved the understanding and the knowledges relative to photosynthetic apparatus of these red microalgae (Bhattacharya et al., 2013; Vanselow et al., 2009).

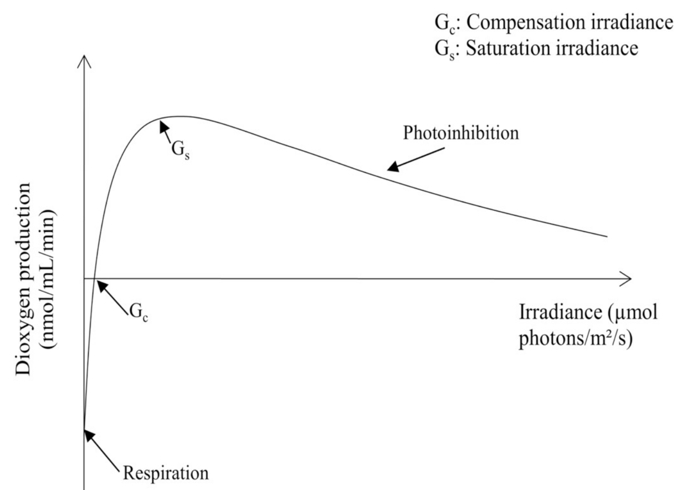

Fig. 3. Effect of light intensity on specific dioxygen production (and then growth rates) of microalgae (Delattre et al., 2016).

3. Production of biomass

3.1. Parameters determining the growth of microalgae

Parameters affecting growth of microalgae can be divided into two main categories which are linked to the culture medium (nutrients) and the operating conditions (light, temperature, pH).

3.1.1. Light

Light energy is an important factor catalyzing photosynthesis in microalgae. It significantly affects their growth kinetics, but this holds true up to a certain threshold. Indeed, many researchers of planktonic red algae have shown that the rate of growth would increase significantly with the increase in light intensity (Cunningham et al., 1989; Dermoun et al., 1992; Gaignard et al., 2018; Levy and Gantt, 1988; Soanen et al., 2016; Villay et al., 2013). However, beyond a threshold value, called the saturation point (Gs), photoinhibition is observed, with cells remaining viable but no longer multiplying (Evans and Maureen, 1974; Ritz et al., 2000; Vonshak and Torzillo, 2004). This threshold value is specific to each species of microalgae. In the dark, respiration occurs, with consumption of intracellular storage sugars. While increasing irradiance, the compensation point (Gc) corresponds to a specific light flux for which dioxygen production by photosynthesis exactly compensate dioxygen consumption by respiration. Between Gc

Table 4
Structural features of mitochondrial, plastid and nuclear genomes of *C. merolae*, *G. sulphuraria* and *P. purpureum*.

	C. merolae	*G. sulphuraria*	*P. purpureum*	References
Nucleus				Matsuzaky et al., 2004; Schönknecht et al., 2013; Bhattacharya et al., 2013
Genome size (Mbp)	16.5	13.7	19.7	
No of chromosomes	20.0	02.0	ND	
No of genes	5335	ND	8355	
Gene containing intron (%)	0.5	50.0	2.8	
GC content (%)	55	ND	ND	
Plastid				Jain et al., 2015; Tajima et al., 2014; Otha et al., 2003
Genome size (kbp)	149.9	167.7	217.6	
No of genes	207.0	ND	224.0	
No of introns	0	0	43	
GC content (%)	37.6	28.5	30.3	
Mitochondrion				Jain et al., 2015; Otha et al., 1998
Genome size (kbp)	32.221	21.428	ND	
No of genes	72.0	28.0	ND	
No of introns	0	0	ND	
GC content (%)	27.1	43.09	ND	

ND: No Data.

and Gs, a light limiting condition occurs, with growth rate linearly increasing with light flux. Therefore, a production of microalgae can be described through the respiration point, the compensation point, and the optimum irradiance corresponding to Gs (Fig. 3). Such a curve can be obtained experimentally by measuring dioxygen production as function of irradiance. For *Porphyridium marinum*, *Rhodella violacea* and *Flintiella sanguinaria*, values of Gs have been respectively determined at 360, 420 and 280 μmol photons.m^{-2}.s^{-1}, confirming differences between species (Gaignard et al., 2018; Soanen et al., 2016; Villay et al., 2013). Nevertheless, these measurements reflect only what happens at the level of "one cell", and phenomena observed at a culture level are quite different. When culturing microalgae in a PBR with artificial light (for which environmental parameters are easily controlled), it is possible to have a predictive approach allowing the maintainance of optimal conditions. More than the incident light flux (q0), the "illuminated volume fraction" (noted γ), is a useful parameter to explain growth kinetics inside PBR. This parameter can be directly deduced by the irradiance distribution as obtained from the radiative transfer model (Cornet et al., 1992; Cornet and Dussap, 2009; Degrenne et al., 2010; Pruvost et al., 2012; Takache et al., 2010), considering the geometry of the reactor. Briefly, the culture volume can be delimited into an illuminated and a dark zone with partitioning driven by the compensation irradiance value (G_C). For eukaryotic cells, a dark zone in the culture volume where respiration is predominant will result in lowering the kinetic rates. Maximum productivity will then require working in the exact condition $\gamma = 1$. These theoretical conditions have been proved experimentally for both Cyanobacteria (Cornet, 2010; Cornet and Dussap, 2009) and *Chlamydomonas reinhardtii* (Takache et al., 2010). At the beginning of a culture, the condition $\gamma > 1$ is usually encountered leading to a loss of efficiency. This is explained by light transmission, which prevents the full exploitation of the light energy received. Using the value of Gs as q0 will prevent this situation with a condition for which γ is close to 1. Nevertheless, due to the increase in biomass, the γ value will decrease progressively to a value below 1 and the increase in the dark volume will then progressively lower the mean volumetric growth rate. Increasing progressively incident light flux will allow maintaining γ close to 1 and then achieve better biomass productivity. Then the condition for which productivity is at its maximum would be maintained as long as there is no other limitation (nutrient…) occurring and a linear growth phase is observed (Muller-Feuga et al., 2003; Pruvost et al., 2012). For solar illumination, such predictions can also be made but difficulties are increased by the strong diurnal/seasonal variations (Pruvost et al., 2012).

Even if some microalgae can tolerate high light intensities (De-Bashan et al., 2008), red microalgae have deep habitat and have low tolerance to high irradiances (Richardson et al., 1983). For example Fuentes et al. (1999) have observed an inhibition of photosynthetic activity of *P. cruentum* from 2000 μmol photons.m^{-2}.s^{-1} in outdoor cultivation.

In general, and at high luminous intensities, microalgae accumulate carbohydrates, used as energy storage molecules during the respiration phase of photosynthesis. For example, Villay et al. (2013) studied the production of EPS in *Rhodella violacea* at different light intensities. The best production was at an irradiance corresponding to the Gs for this strain. Same observations were made for *P. marinum* (Soanen et al., 2016) and *Flintiella sanguinaria* (Gaignard et al., 2018).

The lighting cycle also has an impact on photosynthetic activity and therefore on the metabolism of microalgae. In fact, red microalgae usually grow under a photoperiod (day/night, hours) of 24/0 h, 16/8 h and 12/12 h and under light intensities between 27 and 500 μmol photons.m^{-2}.s^{-1}. Nevertheless, a relation exists between the duration of illumination and the light intensity applied. It seems that microalgae could be exposed to a minimal photon rate per day to have an optimal growth during a long photoperiod. As an example, the study conducted by Oh et al. (2009) on a culture of *P. cruentum* at low irradiance (10 to 25 μmol photons.m^{-2}.s^{-1}) showed that higher biomass concentrations were obtained with 12/12 h and 18/6 h light/dark periods compared with those of 8/16 h and 0/24 h. Another study carried out by Meseck et al. (2005), showed that higher light intensities and longer photoperiods resulted in higher biomass production in less time. Globally, if the light flux applied is lower than the Gs, increasing photoperiod will have a positive effect, while if the light flux is higher, decreasing the illumination duration will drop the photoinhibition impact. Furthermore, the light quality also has an impact on the growth and the pigment composition of red microalgae. Red (600–700 nm) and blue (400–500 nm) lights were used by You and Barnett (2004) to increase cells growth of *P. cruentum*. No significant difference in growth rates or EPS production was observed for blue and red lights, but growth rates were better than for white light.

Quantitative and qualitative photosynthetic pigments contents vary with irradiance, especially for the microalgae possessing a phycobilisome (Cyanobacteria and red microalgae). For these microalgae, a significant decrease of PE was observed in the presence of red light (600–700 nm) and for high irradiances (Akimoto et al., 2012; Marquardt and Rehm, 1995; Rahaoui, 1999), while PC and APC contents increased. This phenomenon suggests an adaptation of the photosynthetic system to the environmental conditions of culture. According to Mihova et al. (1996), the amount of phycobiliproteins in *R. reticulata* was equivalent under white and green lights at three light intensities each. However, when *R. reticulata* was grown under a red light and at low light intensity (18 μmol photons.m^{-2}.s^{-1}), the total supernumerary pigment content increased from 11.35 to 14.5% (w/w). For this microalga the production of biomass was at its maximum for a red light and at high luminous intensity of 215 μmol photons.m^{-2}.s^{-1}. Dupré et al. (1994) confirmed that cells of *Rhodosorus marinus*, as other red algae (Brody and Emerson, 1959; Levy and Gantt, 1988) can adapt their phycobiliprotein content to the amount of incident light. In cells cultured at low irradiances (50 μmol photons.m^{-2}.s^{-1}) PE may account for > 50% of total proteins and is up to twice greater than values obtained at higher irradiance.

3.1.2. Carbon

The atmospheric CO_2 is the main source of carbon since the majority of planktonic algae are autotrophic microorganisms using inorganic carbon through photosynthesis (Masojídek et al., 2013; Rubio et al., 2003; Zaslavskaia et al., 2001). Direct CO_2 sparging into the culture media could then be an efficient method to supply CO_2 for high yield of biomass and control the pH at the same time. At the cellular level, CO_2 can be uptaken by cells through active transport as well as passive diffusion (Amoroso et al., 1998; Badger and Price, 2003; Giordano et al., 2005a, 2005b). Microalgae can also generate CO_2 from bicarbonate (HCO_3^-) by excreting H^+ across their cell membranes, which react with HCO_3^- to yield CO_2 and H_2O. HCO_3^- can also be uptaken by some microalgae, but this requires a transporter system or its prior conversion to CO_2 (Giordano et al., 2005a, 2005b). Evidence for HCO_3^- transport has been demonstrated for some red microalgae such as *P. cruentum* (Colman and Gehl, 1983). Once intracellularly located, HCO_3^- can be interconverted into CO_2 by carbonic anhydrases (CAs). The presence of CA(s) has been found in all of the microalgae from both freshwater and marine environments including Rhodophyceae (Aizawa and Miyachi, 1986). One function of CA is to facilitate the diffusion of CO_2 across the plasma membrane modifying equilibrium between intra- and extra-cellular concentrations. Moreover, as HCO_3^- cannot passively diffuse through cellular membrane, CA allows preventing the leakage of carbon back into the medium. In some microalgae, external CAs are constitutive whereas in others it is induced by absent or low CO_2 levels. The known CAs can be grouped into four types designated the α-, β-, γ-, and ε-CA families (Hewett-Emmett and Tashian, 1996; So et al., 2004). Microalgae and most higher plants exhibit CAs of the β family but this type of CAs has also been identified in archaebacteria (Smith et al., 1999) or yeasts (Götz et al., 1999). All β-

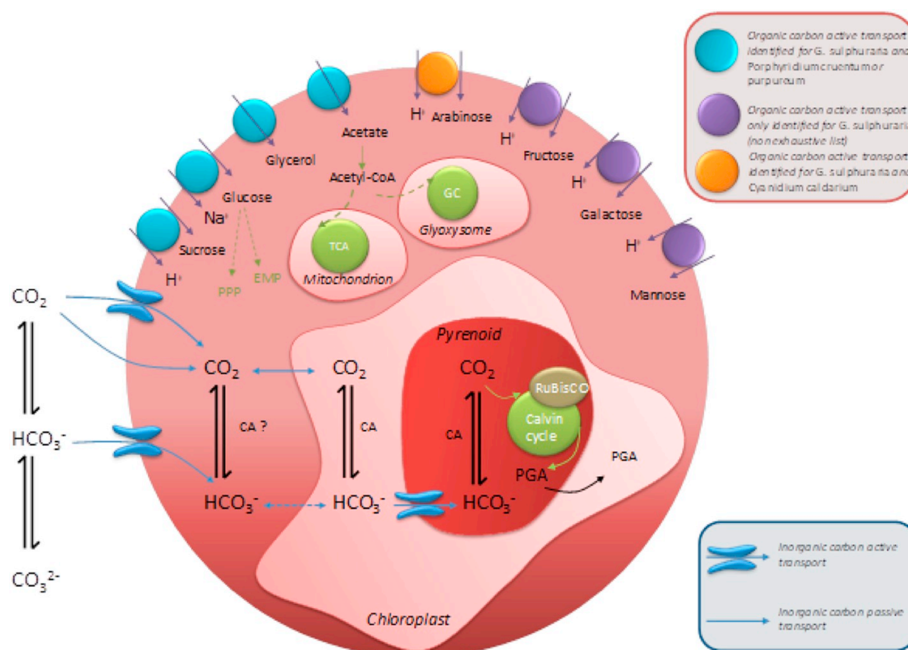

Fig. 4. Overview of organic and inorganic carbon uptake in red microalgae. CA: Carbonic anhydrase; PGA: 3-Phosphoglycerate; TCA: Tricarboxylic Acid pathway; GC: Glyoxylate cycle; EMP: Embden–Meyerhof Pathway; PPP: Pentose Phosphate pathway. Dashed lines: putative pathways.

CAs have a histidine and two cysteine residues that act as zinc ligands (Bracey et al., 1994; Rowlett et al., 1994), and are composed of monomers of about 25 kDa. The β-CA from *P. purpureum* contains two active sites per polypeptide by contrast to the unique site described in higher plants suggesting a gene duplication event (Mitsuhashi and Miyachi, 1996; Mitsuhashi et al., 2000).

Despite its essential part in carbon fixation ability of photosynthetic organisms, RuBisCO remains an unusualy slow enzyme with a low affinity for CO_2. This originates from the fact that O_2 is a competitive substrate of CO_2 in carboxylation and oxygenation reactions (Portis and Parry, 2007). At atmospheric concentrations of CO_2 RuBisCO only functions at about one fourth of its catalytic capacity (Moroney et al., 2007). The existence of this competition causes the redirection of fixed carbon into the photorespiratory cycle leading to the loss of at least 30% of carbon fixed by RuBisCO (Raines, 2011). As a result, many photosynthetic organisms, from Cyanobacteria to higher plants, have developed methods for accumulation of inorganic carbon (Ci) to support RuBisCO in efficient CO_2 capture and CA play important roles in this process. Most researches on eukaryotic CCM have been done using green algae but are not limited to them (Colman et al., 2002; Raven et al., 2002). The role of this CCM is to increase CO_2 level around RuBisCO to alter the CO_2/O_2 ratio at the active site in favor of the carboxylase reaction, thereby achieving better photosynthesis efficiency even at low CO_2 concentrations. Generally, CCM comprises of the following components (Badger, 1987): (i) a transport of Ci species; (ii) a compartment separating RuBisCO from the reactions accumulating the intermediate Ci pool. For algae it appears to be the pyrenoid; (iii) a mechanism for release of CO_2 in the compartment where RuBisCO is localized. This role is played by CA; (iv) a mechanism to prevent CO_2 efflux away from the site of RuBisCO to ensure efficient CO_2 fixation. This can be the starch sheath of the pyrenoid in the case of microalgae. Fig. 4 presents an overview of Ci uptake in red microalgae.

Some microalgae are able to grow heterotrophically in the absence of light (Hu et al., 2018; Liang et al., 2009; Sloth et al., 2017; Xie et al., 2017). These microorganisms possess a 'dark metabolism' similar to that of nonphotosynthetic of eukaryotic organisms. Heterotrophic cultivation of microalgae can be briefly summarized as follow (Morales-Sànchez et al., 2013, 2014): (i) no light requirement; (ii) cell densities, growth rates and storage compounds productivities are increased (Minhas et al., 2016); (iii) classical bacterial reactors are easily operated and maintained under axenic conditions; (iv) cultures can be conducted in fedbatch or continuous modes; (v) cost for biomass harvesting is reduced due to the high cell concentration that can be reached; (vi) air can be used instead of sparging CO_2. However, this mode is not very widespread for production on an industrial scale for several reasons; (i) all microalgae are not able to grow without light source; (ii) the carbon source present can be assimilated by other microorganisms such as bacteria so a strict control of axenicity is needed; (iii) in some cases, the absence of light harms the synthesis of photosynthetic pigments, co-valorization of pigments being therefore not possible; (iv) the production cost could be high. Even if culture systems are classical fermenters, the carbon source expense is often higher than the energy gain due to the absence of light. This is especially true because most autotrophic industrial cultures take place outdoors and free themselves from artificial light sources and privilege the sun. Nevertheless, for a comparable volume of cultures, the spaces needed to install reactors are lower and downstream processes (harvesting) are cheaper due to the high biomass concentration that can be reached.

Mixotrophy is a mode of cultivation, where heterotrophic and autotrophic modes act simultaneously, leading to the utilization of inorganic and organic carbon in the presence of light. In mixotrophic mode, light is not a stringent requirement for growth (Perez-Garcia and Bashan, 2015). The presence of two sources of energy (organic carbon and light), gives microalgae more flexibility, due to complementarity between photoautotrophy and growth on organic carbon, and can help in achieving high growth rates and biomass productivity (Yang et al., 2000). Apart from this, CO_2 released in respiration can be utilized for autotrophic growth, thus making mixotrophic cultivation more efficient than auto- or heterotrophic mode. Mixotrophy also reduces night time biomass lost, as microalgae are not solely dependent on stored carbohydrate for catabolism. In mixotrophy, light not being an obligate requirement for growth, makes photolimitation or photoinhibition less effective in hampering microalgal growth (Chojnacka and Noworyta, 2004; Wang et al., 2014).

Most mixotrophic and heterotrophic studies have been conducted on green microalgae (Cecchin et al., 2018; El-Sheekh et al., 2012;

Poddar et al., 2018). Data available for the red microalgae concern *Galdieria sulphuraria* cultured under mixotrophic conditions with continuous light of 50 μmol photons.m^{-2}.s^{-1} (Fukuda et al., 2018) and *Porphyridium cruentum* grown in mixotrophy under 12 h:12 h light/dark with a light intensity of 234 μmol photons.m^{-2}.s^{-1}. Under these conditions the productivities of PE and EPS increased (Fàbregas et al., 1999a). Based on these observations, it is necessary to study for each microalga the light conditions required to achieve optimal mixotrophy growth and metabolites production. The ability of Rhodophyta to grow on organic carbon sources has been extensively studied for members of Cyanidiaceae family. Cyanidiaceae is a family of evolutionarily conserved, unicellular acidophilic red microalgae, living in hot and acidic environments. It has been considered as the oldest member of the red microalgae lineage (Schilling and Oesterhelt, 2007; Yoon et al., 2002, 2004). Within this group *G. sulphuraria* and *Cyanidium caldarum* are able to grow photoautotrophically, heterotrophically and mixotrophically (Rigano et al., 1977), whereas *Cyanidioschyzon merolae* seems to be an obligate photoautotroph (Raven et al., 1990). *Galdieria sulphuraria* can metabolize > 27 different sugars or polyols, including disaccharides, hexoses, pentoses, deoxysugars, hexitols, pentols as well as aminoacids, TCA cycle intermediates and some organic acids (Gross, 1999; Gross and Schnarrenberger, 1995; Oesterhelt et al., 1999; Rigano et al., 1976, 1977; Schilling and Oesterhelt, 2007). It is to noteworthy that for other microalgae, all carbon sources will not lead to the same growth rates. Among carbon sources tested for several microalgae strains, glucose generally induce a greater growth rate and biomass productivity than acetate at the same concentration (Bouarab et al., 2004; Liang et al., 2009), probably due to differences in substrate uptake and assimilation kinetics. It seems that there is no apparent preference of this microalga for a special group of sugars or sugar alcohols. For example, both D- and L-configurations as well as pentoses or hexoses gave similar growth rates (Gross and Schnarrenberger, 1995; Oesterhelt et al., 1999; Schilling and Oesterhelt, 2007). This ability to grow heterotrophically can lead to very high biomass concentrations. As an example, it was possible to reach > 100 g.L^{-1} dry weight (DW) biomass of *G. sulphuraria* using glucose and sugar beet molasses with a fed-batch approach, with growth rate about 1.44 day^{-1} (Schmidt et al., 2005). Nevertheless, for high concentrations (200 or 300 g.L^{-1} glucose) the specific growth rate was reduced and growth was completely inhibited with 400–500 g.L^{-1} sugars.

Galdieria has then developed efficient uptake and assimilation systems for organic carbon, probably to survive periods of light limitation. The enzymes used for metabolizing these substrates seem to be constitutively expressed in these species (Oesterhelt et al., 1999) while for other microalgae an induction period is often necessary. *In vivo* studies have allowed the identification of at least 14 different sugar and polyol transporters with a pH dependence suggesting a proton symport transport mechanism (Schilling and Oesterhelt, 2007). Additionally, genomic analyses have shown the presence of putative glucose transporters, numerous symporters (at least four for galactose, four for fructose, one for arabinose and sucrose), and four putative glycerol permeases of the aquaporin type. In total, 28 distinct sugar transporter genes have been identified for *G. sulphuraria* (Barbier et al., 2005). Nevertheless, this feature seems quite unique among Rhodophytes (Raven et al., 1990). In the same Cyanidiaceae family, *Cyanidioschyzon merolae* is assumed to be an obligate photoautotrophic organism. Analysis of the *Cyanidioschyzon merolae* genome allowed identifying only a single putative arabinose transporter (Weber et al., 2004).

A large variety of sugar kinases are needed for the assimilation of these organic substrates. A genomic analysis of *G. sulphuraria* has found putative gluco-, galacto-, fructo- glycero-, xylulo-, and ribokinases, with closest similarity to prokaryotic enzymes than with the corresponding plant sugar kinases. Moreover, *G. sulphuraria* possesses many polyol dehydrogenases for introducing sugar alcohols into its heterotrophic metabolism (Barbier et al., 2005; Stein et al., 1997). Overall, *G. sulphuraria* and *C. merolae* exhibit similar enzymatic machinery for sugar and organic carbon metabolisms. The inability of *Cyanidioschyzon* to use sugars and polyols substrates can therefore be attributed to a deficiency in appropriate uptake system rather than a lack of essential enzymes. Nevertheless, this feature has been recently discussed as Moriyama et al. (2015) showed that *C. merolae* is able to grow mixotrophically on glycerol substrate whereas no glycerol uptake system has been identified at genomic level.

Concerning other Rhodophyceae, the ability to grow mixo- or heterotrophically is less detailed. It has been demonstrated that *P. purpureum* can be grown to high cell densities in complete darkness with glucose as the sole carbon source (Oh et al., 2009). In addition to the glucose transporter, four putative sucrose transporters were found in *P. purpureum* genome (Bhattacharya et al., 2013). It is thus possible that *P. purpureum* could also exploit disaccharides, such as sucrose, for heterotrophic growth. This ability has never been demonstrated by growth experiments. Furthermore, *P. cruentum* has been demonstrated to be able to grow using carbon sources such as glucose and acetate (Vazhappilly and Chen, 1998). The possibility of *P. cruentum* to develop mixotrophically on a boiled fraction of *Solanum tuberosum* flour has also been studied (Fàbregas et al., 1999a). In fact, the cell density of *P. cruentum* increased from 4.06 × 10^{6} cells.mL^{-1} under autotrophic conditions to 5.01 × 10^{6} cells.mL^{-1} under mixotrophic conditions. The ability of *Rhodella* to grow heterotrophically on acetate and glucose was also tested but no growth was noticed (Turner, 1970). Fig. 4 presents a non-exhaustive overview of organic carbon uptake in red microalgae.

Apart from reaching high cell densities, both mixotrophic and heterotrophic cultivations generally lead to strong reduction in pigment content. However, some strains of *Galdieria sulphuraria* retain their photosynthetic apparatus when grown in darkness (Gross and Schnarrenberger, 1995; Marquardt, 1998). In fed-batch cultures, PC concentrations of 300–400 mg.L^{-1} can be obtained, corresponding to cellular PC content of 5–10 mg.g^{-1} (Schmidt et al., 2005). This content is quite low in comparison to that of other photosynthetic microorganisms such as Cyanobacteria and notably *Arthrospira platensis*, which may contain up to 120–140 mg.g^{-1} of PC in mixotrophic cultures (Chen et al., 1996; Chen and Zhang, 1997; Marquez et al., 1993). Nevertheless, since very high concentrations of *G. sulphuria* biomass can be obtained, the final PC concentration and the productivity remain interesting. Moreover, light intensities between 0 and 100 μmol photons.m^{-2}.s^{-1} had no influence on PC accumulation in mixotrophic cultures grown on glucose or fructose, while light stimulated PC synthesis in cultures grown on glycerol. In these conditions, the PC content in stationary phase was increased to 20 mg.g^{-1} DW at a light intensity of 80 μmol photons.m^{-2}.s^{-1} (Sloth et al., 2006). Similar results have been obtained for *P. cruentum* mixotrophically cultivated on a boiled fraction of *Solanum tuberosum* flour, with PE and EPS concentrations increasing to 10 μg.mL^{-1} and 330 μg.mL^{-1} instead of 7 μg.mL^{-1} and 129 μg.mL^{-1} respectively, in autotrophic condition (Fàbregas et al., 1999a). Additionally, mixotrophic and heterotrophic cultivation modes can induce accumulation of interesting storage biomolecules. For example, Sakurai et al. (2016) have shown that the glycogen amount in *Galdieria sulphuraria* was significantly increased when grown on glucose, both in mixotrophic and heterotrophic conditions, while lipids content was increased only in heterotrophic condition, especially polyinsturated fatty acids that may be of industrial interest for food applications.

3.1.3. Nitrogen

After carbon, microalgae also require a nitrogen source for their growth, as it is used for the synthesis of chlorophyll and other photosynthetic pigments, amino acids, nucleic acids, and coenzymes (Hu, 2005; Raven and Giordano, 2016). Nitrogen can represent 1 to 10% of the dry biomass.

Nitrogen assimilation is predominantly performed by the glutamine synthetase (GS)–glutamate synthase (GOGAT) cycle, as demonstrated both in green and red algae (Al Amoudi, 1993; Suzuki and Knaff, 2005).

Nitrate is the most common dissolved form of nitrogen in seawater, but many microalgae prefer to use ammonium as a nutrient (Lourenço et al., 2002; Miller and Castenholtz, 2001). All microalgae cells are then assumed to uptake both ammonium and nitrate except *Galdieria sulphuraria* using only ammonium (Reeb and Bhattacharya, 2010). When using nitrates, microalgae need to reduce it back to ammonium, prior to the GS reaction. In higher plant and algal cells, nitrate anions are imported into the cell by specific transporters (Galvan and Fernández, 2001), and reduced to nitrite by NAD(P)H-dependent nitrate reductase (NR) in the cytosol. Nitrite is subsequently reduced to ammonium by ferredoxin-dependent nitrite reductase (NiR) in plastids. Even if the metabolic pathway seems to be the same for all red microalgae, some differences exist. For example, the NR of *Porphyridium aerugineum* differs from the NR of *Cyanidium caldarium* by functioning only with nicotinamide dinucleotide (NADH), whereas the enzyme of *Cyanidium* is functional with both NADH and nicotinamide dinucleotide phosphate (NADPH) (Rigano, 1971). Moreover, the enzyme of *P. aerugineum* is more sensitive to temperature than the enzyme of *Cyanidium* (Rigano and Violante, 1972).

The metabolism of microalgae varies, depending on nitrogen availability and nitrogen source (Gigova and Ivanova, 2015). Differences in growth rate and biochemical composition of *P. cruentum* depending on nitrogen forms are attributed to the fact that microalgae prefer ammonium over nitrate to conserve the energy of the eight electrons that are needed to reduce nitrate (Miller and Castenholtz, 2001; South and Whittick, 1987). Also, concentrations of ammonium over 0.5 to 1.0 μM can inhibit nitrate absorption (Darley, 1987) by repressing NR.

Assimilation of ammonium ions could induce a significant decrease of pH, due to the release of H^+, whereas the use of nitrates induces an increase of pH. This should be considered while conducting microalgae culture in artificial media, in the absence of pH regulation systems such as open ponds. As CO_2 injection into culture induces a decrease in pH, nitrates are often preferred to mitigate the process. Moreover, for alkaline pH, ammonium ions can be interconverted into ammonia (NH_3) with pKa of equilibrium at 9.25 (at 25 °C). As NH_3 is volatile, it can be gassed-off (especially in open ponds), leading to less nitrogen availability for the cells and potential deprivation of media. Another negative consequence of the NH_3 formation is its toxicity. While $NH_4{}^+$ needs a transporter of the ammonium transporter family to pass through cellular membranes, NH_3 diffuses passively (driven by ΔpH), accumulating inside cells (Drath et al., 2008). Moreover, it has been demonstrated for several Cyanobacteria and some microalgae (no data for red microalgae) that high level of NH_3 could induce severe damage on photosynthetic apparatus (Abeliovitch and Azov, 1976; Dai et al., 2014; Markou and Muylaert, 2016; Oyala et al., 2015; Pérez et al., 2013). To avoid this problem, fed-batch strategies can be applied (Carvalho et al., 2004; Converti et al., 2006). Depending on species, other nitrogen sources can be used. Among red microalgae, it has only been evidenced for *Cyanidum caldarium*. In *C. caldarium*, two transport systems for nitrite uptake have been identifieded. One system is linked to the fact that *Cyanidium caldarium* is encountered in very acidic environments, and it corresponds to a passive diffusion of HNO_2 across the cell membranes (Fuggi, 1993). In conditions of higher pH (pH > 5), the assimilation rate of nitrite was comparable to that of nitrate while nitrate was a strong competitive inhibitor (Fuggi, 1989, 1990). It has been proposed that nitrate uptake system can take up both nitrate and nitrite, nitrate having a higher affinity than nitrite.

Some microalgae are also able to use urea (Ellner and Steers, 1955; Williams and Hodson, 1977). Among red microalgae, Naylor (1970) highlighted the lack of urease in *Porphyridium* cells. Nevertheless, further studies have shown that *Porphyridium* cells are able to use urea, but need a significant adaptation time (Naval et al., 1977). Uptake and assimilation mechanism have not yet been demonstrated for red microalgae.

Finally, it has also been shown that *Cyanidium caldarium* was able to directly use glutamate as nitrogen source but uptake mechanism has not been resolved (Rigano, 1971). The different possible uptake and assimilation mechanisms of nitrogen by red microalgae are proposed on Fig. 5.

Assimilation of nitrogen is strongly linked to carbon metabolism. In the presence of nitrogen, the Ci flux is directed toward glycolysis (to the detriment of the synthesis of sucrose, starch or fat) to allow the production of precursors of amino acids (Morot-Gaudry, 1997). Therefore, when the culture medium of microalgae is deficient in nitrogen, the growth of microalgae decreases. They enter in the stationary phase and accumulate lipids (triacylglycerol) and/or carbohydrates (Da Silva et al., 2009; Ho et al., 2013; Li et al., 2011a). This phenomenon induces an increase in the cell volume (Da Silva et al., 2009; Lamers et al., 2012). For red microalgae, a nitrogen starvation could also induce synthesis of EPS, as shown for *Rhodella violacea* (Villay et al., 2013), *Porphyridium marinum* (Soanen et al., 2016) or *Flintiella sanguinaria* (Gaignard et al., 2018). Moreover, when growing on replete media, cells can store nitrogen in phycobiliproteins. Chaloub et al. (2015) demonstrated that the accumulation of phycobiliproteins was stimulated by a 25% increasing of nitrate concentration in the culture medium of *Rhodomonas* sp. Thus, nitrogen starvation decreases the synthesis of photosynthetic pigments, such as chlorophyll and phycobiliproteins. The decrease in pigments in a situation of nitrogen deficiency is not immediate because microalgae adapt their metabolism. The microalgae of the species *Rhodomonas* sp. in a situation of nitrogen deficiency have a content of Chl *a* constant while chlorophyll *c* (Chl *c*) strongly increases the first days following the deficiency and then decreases to return to normal. Once these microalgae are adapted to the conditions of nitrogen deficiency the concentrations in pigments Chl *a*, Chl *c* and PE decline (50% for the chlorophylls and 90% for the PE after 6 days of starvation). This phenomenon leads to a greening of the cultures of red microalgae (Da Silva et al., 2009). Indeed, nitrogen starvation results in the loss of PE color without loss of cell viability, as restoration of nitrogen source promotes repigmentation of the algae (Levy and Gantt, 1990). Even after 5 days under N-starvation, the 'greening' process of *Rhodosorus marinus* was reversible upon addition of nitrate or ammonium and 48 h were necessary to obtain 30% of PE recovery (Dupré et al., 1994).

3.1.4. Phosphorus and sulphur

Although it accounts for only 1% of the total algal biomass, phosphorus is also a major nutrient for microalgae growth. In fact, it participates in several cellular processes such as energy transfer (ATP) and synthesis of nucleic acids and phospholipids. It has been shown that RNA synthesis was the largest biochemical sink for phosphate, accounting for about half of the total phosphate uptake. Phospholipids synthesis accounted for about 20% of the phosphate uptake and 30% were for DNA and ATP (Van Mooy and Devol, 2008). Phosphorous is often one of the most important growth limiting factors in microalgal cultivation as it easily bound to other ions ($CO_3{}^{2-}$, Ca^{2+}, Fe^{2+}/ Fe^{3+}…), especially at high pH, resulting in its precipitation and consequently rendering this essential nutrient unavailable for algal uptake. Orthophosphate ($PO_4{}^{2-}$) is preferred overall for supplying algae and its uptake is controlled by transporters in the cell membrane. Genomic studies have demonstrated that eukaryotic algae typically have several phosphate transporters (Gobler et al., 2011; Read et al., 2013). Nevertheless, they have been rarely functionally characterized (Li et al., 2012, 2006).

Polyphosphate can be present as acid-soluble or acid-insoluble polyphosphate. Acid-soluble polyphosphate is actively involved in metabolism, while acid-insoluble polyphosphate is stored in polyphosphate bodies for when the external phosphate concentration becomes limiting (Miyachi et al., 1964). This storage mechanism is known as the luxury uptake. Luxury uptake of phosphorus is assumed to occur in all microalgae, including red ones (Bertilsson et al., 2003; Capone et al., 1997; Diaz et al., 2008; Gomez-Garcia et al., 2003; Mateo et al., 2006;

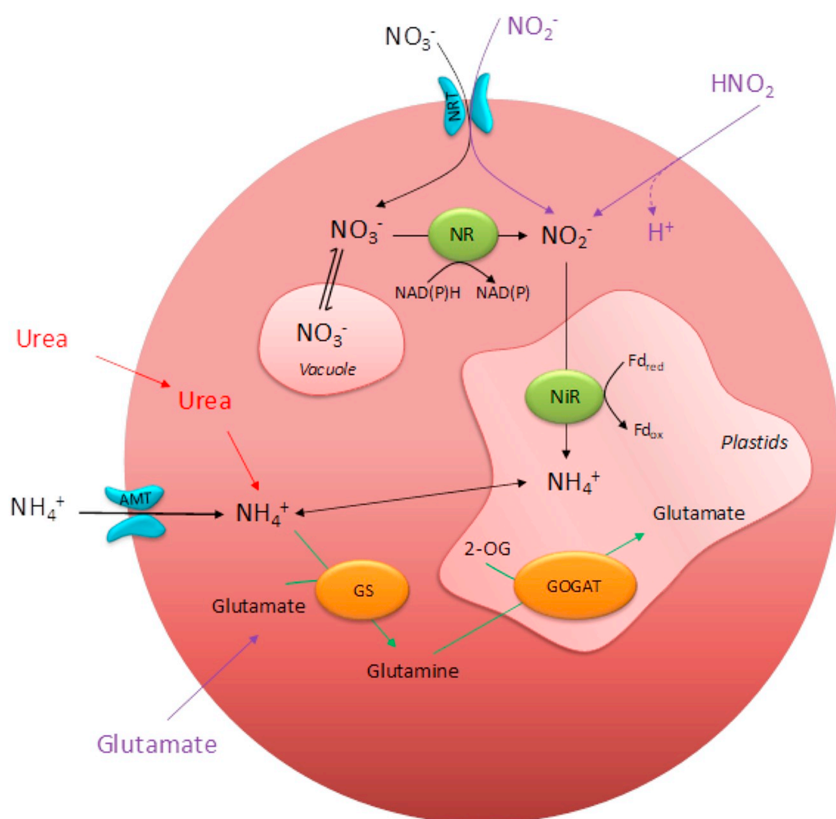

Fig. 5. Possible uptake and assimilation mechanisms of nitrogen by red microalgae. NR: Nitrate reductase; NiR: Nitrite reductase; GS: Glutamine synthetase; GOGAT: Glutamate synthase; AMT: Ammonium transporter; NRT: Nitrate transporter; Fd_{red}: Reduced ferredoxin; Fd_{ox}: Oxydized ferredoxin. In purple, uptake only evidenced for *C. caldarium*. In red, urea uptake only evidenced in *Porphyridium*, but not resolved.

Mazard et al., 2012; Nishikawa et al., 2006, 2009; Orchard et al., 2010; Romans et al., 1994; White et al., 2006).

Sulphur is also important for the development of microalgae cells. It enters in the composition of some amino acids, such as cysteine and methionine, but also in the formation of coenzyme A, involved in the energy metabolism. It is used by the photosynthetic organisms mainly in the form of sulphate (SO_4^{2-}). For Rhodophyta such as *Porphyridium* or *Rhodella* species, effect of sulphate depletion on EPS synthesis has been studied (Arad et al., 1988, 1992). Amounts of polymer released were similar to those for nitrogen deficiency but the level of sulphation was reduced to half in case of sulphate starvation (Arad et al., 1988). These authors suggested that for both nutrient starvations, protein synthesis was reduced, affecting growth and promoting EPS accumulation. Nevertheless, it has been observed that nitrate starvation immediately induced entry in stationary phase, whereas the sulphate depletion induced a delayed response with 2 or 3 generation times needed to stop growth.

3.1.5. Temperature

In addition to nutrients and light, the growth of microalgal cells also depends on temperature. Indeed, there are mesophilic microalgae which develop at temperatures between 20 and 25 °C, thermophilic microalgae which multiply at temperatures above 40 °C and psychrophilic microalgae which grow at temperatures below 17 °C. Each microalgae species is then characterized by an optimal growth temperature. Red microalgae such as *Porphyridium* or *Rhodella* species are mesophilic microalgae whose growth rates are maximum at a temperature around 20 °C. Beyond this value, the growth rate decreases (Ras et al., 2013). This value is strongly strain dependent, and according to photosynthetic activity measurements, optimal temperature of *Rhodella violacea* was 24 °C (Villay et al., 2013) as well as for *Flintiella sanguinaria* (Gaignard et al., 2018), whereas it has been found at 28 °C for *Porphyridium marinum* (Soanen et al., 2016). Due to their specific habitat in hot and acidic environments (pH 1.5–3, temperature 35–55 °C), Cyanidiaceae (*Galdieria*, *Cyanidium* and *Cyanidioschyzon* species) have a much higher optimal temperature of growth and are generally cultivated at temperature around 40 °C (Gardian et al., 2007; Sakurai et al., 2016; Yamaoka et al., 2011).

Besides growth rate, temperature stress also affects chemical composition of red microalgae and may limit nutrient interactions. Lee and Tan (1988) showed that at high temperatures, *P. cruentum* produced large amounts of lipids. However, the production of both PS and pigments was better at a lower temperature (25 °C). The same results were found with *R. reticulata* cultivated between 26 °C and 34 °C. At 34 °C the fatty acids and proteins productivities were higher than those obtained with microalgae cultivated at a lower temperature.

3.2. Technologies for production of red microalgae biomass

The interest in microalgae has increased for many years because of high-value compounds produced by these microorganisms. To improve their industrial exploitation different types of technology were developed for large scale culture (Table 5). These systems were based on the same method: *i.e.* (i) capture of light (artificial or sunlight); (ii) agitation of culture (mechanistic and/or air lift); (iii) carbon application (organic-inorganic carbon and/or gas) for culture and (iv) downstream process (biomass harvesting and purification of the different interest compounds). Culture methods have evolved significantly over the past forty years. At the beginning microalgae were cultivated in open ponds in batch mode and semi-controlled conditions mainly to produce extracts (aquaculture food or health food supplements). Since the production techniques have evolved, photosynthetic microorganisms such as red microalgae can be produced today in continuous and controlled modes in closed systems as PBR, in photoautotrophy or mixotrophy.

3.2.1. Open systems

Open ponds were the first systems developed for industrial microalgae culture. Even now it is the most used system to produce microalgae due to its facility of use and its low cost (Fig. 6). It represents a large part of tools exploited for the global production of microalgae as

Table 5
Tools and culture conditions for red microalgae production.

Microalgae	Bioreactor	Materials	Volume (L)	Dimensions (cm)[a]	Light	Incident light flux (µmolphoton.m^2.s^{-1})	CO_2 (%)	pH	Temperatures (°C)	Medium	References
Porphyridium sp.	Open pond	Polyethylene	25	12.5 (D)	Natural	4–250	ND	ND	9–30	Artificial SeaWater [Jones et al., 1963]	Cohen and Arad (1989)
Porphyridium sp.	Sleeve/Vertical	Polyethylene	25	150 (L) 32 (W)	Natural	7–420	3–4	7–8	11–35	Artificial SeaWater [Jones et al., 1963]	Cohen and Arad (1989)
Porphyridium sp.	Flat-plate/Vertical	Glass	3–72	1.3–30 (T)	Natural	ND	2	7.5	6–27	Modified Artificial SeaWater [Jones et al., 1963]	Singh et al. (2000)
Porphyridium sp.	Bubble column/ Vertical	Polyethylene	19	150 (L) 13 (Dia)	Artificial	150–400	3	ND	23–25	Artificial SeaWater [Jones et al., 1963]	Merchuk et al. (2000)
Porphyridium sp.	Bubble column/ Vertical	Polyethylene	15	150 (L) 13 (Dia)	Artificial	26–1850	3	7.6	31	Artificial SeaWater [Jones et al., 1963]	Luo and Al-Dahhan (2011)
P. aerugineum	Open pond	Polyethylene	25	12.5 (D)	Natural	4–250	ND	ND	9–30	MCYII	Cohen and Arad (1989)
P. aerugineum	Sleeve/Vertical	Polyethylene	25	150 (L) 32 (W)	Natural	7–420	3–4	7–8	11–35	MCYII	Cohen and Arad (1989)
P. cruentum	Flat-plate/Vertical ("V" shape)	Horticultural glass	2	36 (L) 33 (W)	Artificial	75	2.5	7.6	ND	Artificial SeaWater [Jones et al., 1963]	Iqbal et al. (1993)
P. cruentum	Serpentin tubular/ Horizontal	Polymethyl methacrylate	220	9880 (L) 5 (Dia)	Natural	0–4000	ND	7.6	20	Modified Hemerick with SeaWater	Fuentes et al. (1999)
P. cruentum	Serpentin annular tubular/Vertical	Stainless steel	5	1200 (L) 10 (Dia)	Artificial	175–236	ND	ND	25	Enriched Hemerick with Artificial SeaWater	Muller-Feuga et al. (2003)
P. cruentum	Cylindrical/ Vertical	Glass	5	24 (L) 17 (Dia)	Artificial	820	ND	7.6–7.8	20	Enriched Mediterranean SeaWater	Sobczuk et al. (2006)
P. marinum	Cylindrical/ Vertical	Glass	5	ND	Artificial	200–360	0.5–2	8	24–28	Provasoli and modified Provasoli	Soanen et al. (2016)
P. purpureum	Annular column/ Vertical	Stainless steel	3	ND	Artificial	25–50	0.5–2.5	7.6	20	Artificial SeaWater	Fleck-Schneider et al. (2007)
P. purpureum	Bubble column/ Vertical	Glass	9.6	62 (L) 17 (Dia)	Artificial	60–160	2	6.6–8	25	Hemerick	Tebbani et al. (2015)
P. purpureum	Serpentin tubular/ Vertical	Polymethyl methacrylate	600	48,000 (L) 4 (Dia)	Natural	8.2–893.5	ND	ND	11.2–22	f/2	Fuentes-Grünewald et al. (2015)
P. purpureum	Bubble column/ Vertical	Glass	0.9	50 (L) 5 (Dia)	Artificial	100	3	ND	ND	Artificial SeaWater [Jones et al., 1963]	Baer et al. (2016)
Flintiella sanguinaria	Torus/Vertical	Polymethyl methacrylate	1.3	4 (T)	Artificial	280	0.5–2	8	24	FS	Gaignard et al. (2018)
P. purpureum	Bubble column/ Vertical	Glass	9.6	62 (L) 17 (Dia)	Artificial	60–160	2	6.6–8	25	Hemerick	Tebbani et al. (2015)
P. purpureum	Bubble column/ Vertical	Glass	0.9	50 (L) 5 (Dia)	Artificial	100	3	ND	ND	Artificial SeaWater [Jones et al., 1963]	Baer et al. (2016)
R. violacea	Roux flask	Glass	0.7	ND	Artificial	150–420	1	8–8.3	24	f/2 and modified f/2	Villay et al. (2013)
Rhodosorus marinus, *P. cruentem*, *R. violacea*	Tubular/Vertical	Polymethyl methacrylate	55	200 (L) 20 (Dia)	Artificial	240	2	7.1–8.2	20–24	Enriched SeaWater (Walne, 1966)	Dupré et al. (1994)
Galdieria sulphuraria	Cylindrical/ Vertical	Glass	3	13 (Dia)	Artificial	0–395	ND	2	42	Modified medium (Gross and Schnarrenberger, 1995)	Sloth et al. (2006)
Galdieria sulphuraria	ND	ND	4000	ND	Natural	ND	ND	ND	ND	Modified cyanidium medium	Selvaratnam et al. (2015)
Galdieria sulphuraria	Bubble column/ Vertical	Glass	0.9	50 (L) 5 (Dia)	Artificial	100	3	2	ND	Modified medium (Gross and Schnarrenberger, 1995)	Baer et al. (2016)

Abbreviations: ND – Non Described.
[a] L – length, W – width, T – thickness, Dia – diameter, D – depth.

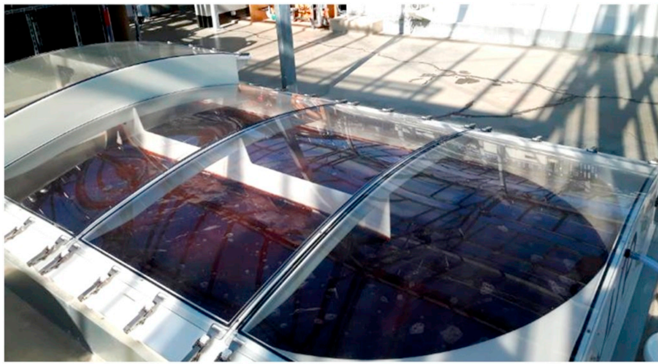

Fig. 6. Industrial production of *Porphyridium cruentum* in closed raceway ponds (AlgoSolis, GEPEA, Université de Nantes, CNRS, France).

compared with closed systems. Early open ponds were without agitation. To improve growth rates, mixing systems were then implemented. Initial shape tested was circular and was used for production of biomass (Fig. 7). This design was later replaced by an elongated shape, the open raceway, which is currently the general design for open ponds (Figs. 6 and 7). Mixing and circulation of the microalgae culture is generally provided by paddlewheels. In open raceways, the sunlight can come only by the top. With mixing, microalgae are less submitted to a dark/light regime and have a better accessibility to light. Moreover, the agitation homogenizes the culture and improves the accessibility to nutrients.

Beside temperature and availability of nutrients, light is the main parameter affecting growth rates of microalgae in outdoor cultures. High incident irradiance level generally increases raceway productivity, as a high surface irradiance generally means a larger illuminated culture volume. The light energy received by cells is represented by photon flux density (PFD), which should be expressed within the range of photosynthetic active radiation (PAR). This PAR corresponds to the part of the sunlight that can be used by microalgae for photosynthesis, then within the wavelength range of 400–700 nm.

Another important parameter for the productivity in these systems is the availability of CO_2 necessary for the growth of microalgae (carbon source) in autotrophy. CO_2 present in the atmosphere is generally not sufficient for optimal growth and addition of CO_2 in the culture is sometimes performed by bubbling. The depth of the open raceway is also an important parameter for carbon availability. Deeper is the pond, greater is the carbon dioxide storage and lower is the loss. However, the depth of these systems is always insufficient due to light constraints and limits the CO_2 transfer into the liquid phase.

The cultivation of red microalgae in open ponds has been poorly described in literature. Vonshak et al. (1985) have studied the outdoor cultivation of *Porphyridium cruentum* in 130 L open ponds. They have found an average daily output rate of 10–12 $g.m^{-2}.d^{-1}$ which could be increased to 22 $g.m^{-2}.d^{-1}$ (~0.17 $g.L^{-1}.d^{-1}$) when the culture was harvested more often, keeping a stable cell biomass concentration. This increasing biomass productivity was possible because of the reduction of the light limitation. Their results may suggest an output of 40–50 $tons.ha^{-1}.year^{-1}$ of dry biomass, with approximately 20–25 tons of $PS.year^{-1}$ which appear to be an acceptable production for red microalgae. Nevertheless this study has been done at laboratory scale with a 1 m^2 pond. As volumes increase in the large-scale cultivation open ponds, the productivity decreases and the risk of contamination increases. Added to an imprecise control of culture parameters (as temperature, pH, CO_2 availability, etc.), cultures of red microalgae in industrial open ponds is not really developed. Consequently the use of closed systems is privileged (Cohen and Arad, 1989).

3.2.2. Closed systems

Compared to open ponds, the closed systems allow better control of the culture and are adapted for fragile microalgae being more demanding for their biotic and abiotic culture conditions. However, biomass production in closed systems is more expensive (cost of the equipment, operating costs and maintenance). Moreover, during cultures in closed systems, important adhesion of cells could be observed on surface of culture vessels. In open ponds, the main drawback is linked to improved difficulties in properly cleaning the pond between two productions. In closed PBR, this biofouling may be catastrophic, reducing light transmission inside the culture, decreasing photosynthetic rate, then biomass productivity, and possibly leading to the early stop of the culture due to cellular death. This biofouling

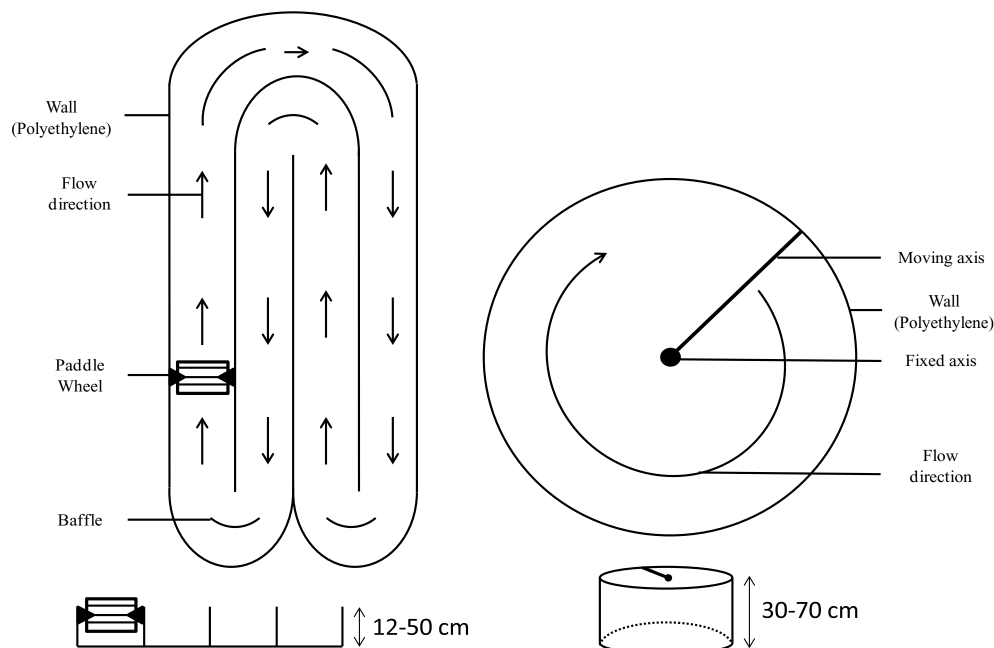

Fig. 7. Schematic drawing of two different open ponds for mass culture of microalgae, sky view (top) and horizontal view (down), raceway shape (left) and circular shape (right).

Fig. 8. Culture of *Porphyridium cruentum* in polyethylene sleeves (Greensea, Mèze, France).

phenomenon is dependent on several parameters, such as the microalgae strain, the type of material used for manufacturing the PBR, the culture time (biofouling increases with culture duration), and the fluid velocity (and shear stress) at the vicinity of the wall. EPS, that are significantly excreted by red microalgae, may increase this phenomenon. The review of Zeriouh et al. (2017) summarized knowledges on biofouling of microalgae inside PBR, showing that the mechanism of cells adhesion is similar to the well-known bacteria biofilm formation mechanism, with the initial step linked to hydrophobic/hydrophilic interactions, followed by an expansion of the biofilm thickness in which EPS would play a major role. Strategies to decrease this biofouling generally target the preliminary step of adhesion by modifying the surface charge of the wall material (Zeriouh et al., 2017). Nevertheless, closed systems have some advantages including (i) a better control of the culture environment, *i.e.* a higher gas and light transfer, (ii) a protection against environmental contaminations, (iii) a limitation of evaporation and then result in better biomass productivities. Closed systems have been designed on a variety of structures including sleeves (Fig. 8) and PBR (bubble column, cylindrical, torus, vertical tubular, horizontal tubular, flat-plate, annular tubular) (Figs. 9 and 10).

The use of closed system for the mass culture of red microalgae dates from 1989 with the study of Cohen and Arad about the comparison between cultures of *Porphyridium* sp. in open ponds and closed system, a polyethylene sleeve which prevents culture from

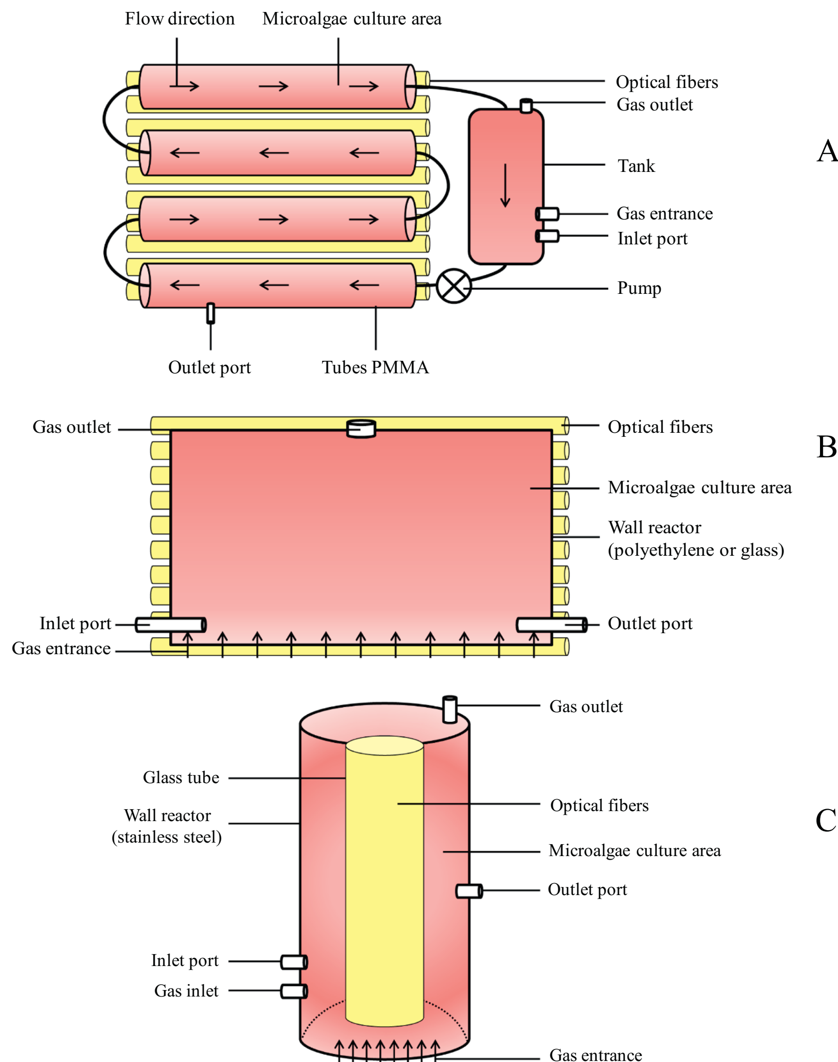

Fig. 9. Schematic drawing of culture of red microalgae in a vertical tubular photobioreactor illuminated with artificial lights (A), vertical flat-plate photobioreactor illuminated with artificial lights (B), vertical annular photobioreactor illuminated with artificial lights (C).

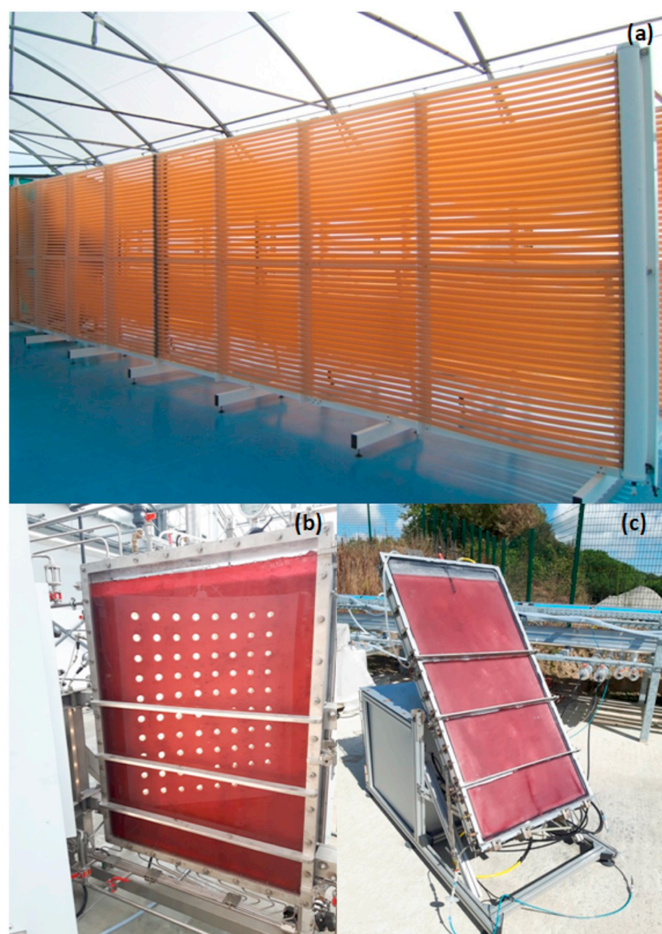

Fig. 10. Cultures in different photobioreactors from different companies. (a) *Porphyridium cruentum*, vertical tubular photobioreactor (Greensea, Mèze, France); (b) *Porphyridium cruentum*, flat plate photobioreactor with artificial light and (c) *Porphyridium cruentum*, inclined flat plate photobioreactor with natural light (AlgoSolis, GEPEA, Université de Nantes, CNRS, France).

contamination and evaporation (Cohen and Arad, 1989). With this system, cultures of *Porphyridium* sp. and *P. aerugineum* were achieved outdoor and the results showed a better growth and biomass production for each species compared with the cultures performed in classic open ponds. The sole biomass production was higher by almost 300% and 60% for *Porphyridium* sp. and *P. aerugineum*, respectively. Logically this increase in biomass production was correlated with the PS content (both bound PS (BPS) and released PS (RPS)) which was better (6 times higher for BPS and 3 times higher for RPS of *Porphyridium* sp.) with polyethylene sleeves compared to open ponds. This is mainly due to better mixing, higher light availability, control of contamination and prevention of evaporation that can lead to an increase in salinity. They demonstrated that the utilization of closed systems can be an alternative method for culturing microalgae outdoor but these yields for both biomass and compounds are not sufficiently attractive compared to those obtained with instrumented PBR.

For industrial production the red-microalgae are mainly cultivated in horizontal and vertical tubular or flat-plate PBR with sunlight in order to reduce the costs (Fig. 10). The major drawback of this method is the absence of control on the light intensity, the daily variation of light due to solar energy and the dependence of climatic conditions and seasonality effects. Studies on daily cyclic variations on growth of *P. cruentum* in a horizontal tubular PBR showed an inhibition of photosynthetic activity during higher light intensity of the sun (Fuentes et al., 1999). These drawbacks can be reduced with PBR in greenhouses but they require a large cooling system to compensate the rise of temperature. It is also possible to work under artificial light but it will generate more costs (Fig. 9a).

In a flat-plate PBR with artificial light (Fig. 9b), the growth rate and PS production of *P. cruentum* increased with light intensity (Liqin et al., 2008). The best productivities (PS) of *Porphyridium* sp. were observed with light path length of 1.3 cm but dropped for higher path lengths suggesting a lack of light availability. However, a 20 cm light path reactor was described as the best solution for microalgae culture because of its surface to volume ratio which confers to it an advantage for industrial applications (Singh et al., 2000). Design of PBR thus evolved over time to increase photosynthetic efficiency. Optical fibers can be fixed vertically to culture flow, inside the reactor which has an annular shape (Fig. 9c). Microalgae are thus submitted to a constant incident light flow (Fleck-Schneider et al., 2007) or short light:dark cycles (Muller-Feuga et al., 2003). In this study, the short light:dark cycles resulted in significant increase in efficiency as compared with continuous illumination for a *P. cruentum* culture. Nevertheless, the authors did not specify if these results can be extrapolated to different PBR geometries and/or other strains that have not been validated by comparison of experimental and theoretical data. This “flashing light” method can probably be considered as a continuous illumination because of the low light/dark frequency.

In 2013, Zhang et al. developed a novel flat-plate PBR composed of inclined baffles inside (Zhang et al., 2013). One year later novels flat-plate PBRs with special mixers were developed by Huang et al. (2014). Performances of the PBR culture were upgraded thanks to mixers improving significantly the fluid mixing along the light gradient. Authors demonstrated that fluid velocity along light gradient and the light:dark cycle were the key factors for microalgae growth. The photosynthetic activity and the growth of microalgae can be influenced by fluid velocity, including mixing and shear stress (Leupold et al., 2013). The results obtained by these authors indicate that hydrodynamic forces have a stimulating effect on the physiology of microalgae, depending on species. Nevertheless, too much agitation can have a negative impact on microalgae growth, decreasing the photosynthetic activity especially for red microalgae that have only a layer of mucilage surrounding their cytoplasmic membrane.

Design of tubular PBR has also evolved for culturing delicate microalgae species. Cells of *P. cruentum* were deposited on a disk and cultivated like a biofilm (Lutzu et al., 2017). Under optimal conditions, the carbohydrate content was increased with attached cultivation system (up to 42% of the DW) compared to suspended cultivation system (up to 35% of the DW). Lipid and protein contents were not influenced by the culture mode. Despite the good results obtained on the production of PS, this method is not yet usable for industrial production of PS and biomass and further investigations remain necessary for scale up and commercial applications. Some technologies for microalgae production have also been developed and patented. In 1999 a new design of PBR was developed for promoting microalgae culturing with high concentration of pigments, PUFAs and PS (Hirabayashi et al., 1999). This invention made with a transparent material had a shape of dome, cone or cylinder. A gas input allowed the movement of the culture around a light source and optimized the yield of biomass production. Inventors claimed the possibility to cultivate a large range of microalgae but did not give information about the species.

3.2.3. Auto- vs hetero- vs mixotrophy

Autotrophic cultivation mode is the oldest and the most used in the industrial production of microalgae for algae extracts (aquaculture food or health food supplements) because of the low-cost technology used (open ponds). However, as previously discussed, some unicellular algal species are able to develop in heterotrophy. In the absence of light, the microalgae must draw on its external environment to obtain an organic carbon source necessary for its development and/or catabolize its storage carbohydrates or lipids. Glucose, acetate and glycerol are the most used carbon sources. It has been shown that the heterotrophic culture

can significantly improve the production of cellular biomass, as well as some compounds for some species (Chojnacka and Noworyta, 2004; Liu et al., 2011; Miao and Wu, 2004). Some companies have observed that species belonging to the genus *Porphyridium* are the most studied and exploited and thus try to develop new strategies and cultivation methods for other strains of red microalgae. This is the case of the French company Fermentalg which has recently deposited a patent concerning the cultivation of *Galdieria sulphuraria* (Cagnac et al., 2015). The patented culture process is characterized by specific conditions of the culture in terms of illumination and nutrients allowing to obtain an improved amount of red microalga, rich in proteins and pigments, especially carotenoids: β-carotene and zeaxanthin. The culture is carried out in mixotrophy with a carbon source selected from glucose, sucrose, acetate or glycerol and whose concentration is between 50 mM and 800 mM. In addition, the P/C ratio expressed as mole of phosphorus/mol of carbon must be close to 0.00131. The ideal light wavelength for culture is preferably 455 nm, which corresponds to the maximum absorption of β-carotene improving pigment accumulation in the biomass. The developers of the process valued that under all these very strict conditions of cultivation it would be possible to obtain between 90 and 150 g.L^{-1} of DW. Moreover, Fermentalg claims that such conditions can promote the *Galdieria sulphuraria* protein production up to 51% of the dry matter or more. To our knowledge no industrial production in bioreactor in mixotrophy of strains of this type could be found at an earlier date. This patent is thus the first example of innovation concerning the cultivation and the valorization of extreme red microalgae belonging to the Cyanidiophyceae class, and more specifically *Galdieria sulphuraria*.

4. Valuable metabolites from red microalgae and their extraction/purification (downstream processes)

4.1. Pigments and proteins

As previously stated, microalgae are a reservoir of natural substances. The production of valuable biochemicals is also based on the exploitation of their proteins and pigments content. Ten years ago, Becker (2007) published a research review paper introducing the so called “protein gap”. Nowadays, we can get a feel for the reality of this critical point, especially considering the significant demand of “old” and “new” proteins for health food, cosmetics, animal and probably human feed in the Horizon 2050 (growing world population estimated to 10 billion persons). Since this statement, some works have tried to deal with this challenge, *e.g.* the works of Graziani et al. (2013) with *Galdieria sulphuraria* which is rich in proteins (26–32%) or of Dere et al. (2003) who investigated 175 species including Rhodophyta for their proteins content. The recent reviews of Cian et al. (2015), revisited by Wells et al. (2017) highlighted the potential of proteins from red seaweeds, including microalgae, as functional foods. As an example, protein content of *P. aeruginum* biomass has been estimated to 31.6% (w/w), and between 28 and 39% (w/w) for *P. cruentum* (Becker, 2007; López et al., 2010; Tibbetts et al., 2015). Moreover, beside this high protein content, Tibbets et al. 2015 have also analyzed the amino acid profile from *P. aeruginum*, highlighting a high essential amino acids amount, with nutritional quality potentially greater than egg albumin. Nevertheless, protein efficiency ratio, digestion coefficient, net protein utilization, color and taste must also be considered to show the nutritional potential of proteins extracted from microalgae. Yet, many issues are still persistent such as high production cost, technical problems to use algal material in food preparations, ethics linked to Nagoya protocol, etc. To our knowledge, there are still no significant applications for proteins from red microalgae as (human) food. In fact, the commercial use of red microalgae for their proteins and/or pigments content is much more associated to niche markets such as fluorescent tags (Glazer and Stryer, 1984) and natural dyes (Arad and Yaron, 1992). Proteins and pigments from red microalgae can thus show health, food and cosmetic applications as reported for some examples in Table 6. High-value metabolites will be looked for red microalgae and pigments meet these expectations for designing and/or developing natural, new and healthy functional ingredients (Cuellar-Bermudez et al., 2015). Main pigments, *i.e.* (i) chlorophyll, (ii) carotenoids and other photosynthetic accessory pigments, *i.e.* (iii) phycobiliproteins, were identified among red microalgae. Overall, the pigments are not distributed in the same way over the photosystems I and II. Chlorophylls are green pigments with polycyclic planar structures esterified by a phytol side-chain (Pignolet et al., 2013). They are located in the thylakoïds of chloroplasts (Lehninger et al., 2005). The distribution of chlorophylls is quite simple for Chl *a* since all photoautotrophic algae possess it. Chl *b* is present in green algae and various types of Chl *c* are found in brown algae (Stengel et al., 2011). Chlorophyll *d* (Chl *d*) was identified in Rhodophyta as well as other main accessory pigments such as R-phycocyanin (R-PC), APC or α/β carotens (Schubert et al., 2006). Carotenoids can be α, β and γ carotenes or oxygen-containing compounds called xanthophylls. The latter group is composed of varying amounts of lutein, violaxanthin, spirilloxanthin, neoxanthin and fucoxanthin. Their distribution patterns vary depending on the species, which is of first help for determining taxonomic status. As example, alloxanthin, lutein, neoxanthin, prasinoxanthin, violaxanthin, fuxoxanthin are not described in Rhodophyta on the contrary to zeaxanthin. Serive et al. (2017) recently published the description of pigment communities among 37 microalgae strains. They identified specific pigments (excluding phycobiliproteins) corresponding to the red lineage, including 3 Rhodophytes and 1 Glaucophyte. Common synthesis of zeaxanthin, Chl *a* epimer, Car36 and Car29 was identified. They also detected β-cryptoxanthin which is the biosynthetic intermediate between β-carotene and zeaxanthin. Carotenoids (Car45, Car50, Car57) and specific porphyrins (P4, P21 and P22) were also pointed out depending on these species. Phycobiliproteins, which are blue or red pigments, are especially found in Rhodophyta, Cryptophyta and Cyanophyta. Phycobiliproteins have a linear tetrapyrrole prosthetic group, covalently linked

Table 6
Some applications of red microalgae pigments and proteins.

Applications		Proteins/pigments	References
Food	Natural dye	Phycocyanin	Arad and Yaron (1992)
	Natural dye for micelle solubilization	C-phycocyanin	Bermejo et al. (2000)
	Food marker	Phycoerythrin	Pulz and Gross (2004)
	Health food supplement	Proteins/enzymes	Rasmussen and Morrissey (2007)
Diagnostic, Biomedical research, Biosensors	Fluorescent markers	Phycobiliproteins	Glazer and Stryer (1984); Glazer (1994); Perez-Garcia et al. (2011)
	Immunomodulator	Phycobiliproteins	Iijima and Shimamatsu (1982)
	Anticarcinogen		
	Light-sensing element	B-phycoerythrin	Ayyagari et al. (1995)
	Anti-oxidant	Phycocyanin	Datla (2011)
	Anti-inflammatory	Phycocyanin	Datla (2011)
Cosmetic	Eye shadow	Phycocyanin	Arad and Yaron (1992)
	Natural dye	Phycocyanin	Dainippon InK and Chemicals Inc (1987)

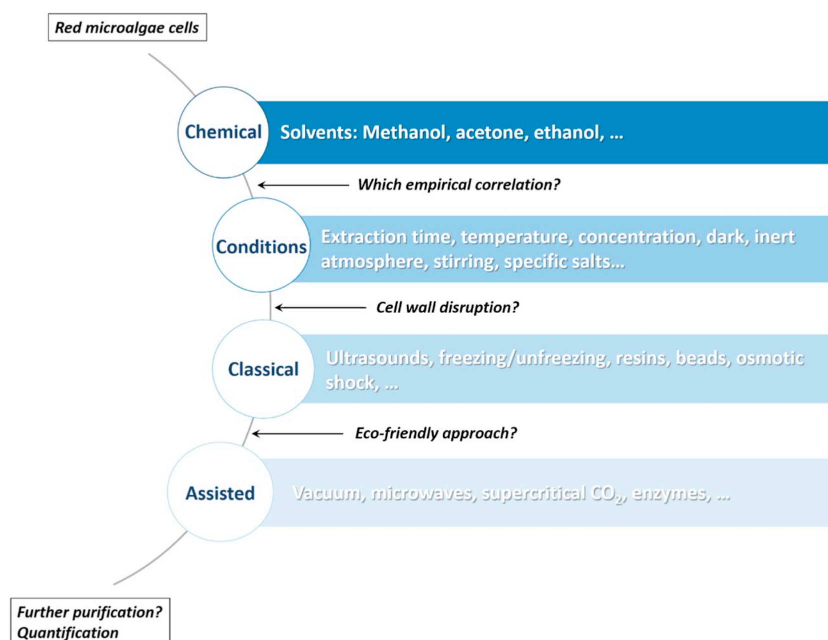

Fig. 11. Main strategies for the extraction of red microalgae pigments.

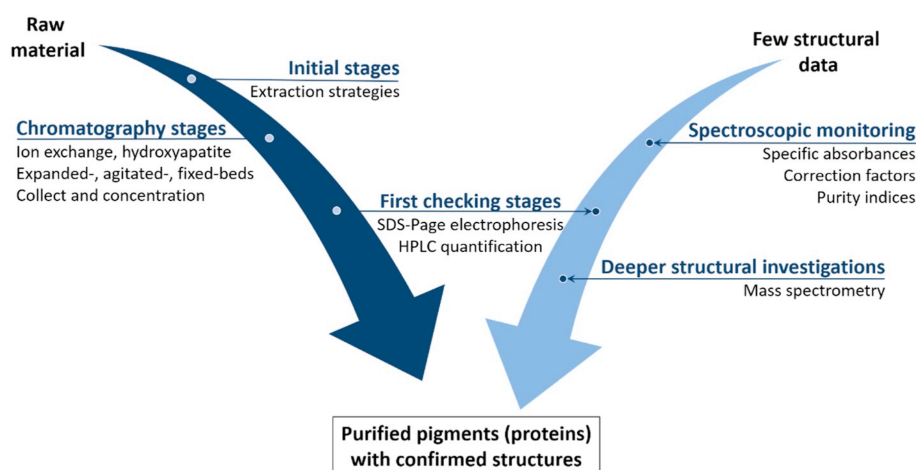

Fig. 12. How purifying pigments and investigating their structures?

in their functional state to proteins (*via* cysteine residues) (Bermejo et al., 2002). They are associated in complexes called phycobilisomes (Fig. 2) which are attached in regular arrays to the outer surface of thylakoïds (Beale, 1993). As example, the phycobilisomes from *P. cruentum* are hemiellipsoidal with a tricylindrical core subassembly in the semi-spherical center, and numerous peripheral rods (Pignolet et al., 2013). Molecules such as APC, PC or PE are an integral part of these structures. As reported by Arad and Yaron (1992), these pigments can absorb light from 450 to 650 nm and transfer it to the photosynthetic reaction center. This energy chain thus involved red PE (λ_{max} ~ 540–570 nm), blue PC (λ_{max} ~ 610–620 nm) and APC (λ_{max} ~ 650–655 nm). PEs are composed of major classes called B-PE (λ_{max} ~ 565, 546 and a shoulder at ~499 nm), R-PE (λ_{max} ~ 568, 499 and a shoulder at ~545 nm) and C-PE (λ_{max} ~ 565 nm) (Bermejo et al., 2003). C and R prefixes characterize the origin of the pigments, respectively Cyanophyta or Rhodophyta. B and b prefixes design other phycobiliproteins. We can also note that the dilution rates, resident time, solar irradiance parameters and more generally culture conditions (see Section 3) greatly change the protein content profile in PBR systems as reported by Fuentes et al. (2000) for red microalgae. Since the pigment content is often a specific feature of each microalgae (Henriques et al., 2007), we can easily understand that a specific extraction method should be use for each investigation. During the last decades, numerous protocols were proposed but also comparisons of different methods to extract and quantify pigments. In general, the extraction techniques involve chemical, mechanical and/or enzymatic procedures (Fig. 11). New protocols, more efficient, eco-friendly and non-damaging for pigments are emerging in the literature. As example, Serive et al. (2017) developed an extraction strategy consisting in suspending a pellet of algae cells in ethanol and mixing the suspension with 500 µm glass beads during 30 min at 30 Hz frequency. The pigments were collected after centrifugation and filtered on 0.2 µm prior to immediate analysis. It is noteworthy that purification steps of pigments/proteins are sometimes needed, in particular for industrial applications. The processes to obtain these purified biomolecules but also to confirm their structures are described in Fig. 12. Regarding the literature, the extraction and purification methods of phycobiliproteins have evolved and the purification of B-PE was the most investigated from red microalgae, in terms of recovery, purification and characterization.

B-PE, R-PC and APC concentrations and the purity index (PI) of B-PE were evaluated as described by Bermejo et al. (2002) measuring absorbances at 280 ($A_{280\ nm}$), 545 ($A_{545\ nm}$), 565 ($A_{565\ nm}$), 620 ($A_{620\ nm}$) and 650 nm ($A_{650\ nm}$) and using the eqs. (1) to (4):

$$[R-PC] = \frac{(A_{620\ nm} - 0.7A_{650\ nm})}{7.38} \quad (1)$$

$$[APC] = \frac{(A_{650\ nm} - 0.19A_{620\ nm})}{5.65} \quad (2)$$

$$[B-PE] = \frac{(A_{565\ nm} - 2.8[R-PC] - 1.34[A-PC])}{12.7} \quad (3)$$

$$PI = \frac{A_{545nm}}{A_{280nm}} \quad (4)$$

Among the numerous protocols published between 1950s and 2000s to purify B-PE, the most used are those of Glazer and Hixson (1977), Thepenier et al. (1987), Tcheruov et al. (1993) and Bermejo et al. (2003, 2002). All these protocols included a step of biomass lysis in an adapted buffer (congelation, sonication, bead mill or high pressure treatments), a step of insoluble particles removing (centrifugation or microfiltration), a step of purification by chromatography (hydroxyapatite, size exclusion or ion exchange) with or without a preliminary treatment of B-PE extracts with ammonium sulphate $(NH_4)_2SO_4$ to concentrate the B-PE. The IPs of purified B-PE obtained with these methods were between 0.8 and 5. Bermejo and coauthors published details of their investigations about the methodology applied to extract this major light-harvesting pigment from *P. cruentum* (Bermejo et al., 2002, 2003). The most interesting point is probably the preliminary cost analysis performed to better understand the process, especially for a scale up production. The flow chart of B-PE (and R-PC) purification from the biomass consisting in (i) a cell disruption (sonication) followed by a centrifugation, (ii) a filtration of the supernatant prior to a precipitation with $(NH_4)_2SO_4$ (65%, 12 h), (iii) a centrifugation (4500 g, 10 min) to get a pellet which was resolubilized in specific buffers (acetic acid-sodium acetate, pH 5.5) then dialyzed, (iv) other centrifugations to finally obtain a supernatant injectable in ion exchange chromatography (DEAE-cellulose columns, *i.e.* 15 × 2.5 cm for small scale and 15 × 9 cm for large scale), (v) a collect of PE and PC fractions (Bermejo et al., 2002).

To limit the number of unitary operations during the purification of B-PE Benavides and Rito-Palomares (2006) have developed two-phase processes to avoid chromatography and $(NH_4)_2SO_4$ precipitation steps. The method involved cell lysis by sonication and B-PE recovery by aqueous two-phase partition using poly(ethylene glycol) (PEG). Overall the proposed aquous two phases system process comprising 29% (w/w) of PEG, 9% (w/w) of potassium phosphate, 40% (w/w) B-PE crude extract obtained after sonication of biomass and a pH of 7 was efficient to recover 90% of B-PE in the PEG-rich phase with a purity of 3.2. EPS and cell attached polysaccharides are the main drawback limiting these B-PE purification processes as they change the solubility of this pigment and the behavior of red microalgae during their lysis (Jubeau et al., 2013). To avoid this phenomenon only cells in exponential growth phase are generally used to extract and purify B-PE to limit the presence of polysaccharide (Jubeau et al., 2013). Therefore, with the objective to give value in the same time to cell-attached polysaccharides and B-PE from *Porhyridium* species, at all phases of growth, a two-step membrane process was developed by Marcati et al. (2014). With this method authors co-extracted EPS and B-PE using tangential ultrafiltration with several molecular weight cut off. This procedure enabled authors to extract B-PE with a PI of 2.3 and a yield of 48%.

4.2. Polysaccharides

So far, polysaccharides (PS) from microalgae have been poorly explored despite their original monosaccharide compositions and structures in a very close relationship with remarkable physico-chemical and biological properties. This paragraph gives an overview of the different PS from red microalgae and also of the main strategies developed for their extraction and purification.

The composition of PS from microalgae has been largely described in scientific literature (for a review see Delattre et al., 2016). Generally, two families of PS are described: *i.e.* (i) the storage PS constituting intracellular PS (IPS) and, (ii) the structural PS (including EPS, RPS and BPS and cell wall PS).

Starch and starch-like PS represent the main storage IPS of microalgae. As well-documented in literature, storage IPS of green algae and blue-green algae are starch and glycogen respectively (Chao and Bowen, 1971; Patron and Keeling, 2005). Concerning red microalgae, floridean starch is described as storage IPS. Also called "floridean glycogen" this is an *α*-polyglucan structure similar to starch but without amylose (Usov, 2011). Nevertheless, presence of amylose has been detected for some red microalgae such as *P. aeruginum*, *P. purpureum*, *P. sordidum*, *Rhodosorus marinus*, *Rhodella violacea*, *Flintiella sanguinaria*, (McCracken and Cain, 1981; Shimonaga et al., 2007, 2008) but to a lesser extend than for green microalgae and higher plants. As an example, amylose content of starch from *P. purpureum* was found to be < 10% (Shimonaga et al., 2007), but may account up to 23% for *F. sanguinaria* (McCracken and Cain, 1981). Moreover, analyses of chain length, crystallinity, and level of branching have highlighted significant differences with the storage carbohydrates of green microalgae and with floridean starch (Shimonaga et al., 2008). For these species, the authors recommend to use the denomination of "semi-amylopectin" type polysaccharide instead of floridean starch. It is also to mention that for 2 members of the cyanidiales family (*Cyanidium*, *Galdieria*), no amylose was found, but a higher level of branching was encountered, leading to a structure assimilated to glycogen (Shimonaga et al., 2008, 2007), whereas for *Cyanidioschyzon merolae*, the structure was closer to floridean starch (Hirabaru et al., 2010). The number and the length of the branching could be altered in function of the microalgae species (Chao and Bowen, 1971). Then, structurally, floridean starch is identical to glycogens and amylopectins with a transitional branching degree and an average length generally described in the range between 9 and 13 glucose residues in the linear chain (Hirabaru et al., 2010; Shimonaga et al., 2008). The main difference between starch, glycogen and floridean starch is the cells localization. Starch from green algae is present (formation and storage) in plastid whereas floridean starch and glycogen are localized in the cytosol (Usov, 2011; Yu et al., 2002).

Contrary to other microalgae, microfibrillar cellulose is not present in the cell walls of red microalgae. As related by Arad and Levy-Ontman (2010), red microalgae have the particularity of possessing the cell walls like encapsulated within a gel matrix constituted of PS. One part of these PS is excreted in the medium as soluble EPS (sometimes called RPS) and the other one concerns EPS bounded to the cytoplasmic membrane and called BPS (Geresh and Arad, 1991). Some author related that around 50 to 70% of PS remain bounded to the red microalgae during their growth (Singh et al., 2000). The physiological function of EPS is still ambiguous even if the main role described in literature seems to be the prevention of microalgae against dessication phenomenon and the protection against a multitude of everyday environmental conditions (Arad and Levy-Ontman, 2010; Delattre et al., 2016; Pignolet et al., 2013; Ramus, 1981). The most studied red microalgae (Rhodophyta) EPS were produced and extracted from *R. reticulata*, *Porphyridium* sp., *P. aerugineum* and *P. cruentum* (Delattre et al., 2016; Geresh and Arad, 1991; Geresh et al., 1990, 1992, 2009; Gloaguen et al., 2004). *P. cruentum* is industrially exploited for the production of EPS (Arad and Levy-Ontman, 2010; Pignolet et al., 2013). Currently, the specific structures of these high molecular weight (MW) complex heteropolysaccharides are not totally defined. These anionic EPS are mainly composed of anionic sulphated PS made up of glucuronic acid and several major neutral sugars such as xylose, galactose and glucose (Arad and Levy-Ontman, 2010; Geresh and Arad, 1991;

Gloaguen et al., 2004). Other minor sugars such as 2-*O*-methyl-GlcA, 4-*O*-methyl-Gal, 4-*O*-methyl-Xyl, 3-*O*-methyl-Xyl, 2,3-di-*O*-methyl-Rha, 2,3-di-*O*-methyl-Fuc, di-*O*-methyl-Hex and 6-*O*-methyl-Man could be found in EPS (Geresh and Arad, 1991; Geresh et al., 1992). It is generally admitted that EPS from red microalgae are sulphated, with sulphate content ranging between 1 and 14% (w/w) (de Jesus Raposo et al., 2013; Geresh and Arad, 1991; Roussel et al., 2015; Soanen et al., 2016; Villay et al., 2013). However, it is noteworthy that only very few studies have been conducted on species other than *Porphyridium* and *Rhodella*. Thus, these structural features may be quite different for other red microalgae. As an example, the recent characterization of the EPS from *Flintiella sanguinaria* has shown no sulphate groups (< 0.6% w/w), but a 5.1% (w/w) methylated and 3.2% (w/w) acetylated polymer (Gaignard et al., 2018). Till now, only few studies published by some authors have elucidated the partial structure of EPS from red microalgae (Delattre et al., 2016; Geresh et al., 1990, 2009; Gloaguen et al., 2004). A specific partial hydrolysis of EPS from *Porphyridium* sp. using acid leads to the identification of glucose in position *O*-6 and galactose in position *O*-6 and *O*-3 (Lupescu et al., 1991). Moreover, by using chemical fragmentation of EPS from *Porphyridium* sp. with degradation of glucuronic acid by lithium/ethylenediamine treatment, Gloaguen et al. (2004) produced two neutral oligosaccharides fragments which were the main glycosidic repetition units of the PS. These two oligosaccharides fractions were respectively: β-D-Gal$_p$-(1,3)-β-D-Glc$_p$-(1,3)-β-D-Xyl$_p$-(1,4)-β-D-Xyl$_p$-(1,4)-L-Gal$_p$-(2,1)-β-D-Xyl$_p$ and β-D-Xyl$_p$-(1,4)-β-D-Xyl$_p$-(1,4)-L-Gal$_p$-(2,1)-β-D-Xyl$_p$. However all the EPS from red microalgae described in the literature are very different for their structure and rheological properties compared with those from red macroalgae. Indeed, red marine macroalgae are producers of sulphated or not sulphated galactans highly exploited as hydrocolloids and more recently as biological agents (Pierre et al., 2014). They are constituted of a linear backbone of 3-linked β-D-Gal*p* residues (A units) and 4-linked α-D/L-Gal*p* residues (B units) forming an alternated sequence (AB)n. The B unit can be replaced at various percentages by 3,6-anhydro derivatives. Hydroxyl groups of this backbone can be substituted by sulphates, methyl and/or pyruvates. Depending on L or D series of B units, on amount of 3,6-anhydrogalactose residues and on sulphate content several families of sulphated galactans from macroalgae have been described including agarans, carrageenans and complex sulphated galactans (Pierre et al., 2014). The annual market for PS from seaweed is around 9600 t for agars, 26,500 t for alginate and 50,000 t for carrageenans. The seaweed PS market represented around 1 billion US$ in 2010. The industry uses almost 20 million tons of wet seaweed annually (FAO, 2012 - http://www.fao.org/fishery/statistics/global-aquaculture-production/en), harvested either from naturally growing (wild) seaweed or from cultivated (farmed) crops. European output of phycocolloids is estimated to have an annual wholesale value of around €130 million which is 97.5% of the total for all algal products in Europe. The phycocolloid market is not saturated and is driven mainly by novel food, pharmaceutical and cosmetic industries as shown by the recent success of fucoidan and ulvan, two recently commercialized PS from macroalgae (Kraan, 2012). Indeed, a very intriguing feature that distinguishes algal biomass from other resources is that it contains large amounts of sPS, whose beneficial biological properties have prompted scientists to increase their use in the biomedical fields. The presence and the distribution of sulphate groups in these PS are reported to play an important role in the antiviral, anticoagulant, antioxidant and anticancer activity of these materials.

Due to its highly complex structure and rheological properties, sPS from *Porphyridium* sp. have been largely described for prospective applications in food, cosmetic and pharmaceutical fields as antioxidant, anti-inflammatory, anti-bacterial, anti-viral, anti-tumour and hypolipidemic agents (Arad and Levy-Ontman, 2010; Geresh et al., 2002; Guzman-Murillo and Ascencio, 2000; Huleihel et al., 2002; Jiao et al., 2011; Matsui et al., 2003; Minkova et al., 1996; Talyshinsky et al., 2002; Tannin-Spitz et al., 2005).

A very small number of PS from microalgae have been successfully extracted and purified before structural characterization. In an industrial point of view, it is therefore necessary to optimize and propose the most efficient extraction processes to produce high yield microalgae PS (EPS, IPS…).

To produce better microalgae PS yields it is crucial to distinguish the best growth and culture parameters (Delattre et al., 2016). Once the cultivation conditions are well-optimized, researchers have to investigate environmental friendly and very cost-effective selective extraction processes to extract and highly purify PS from microalgae (Azeredo et al., 2003; Bertocchi et al., 1990; Delattre et al., 2016; Liu and Fang, 2002; Takahashi et al., 2009). Focusing on red microalgae, lots of works have investigated the optimization of extraction processes. Ramus proposed for the first time to fractionate and purify selectively the PS from *P. aerugineum* (Ramus, 1972). Briefly, his idea was to dissolve the BPS by using hot water treatment after a first round depigmentation of red microalgae with an acetone/ethanol solvent. After that, a specific EPS precipitation step was performed using cetyl pyridinium chloride solution. In the final step, EPS was selectively purified by ethanol treatment. In the same way as in 2004, Gloaguen and coworkers used depigmented ground *Porphyridium* sp. powder to extract acidic PS with hot distilled water (under reflux during 1 h at 100 °C) following by precipitation using hexadecyl trimethyl ammonium bromide (3% *w/v*). The PS was then purified by KCl and ethanol/water solutions. Finally PS was dialyzed against water and lyophilized.

Recently, Delattre et al. (2016) gave a deep overview of the main general processes used to extract and fractionate PS (EPS and BPS) from microalgae cultures. As generally described in literature, after the first step of microalgae cultivation using the optimized conditions (salinity, irradiance, temperature, pH, … as described in Section 3.1), the best concentration of EPS fraction was solubilized in the medium. A second step (harvesting) involving the recovery of PS was developed by using microfiltration or centrifugation in order to remove microalgae biomass (Delattre et al., 2016; Li et al., 2011b; Liu and Fang, 2002; Patel et al., 2013; Ye et al., 2005). Finally, EPS was concentrated under vacuum from filtrate and/or supernatant and selective recovery/purification of EPS was performed by precipitation with isopropanol or ethanol (Delattre et al., 2016; Patel et al., 2013; Usov, 2011).

Even if this alcoholic precipitation process offers considerable advantages in industry (solvent recycling, reduction of cost production, …), still there remain questions about the co-precipitation of salt in the case of EPS produced in culture media with high salinity as in the case of those from *Porphyridium* sp. (Delattre et al., 2016; Patel et al., 2013). Thus another efficient approach using dialysis or tangential ultrafiltration (TUF) to desalt EPS extracted from red microalgae (Eteshola et al., 1998; Gloaguen et al., 2004; Patel et al., 2013). Works from Patel et al. (2013) well-illustrated the impact of TUF onto the final EPS purification from *P. cruentum*. In this study, the authors have shown that TUF performed with polyethersulfone (PES) filtration membrane with molecular weight cut off (MWCO) of 300 kDa was a more efficient approach to extract and purify EPS from *P. cruentum* compared to alcoholic precipitation which is not successful enough to remove salts. Based on these results, an optimized EPS (including BPS) purification process was proposed by Marcati et al. (2014) by using two ultrafiltration steps with PES membranes having MWCO of 10 and 300 kDa. More broadly, it is universally accepted by several studies that TUF technology allows to add vaue and purify others high-value added biomolecules such as pigments or proteins along with EPS purification (Delattre et al., 2016; Pignolet et al., 2013). Nevertheless, it is important to notice that these processes (alcoholic precipitation and TUF treatment) performed on supernatant/filtrate from red microalgae did not allow the specific extraction of > 50% of PS, 50% remaining bounded to microalgal cell (Arad and Levy-Ontman, 2010). Consequently, to increase the extraction of bound PS several methods could be investigated on the red microalgal biomass such as treatments with sodium hydroxide, formaldehyde, sonication, ionic resins or ethylene

diamine tetracetic acid (for review see Brown and Lester, 1980; Comte et al., 2006; Delattre et al., 2016; Frølund et al., 1996; Takahashi et al., 2009).

4.3. Lipids

Microalgae contain a wide variety of fatty acids, the composition of which varies according to the species and growth conditions. They produce specific fatty acids that can find different application destinations in several areas. The most studied microphytes today, in particular for the production of biofuels, are green microalgae (*Chlorella*, *Chlamydomonas* …), cyanobacterium *Arthrospira platensis* and microalgae known as oleaginous such as *Botryococcus braunii*, *Dunaliella* sp. or *Nannochloropsis* sp. Many microalgae strains have high lipid content (20–50% dry weight). It can be enhanced by optimising the growth determining factors. In natural environment algal cells can rarely synthesize > 30% oil, therefore for fuel production yields of 50–60% oil from dry weight algal cells should be considered as excellent. Solazyme's algal strains have been designed to synthesize and store 75% oil of dry weight of algal cells (Harun et al., 2010; Nigam and Sing, 2011).

In parallel with the work carried out on green microalgae, increasing investigations on the bio-refining of red microalgae are carried out for the production of biomolecules. Surprisingly, most studies are limited to the red microalga belonging to the genus *Porphyridium* and the products from other red microalgae are only poorly documented. The lipids of *Porphyridium* share with fish oils the presence of ω3 long chain PUFAs such as eicosapentaenoic (EPA - 20: 5 ω3) and docohexaenoic (DHA - 22: 6 ω3) acids (Becker, 1994; Borowitzka, 1988), which represent the main composition of the total fatty acids. They are distinguished either by a mono-specific composition or by the presence of compounds belonging to other series such as the ω6 series with arachidonic acid (AA - 20: 4 ω6) present in *P. cruentum* (Ahern et al., 1983; Cohen et al., 1995). The oils derived from microalgae are of commercial interest due to their low cholesterol level and the diversity of their PUFAs, which have the advantage of being odorless (Sato et al., 2017). There are two types of red microalgae that produce different types of oil. The majority of red and marine algae (and some Cyanidial species) produces oil contents containing very high amounts of PUFAs, while *C. merolae* oil does not contain PUFAs and is suitable biofuels (Sato and Moriyama, 2007).

For biofuels production, microalgae are first grown under optimal conditions in order to maximize cell concentration. Then the culture is subjected to one or many changes in growth conditions (temperature, pH, dark/light cycle, etc.) during the stationary phase which works as induction factor for maximum PUFA biosynthesis. Cohen et al. (1988) suggested a relationship between the fatty acid composition and the environmental conditions in *P. cruentum*. In fact under optimal conditions providing the fastest exponential growth, the dominant PUFA was the EPA. In contrast, under growth limiting conditions (very low light intensity, high temperature (30 °C) and increased pH), the ratio of % 20:4 to % 20:5 was increased from 0.8 up to 5.4, and could be increased up to 11 in stationary phase regardless of the light intensity in *P. cruentum*. Therefore the 20:4 and the 18:2 acids became dominant. In contrast Ahern et al. (1983) suggested that the content in AA increased strongly in *Porphyridium cruentum* especially at temperatures lower than 20 °C. Nevertheless these changes in PUFAs profiles as a function of temperature appear to be strain dependent since in the case of *Porphyridium sordidum*, the EPA content of the total fatty acid increased at low temperature. In fact this one increases progressively from 14 to 28% at low light intensity (150 µmol photons.m^{-2}.s^{-1}) when the temperature was decreased from 33 to 17 °C, respectively (Mihova et al., 1997).

Temperature is not the sole culture parameter affecting lipid production by red microalgae, as photoperiod and light intensity can also have an impact on it. In 2009, Oh et al. demonstrated that a maximum of 19.3% (w/w) lipid can be obtained by growing *Porphyridium cruentum* in batch mode under 18 h/12 h, then 12 h/12 h light/dark photopreriods. This rate can rise up to 20.5% (w/w) in fed-batch, probably due to a lower impact of limiting environmental factors on the synthesis of concerned PUFAs. In addition, the lipid profile produced by *P. cruentum* is composed of approximately 30% C16 and C18, making this microalgae a good candidate for biodiesel production (Oh et al., 2009). As shown by Ivanova et al. (2015), the EPA content of *Rhodella reticulata* can reached 46% (w/w) of the total fatty acids when it cultivated at 27 °C, which is close to *Porphyridium cruentum* (Cohen and Cohen, 1991).

The composition of the culture medium may also have an impact on the lipid level. In *Poprhyridium purpureum*, the study of Kavitha et al. (2016) demonstrated that a moderate increase in chloride, sulphate, nitrate, and phosphate levels favored better biomass production yield, but also that much higher concentrations of these components led to both a maximum total lipid production and EPA content (Kavitha et al., 2016).

After an enrichment step, the lipids are extracted by a usual organic solvent such as hexane or chloroform, by supercritical CO_2 and by subcritical water. PUFAs such as eicosapentaenoic acids (20:5 or EPA) and arachidonic acids (20:4 or AA) are more suited to nutrients or healthy products rather than biofuels. Understanding the entire pathways of lipid biosynthesis, including TAG, was quite limited in red algae until recently (Sato et al., 2017).

In lipid biosynthesis, the most important characteristic of red algae is the absence of stearoyl acyl-transporter-protein (ACP) desaturase which is omnipresent in green algae and terrestrial plants, as well as some actinobacteria (Sato, 2009). This enzyme has been detected in the plastid stroma and the primary products of fatty acid synthesis (FAS) are palmitic (16: 0) and oleic (18: 1) acids in green algae and plants. Rhodophyceae produce saturated acids, such as 16:0 and stearic acid (18:0), which are transported to the cytoplasm and activated to become Acyl CoAs. Acyl CoAs are then used in the synthesis, desaturation or elongation of acyl lipids in endoplasmic reticulum (Guschina and Harwood, 2006).

5. Economic valorization

Among the Rhodophytes, species belonging to *Porphyridium* and *Rhodella* are the sole microalgae exploited for the production of high value compounds. The value of these molecules has been enhanced in some industrial areas often by small companies (Table 7). The research of patents in science finder scholar (https://scifinder.cas.org/) using the sole keyword "*Porphyridium*" found 307 patents, the major part of them claiming for applications of high value biomolecules in the field of food, feed, nutraceutic, cosmetic, diagnostic and therapy.

5.1. Nutraceutical, food and feed markets

The nutraceutical market concerns all the biomolecules having a positive effect on the health, either biomolecules incorporated in more complex preparations or directly as a dietary supplement. This market has been growing for the last twenty years (Bernal et al., 2011). The red microalgae can find a place in this market notably as a source of proteins, PUFAs and functional PS. The principal PUFAs produced by microalgae are the EPA (20:5 ω 3, 6, 9, 12, 15), DHA (22:6 ω 3, 6, 9, 12, 15, 18), γ-Linolenic acid (GLA - 18:3 ω 6, 9, 12) and the AA (20:4 ω 6, 9, 12, 15). A recent study claims that PUFAs (450 mg.day^{-1}) are not being met as most people consider that a current intake of PUFAs by adults of about 282 mg.day^{-1} (EPA and DHA being about 244 mg.day^{-1}) (Givens and Gibbs, 2006). Only EPA and AA are produced by red microalgae, especially *Porphyridium* sp. (Spolaore et al., 2006). The most interesting specie for both commercial production and application of EPA and AA is *P. cruentum* (de Jesus Raposo et al., 2013) as these two PUFAs can represent up to 23.9% and 23% of the total

Table 7
Red microalgae exploitation by industry.

Species	Company and location	biomolecules	Applications and markets
Porphyridium sp.	Greensea (France) http://greensea.fr/en/	Pigments	Medical diagnostics, molecular biology and fluorescence techniques
Porphyridium sp.	Greensea (France) http://greensea.fr/en	Living phytoplankton	Aquaculture
Porphyridium sp.	Frutarom (Israel) http://www.frutarom.com	Sulphated Polysaccharides	Oxidative cell protection, immune photo-protection, anti-inflammatory, anti irritant (cosmetic)
P. cruentum	AlgoSource (France) http://www.algosource.com	Sulphated Polysaccharides	Cellular regeneration, moisturizing gent (cosmetic)
P. cruentum	Micoperi Blue Growth (Italy) http://www.micoperibg.eu	Sulphated Polysaccharides	Antioxidant, anti-inflammatory, antimicrobial agent (cosmetic)
P. cruentum	Greensea (France) http://greensea.fr/en/	Oligosaccharide	Vasoconstriction of blood vessels (cosmetic)
P. cruentum	Phyco-Biotech (France) http://phyco-biotech.com/fr	B-phycoerythrin	Medical diagnostics, molecular biology and fluorescence techniques
P. cruentum	Asta Technologies (Israel) https://www.asta.co.il	Sulphated polysaccharides	Sun-shielding, anti-inflammatory, anti-ageing, protecting againts irritants (cosmetic)
P. cruentum, *Porphyridium* sp.	Solazyme (USA) http://solazymeindustrials.com	Sulphated polysaccharide	Health and appearance of skin (cosmetic, nutraceutic)
P. cruentum	Isua® Biotechnologie & Compagnie (France) http://isua.eu	Biomass	Aquaculture
P. cruentum	Phyco-Biotech (France) https://phyco-biotech.com	Phycobiliproteins	Medical Diagnostic

fatty acid content of this microalgae, respectively (Handayani et al., 2011). They could be included in functional foods or feed for animals (notably for aquaculture) but also in turn, supplying milk and eggs supplemented in those PUFAs. However, the production costs are always too expensive and not competitive compare to other sources and limit their added value.

Concerning PS, there is a lot of information in literature about their different nutraceutical activities. In 2009 the Solazyme company has developed nutraceutical composition with antioxidant properties based on the utilization of PS produced by red microalgae belong to *Porphyridium* genus (Dillon et al., 2006b). This formulation allowed reducing inflammation and preventing oxidative damage in mammalian tissues. Note that one future way of valorization for PS of red microalgae could be their use as thickener agent when their obtaining costs will be competitive to that of plant or macroalgae PS (Delattre et al., 2016; Liu et al., 2016). Indeed, the phycocolloid market is not saturated and is driven mainly by novel food.

The functional properties of *P. cruentum, i.e.* defatted biomasses have been investigated and compared with those of soybean flour (Guil-Guerrero et al., 2004). Results showed that *P. cruentum* biomass had functional properties comparable to those of soybean flour. The nutritional potential of red microalgae proteins has been poorly explored even if comprehensive analyses and nutritional studies have demonstrated that algae proteins are of high quality and comparable to conventional vegetable ones (Becker, 2007). It equally depends on amino acids composition and digestibility of these proteins. One advantage to use red marine microalgae as a source of proteins for food or feed is the lack of rigid cell walls in these species, leading their proteins more accessible and so more digestible compared to those of microalgae which have a cellulosic cell wall. The role of *Porphyridium* sp. used as feed supplement on metabolism of chicken was studied by Ginzberg et al. (2000). It was found that cholesterol of egg yolk was reduced of about 10% (Ginzberg et al., 2000). In addition, it modifies the profile of fatty acids and contributes to the increase in carotenoid content in egg yolk. The same result was found by Dvir et al. (2000) who showed that the addition of the biomass of *Porphyridium* and *R. reticulata* in the diet of rats reduced the levels of cholesterol, triglycerides and very low density lipoproteins (VLDL) in their serum while improving the hepatic cholesterol levels (Dvir et al., 2000). These antihyperlipidemic activities are due to the dietary fibers and the sPS contained in the algal biomass. This activity was the subject of a patent application in 2006 (Dillon et al., 2006a). All of these results should encourage the use of red microalgae as functional foods.

5.2. The cosmetic market

The cosmetic market of compounds from red microalgae mainly concerns the PS and pigments. Even if PS of red microalgae could be used in the cosmetic field for their rheological properties (Arad and Levy-Ontman, 2010), their production costs are not competitive compared to those of hydrocolloids from macroalgae and terrestrial plants (Delattre et al., 2016) as written above. PS from red microalgae are mainly used in cosmetic for their biological activities, especially antioxidant, anti-inflammatory, antimicrobial, activities but also as strengthening skin barrier, or hydrating agent (Chen et al., 2010; Potter et al., 2013; Tannin-Spitz et al., 2005; Yanhui, 2017) (Tables 7 and 8). Bayona et al. (2012) suggested that sPS produced by *P. cruentum* can be used as active ingredient in cosmetic and pharmaceutical compositions due to its activity. After the production and purification of a PS using a bubble column PBR, the authors tested its activity on three enzymes involved in some key substrates responsible of the good appearance of the skin. The PS was able to decrease significantly the activity of hyaluronidase from about 96.6 ± 0.3% with a concentration ranging from 0.25 to 2.5 $mg.mL^{-1}$. This PS was also able to reduce the elastase activity from about 46.0 ± 7.1% at a concentration of 5 $mg.mL^{-1}$. The authors then suggested that sPS from the red microalgae *P. cruentum* can be used as active ingredient in cosmetic formulations in order to maintain a good appearance of the skin. This activity is now patented by the Solazyme Company since 2014 (Dillon et al., 2006c). This patent concerns three principal properties of the PS including the stimulation of both elastin and collagen synthesis and the inhibition of collagenase. Another patent was found on the skincare market concerning the utilization of sPS to improve the barrier function and/or hydration of the skin. This patent developed by the L'Oréal Company (Potter et al., 2013) is a mixture of a sPS from *Porphyridium* sp., sPS from marine bacteria and ulvan, associated to C-glycoside. The L'Oréal company developed another patent based on the utilization of sPS as anti-dandruff agent (Potter et al., 2011). Dandruff corresponds to excessive and visible desquamation of the scalp resulting from a problem of multiplication of the epidermal cells due to a disorder of the microbiote on the scalp. The formulation comprising a sPS from *Porphyridium* sp. was used as cosmetic treatment process intended to eliminate and/or reduce

Table 8
Main applications of EPS from red microalgae.

Activities	Species	Functions	Mode of action	References
Antivirus	*Porphyridium* sp.	Retroviruses	(1) Anionic nature of EPS against viruses	Huleihel et al. (2001); Fàbregas et al. (1999b); Hasui et al. (1995); Martinez et al. (2005); Kim et al. (2012)
	Porphyridium aerugineum	Herpes simplex virus (HSV)	(2) Inhibition of attachment/adsorption	
	Rhodella reticulata	Varicella zoster viruses (VZV)	(3) Inhibition of the penetration of viral particles into host cells	
	Porphyridium cruentum	Viral hemorrhagic septicemia (VHS) for *Salmonid* fish and mammalian viral diseases	(4) Inhibition of virus replication during the early phases of the virus infection (5) Prevention of the formation of various retroviral reverse transcriptase without cytotoxic effects against the host cells	
Anti-inflammatory	*Porphyridium*	Anti human skin inflammation	Inhibition of the migration/adhesion of polymorphonuclear leukocytes	Matsui et al. (2003)
Antibacterial	*Porphyridium cruentum*	Antibacterial activity againt *Salmonella enteritidis*	Antibiofilm formation block adhesion of pathogens to host cells	de Jesus Raposo et al. (2014)
Antioxidant	*Porphyridium cruentum*	Prevent the accumulation and the activity of free radicals and reactive chemical species	Scavenging the free radicals and transporting them from the cells to the medium	Tannin-Spitz et al. (2005)
Antitumor	*Porphyridium cruentum*	Antitumor cell proliferation on S180 tumors in mice models	(1) Reinforcement of immune system induced by the EPS (2) Regulate the cell signaling pathways (3) Block the interaction between cancer cells and the host cells	Sun et al. (2012)
Biolubricant	*Porphyridium*	Good lubrication capacity: substitute hyaluronic acid		Arad and Weinstein (2003); Arad et al. (2006)
Antiparasitic	*Porphyridium purpureum* *P. marinum* *Rhodella violacea* *R. maculata*	Protect honeybees against *Nosema* species		Roussel et al. (2015)
Cosmetic	*Porphyridium* sp. *P. aerugineum* *R. reticulata*	Stabilize the network structure of aloe vera gel and extend its shelf life		Yaron et al. (1992)
Wastewater treatment	*R. reticulata*	Remove toxic ions from industrial wastewater	The negative charge (due to the presence of glucuronic acid and half ester sulphate groups) was used to prepare ion exchangers	Geresh et al. (1997)

dandruff compare to other treatment (*e.g.* of zinc pyrithione). Another patent was developed in 2009 concerning a composition comprising a red microalgae PS and a heavy metal (Arad, 2009). This one can be used as cosmetic, diagnostic or pharmaceutical formulation. Its pharmaceutical interest is further developed in the next part. Arad and Levy-Ontman (2010) cited a heading cosmetic company that uses PS in a wide range of cosmetic products because of their biological activities. In addition, vertical polyethylene bioreactors have been developed by Arad and Levy-Ontman (2010) to facilitate the commercial production of PS. These bioreactors are currently used by the multinational company Frutarom, located in the Negev region of Israel (Table 7).

5.3. Pharmaceutical and/or therapeutic markets

There exist many biological activities promoted by red microalgae PS which could be a source of value-added in the pharmaceutical area but only a few are effective at this time. EPS from red microalgae have many potential applications such as: antiviral, anti-tumor, antibacterial, biosurfactant, bioemulsifier… Indeed, this review puts up-to-date and summarizes the importance of EPS from red microalgae and their high-value applications in pharmaceutical and/or therapeutic fields (Tables 7 and 8). All these activities demonstrated by several researchers depend on the physico-chemical characteristics of the PS such as degree of sulphation, molecular weight, nature of sugars and glycosidic branching. For example, the PS and their oligomers obtained by microwave degradation from *P. cruentum* (Sun et al., 2009) and *Porphyridium* sp. (Tannin-Spitz et al., 2005) have an antioxidant power. They fight reactive oxygen species produced under the influence of a strong light radiation. The EPS of *P. cruentum* are also described for their anti-tumor and immunomodulatory activity (Sun et al., 2012). Indeed, they improve the immune response by stimulating the proliferation of macrophages and lymphocytes. These biological activities are more important for small molecules (Mw = 6.55×10^4 g.mol^{-1}) compared with higher ones (Mw = 2.56×10^5 g.mol^{-1}). Moreover, Arad's team confirmed that there was a linear relationship between the percentage of sulphate in the PS and its antiviral activity against the Herpes virus (Arad and Levy-Ontman, 2010). These PS exhibited also chelating activities. A formulation comprising a red microalgae PS and a heavy metal was patented by Arad in 2009 (Arad, 2009). The metal deficiencies are becoming a real problem in society because of the rising of people suffering from them. PS such as hyaluronate can be used as chelates in pharmaceutical formulations to deliver metals (Nimrod and Greenman, 1986). The patent US20110070159A1 (Arad, 2009) was based on the same principle. PS produced by red microalgae can also be exploited as active biomolecules. The patent WO1997000689A1 (Arad et al., 1995) claims that PS from red microalgae are effective antiviral agents protecting against viral infection, especially the Varicella Zoster virus. This patent also claimed a formulation of one or several PS from *Porphyridium* sp., *P. aerugineum* and *Rhodella reticulata* preventing viral infection and replication. sPS from red microalgae belonging to *Porphyridium* and *Rhodella* genus were also employed successfully to prevent infection by microsporidia in humans or animals but especially in bees (Michaud et al., 2012).

The other high value biomolecules produced by Rhodophyta and used in pharmaceutical area are pigments. Microalgae synthesize fluorescent pigments which are coupled with chlorophyll in order to realize their photosynthesis. These fluorescent molecules, after extraction and purification are used as fluorescent markers in the medical diagnostic industry and offer a good alternative to radioimmunological tracers with the same sensitivity of detection. Their applications are valuable in different areas including the medical diagnostics, molecular biology, and fluorescence techniques, like the fluorescence activated cell sorting (FACS), enzymatic markers, immuno-detection or assay by fluorescence resonance energy transfer (FRET). Among phycobiliproteins produced by red microalgae, B-PE from the genus *Porphyridium*, is commercially available as fluorescent agent for flow cytometry and immunofluorescent staining (*e.g.* Invitrogen, Columbia Bio-sciences, AnaSpec) (Ibanez-Gonzáleza et al., 2016). Bermejo et al. (2002) estimated the cost of production of B-PE from *P. cruentum* at 55000 US$ per Kg. Furthermore, Richa et al. (2011) mentioned in their review the potential commercial applications of phycobiliproteins such as biomedicine as antioxidant, anti-inflammatory, neuroprotective and hepatoprotective, and fluorescence-based immunity assays. Moreover, PE extracted from the seaweeds *Porphyra haitanensis* has recently been described as having cancer-preventing potential due to its antioxidant properties, Pan et al., 2013, opening new high added value opportunities for PE from microalgae.

6. Conclusions

Red microalgae form a large taxonomic group of benthic marine microalgae. They are uni-and pluricellular photosynthetic microorganisms belonging to Cyanidiophytina and Rhodophytina subphyla. Their main characteristics are to possess a polysaccharidic mucilage instead of a classical cell wall and a complex phycobilisome permiting their adaptation to depths up to 250 m. Even if the potential of some of them, such as those belonging to *Porphyridium* or *Rhodella* genera, has been fully explored by academic laboratories and some companies, other are still poorly exploited despite their potential, as shown by the very low number of patents claiming on them. Considering the industrial exploitation of the genus *Porphyridium* and *Rhodella*, parameters affecting their growth in photoautotrophy in PBR (rather than in open ponds) with several geometries ans structures have been abundantlty investigated. Theses works have mainly focused on elaboration of specific culture media and on characterization of the best physico-chemical parameters of culture. The recent analysis of available nuclear genomes of some red microalgae showed structural differences on sizes of genomes as well as on number of chromosomes. The exploration of these genomes but also of metabolites production by poorly described species could be a source of innovation. As an example, recent works in our group on the EPS production by the strain *Flintiella sanguinaria* led to the identification of an undescribed methylated and acetylated galactoxylan having some structural similarities with EPS from *Porphyridium* or *Rhodella* species but including significant amounts of rhamnose and glucuronic acid in its structure and exhibiting no sulphate groups (Gaignard et al., 2018). The low number of research programs focusing on the screening of microalgae species for the production of high value products and not only for the production of biofuels but also their costs of production, are probably at the origin of this underexploitation of red microalgae. Indeed, in their review published in 2010 and entitled “Red microalgal cell-wall PS: biotechnological aspects”, Arad and Levy-Ontman concluded that an alternative to PS from red and brown macroalgae usually harvested from their natural habitat could be the sPS from red microalgae. It was possible at this time to extend this assessment to other high value compounds such as pigments, enzymes, or PUFAs detected in red microalgae. Eight years later nothing has really changed as the cost for red microalgae production in photoautotrophy in diluted media has severely limited their applications in large scale markets such as food, feed, or hydrocolloids. The sole examples of real access to niche markets (medical diagnostic and cosmetic) are those of B-PE and sPS from *Porphyridium* strains. So what is the future for red microalgae? It can probably be divided into three sections; *i.e.* (i) the screening of natural ecosystems for the identification of new species and better comprehension of their physiology/metabolism; (ii) the development of bioengineering approaches to modify the metabolism of these microorganisms; and (iii) process intensification and improvement for their production in photoautotrophy at high cellular density is a difficult but necessary journey towards large scale production with low costs.

Acknowledgements

This work was supported by the ANR POLYSALGUE project, grant ANR-15-CE21-0013 of the French Agence Nationale de la Recherche.

References

Abeliovitch, A., Azov, Y., 1976. Toxicity of ammonia to algae in sewage oxidation pounds. Appl. Environ. Microbiol. 31, 801–806.
Ahern, T.J., Katoh, S., Sada, E., 1983. Arachidonic acid production by the red alga *Porphyridium cruentum*. Biotechnol. Bioeng. 25, 1050–1070.
Aizawa, K., Miyachi, S., 1986. Carbonic anhydrase and CO_2 concentrating mechanisms in microalgae and cyanobacteria. FEMS Microbiol. Rev. 39, 215–233.
Akimoto, S., Yokono, M., Hamada, F., Teshigahara, A., Aikawa, S., Kondo, A., 2012. Adaptation of light-harvesting systems of *Arthrospira platensis* to light conditions, probed by time-resolved fluorescence spectroscopy. Biochim. Biophys. Acta Bioenerg. 1817, 1483–1489.
Al Amoudi, O.A., 1993. Ammonium assimilation in the red microalga *Porphyridium purpureum*. J. Univ. Kuwait (Sci.) 20, 251–258.
Amoroso, G., Sultemeyer, D., Thyssen, C., Fock, H.P., 1998. Uptake of HCO_3^- and CO_2 in cells and chloroplasts from the microalgae *Chlamydomonas reinhardtii* and *Dunaliella tertiolecta*. Plant Physiol. 116, 193–201.
Arad, S., 2009. Compositions Comprising Red Microalgae Polysaccharides and Metals, US20110070159A1.
Arad, S.M., Levy-Ontman, O., 2010. Red microalgal cell-wall polysaccharides: biotechnological aspects. Curr. Opin. Biotechnol. 21, 358–364.
Arad, S., Weinstein, Y., 2003. Novel lubricants from red microalgae: interplay between genes and products. Biomedica 1, 32–37.
Arad, S.M., Yaron, A., 1992. Natural pigments from red microalgae for use in foods and cosmetics. Trends Food Sci. Technol. 3, 92–97.
Arad, S.M., Friedman, O.D., Rotem, A., 1988. Effect of nitrogen on polysaccharide production in a *Porphyridium* sp. Appl. Environ. Microbiol. 54, 2411–2414.
Arad, S., Lerental, Y., Dubinsky, O., 1992. Effect of nitrate and sulfate starvation on polysaccharide formation in *Rhodella reticulate*. Bioresour. Technol. 42, 141–148.
Arad, S., Huliheil, M., Tal, J., 1995. Antiviral Agents, WO1997000689A1.
Arad, S., Rapoport, L., Moshkovich, A., Van Moppes, D., Karpasas, M., Golan, R., Golan, Y., 2006. Superior biolubricant from a species of red microalga. Langmuir 22, 7313–7317.
Ayyagari, M., Pande, R., Kamtekar, S., Gao, H., Marx, K., Kumar, J., Tripathy, S., Akkara, J., Kaplan, D., 1995. Molecular assembly of proteins and conjugated polymers: toward development of biosensors. Biotechnol. Bioeng. 45, 116–121.
Azeredo, J., Henriques, M., Sillankorva, S., Oliveira, R., 2003. Extraction of exopolymers from biofilms: the protective effect of glutaraldehyde. Water Sci. Technol. 47, 175–179.
Badger, M.R., 1987. The CO_2 concentrating mechanism in aquatic phototrophs. In: Hatch, M.D., Boardman, N.K. (Eds.), The Biochemistry of Plants: A Comprehensive Treatise. Academic Press, San Diego, pp. 219–274.
Badger, M.R., Price, G.D., 2003. CO_2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. J. Exp. Bot. 54, 609–622.
Baer, S., Heining, M., Schwerna, P., Buchholz, R., Hübner, H., 2016. Optimization of spectral light quality for growth and product formation in different microalgae using a continuous photobioreactor. Algal Res. 14, 109–115.
Barbier, G., Oesterhelt, C., Larson, M.D., Halgren, R.G., Wilkerson, C., Garavito, R.M., Benning, C., Weber, A.P.M., 2005. Comparative genomics of two closely related unicellular thermo-acidophilic red algae, *Galdieria sulphuraria* and *Cyanidioschyzon merolae*, reveals the molecular basis of the metabolic flexibility of *Galdieria sulphuraria* and significant differences in carbohydrate metabolism of both algae. Plant Physiol. 137, 460–474.
Barsanti, L., Gualtieri, P., 2018. Is exploitation of microalgae economically and energetically sustainable? Algal Res. 31, 107–115.
Bayona, K.C.D., Gallón, S.M.N., Estrada, A.L., Rios, J.C., Garces, L.A., Martínez, A.M., 2012. Activity of sulfated polysaccharides from microalga *Porphyridium cruentum* over degenerative mechanisms of the skin. Int. J. Sci. Adv. Technol. 2, 85–92.
Beale, S.I., 1993. Biosynthesis of phycobilins. Chem. Rev. 93, 785–802.
Bean, R.C., Hassid, W.Z., 1955. Assimilation of $^{14}CO_2$ by photosynthesizing red algae *Iridophycus flaccidum*. J. Biol. Chem. 212, 411–425.
Becker, E.W., 1994. Microalgae: Biotechnology and Microbiology. Cambridge University Press, Cambridge.
Becker, E.W., 2007. Micro-algae as a source of protein. Biotechnol. Adv. 25, 207–210.
Benavides, J., Rito-Palomares, M., 2006. Simplified two-stage method to B-phycoerythrin recovery from *Porphyridium cruentum*. J. Chromatogr. B 844, 39–44.
Bermejo, R., Talavera, E.M., delValle, C., Alvarez-Pez, J.M., 2000. C-phycocyanin incorporated into reverse micelles: a fluorescence study. Colloids Surf. B. 18, 51–59.
Bermejo, R.R., Alvarez-Pez, J.M., Acien Fernandez, F.G., Molina Grima, E., 2002. Recovery of pure B-phycoerythrin from the microalga *Porphyridium cruentum*. J. Biotechnol. 93, 73–85.
Bermejo, R., Acién, F.G., Ibáñez, M.J., Fernández, J.M., Molina, E., Alvarez-Pez, J.M., 2003. Preparative purification of B-phycoerythrin from the microalga *Porphyridium cruentum* by expanded-bed adsorption chromatography. J. Chromatogr. B 790, 317–325.
Bernal, J., Mendiola, J.A., Ibáñez, E., Cifuentes, A., 2011. Advanced analysis of nutraceuticals. J. Pharm. Biomed. Anal. 55, 758–774.
Bertilsson, S., Berglund, O., Karl, D., Chisholm, S.W., 2003. Elemental composition of marine *Prochlorococcus* and *Synechococcus*: implications for the ecological stoichiometry of the sea. Limnol. Oceanogr. 48, 1721–1731.
Bertocchi, C., Navarini, L., Ceshro, A., 1990. Polysaccharides from cyanobacteria. Carbohydr. Polym. 12, 127–153.
Bhattacharya, D., Price, D.C., Chan, C.X., Qiu, H., Rose, N., Ball, S., Weber, A.P., Arias, M.C., Henrissat, B., Coutinho, P.M., Krishnan, A., Zäuner, S., Morath, S., Hilliou, F., Egizi, A., Perrineau, M.M., Yoon, H.S., 2013. Genome of the red alga *Porphyridium purpureum*. Nat. Commun. 4, 1941.
Borowitzka, M.A., 1988. Microalgae as sources of essential fatty acids. Aust. J. Biotechnol. 1, 58–62.
Bouarab, L., Dautab, A., Loudikia, M., 2004. Heterotrophic and mixotrophic growth of *Micractinium pusillum* Fresenius in the presence of acetate and glucose: effect of light and acetate gradient concentration. Water Res. 38, 2706–2712.
Bracey, M.H., Christiansen, J., Tovar, P., Cramer, S.P., Sue, G., Bartlett, S.G., 1994. Spinach carbonic anhydrase: investigation of the zinc-binding ligands by site-directed mutagenesis, elemental analysis, and EXAFS. Biochemistry 33, 13126–13131.
Brody, M., Emerson, R., 1959. The effect of wavelength and intensity of light on the proportion of pigments in *Porphyridium cruentum*. Am. J. Bot. 46, 433–440.
Brown, M.J., Lester, J.N., 1980. Comparison of bacterial extracellular polymer extraction methods. Appl. Environ. Microbiol. 40, 179–185.
Burger, G., Saint-louis, D., Gray, M.W., Lang, B.F., 1999. Complete sequence of the mitochondrial DNA of red alga *Porhyra purpurea*. Cyanbacterial introns and shared ancestry of red and green algae. Plant Cell 11, 1675–1694.
Cagnac, O., Richard, L., Labro, J., 2015. Novel Method for the Culture of Unicellular Red Algae, WO2017050917A1.
Capone, D.G., Zehr, J.P., Paerl, H.W., Bergman, B., Carpenter, E., 1997. *Trichodesmium*, a globally significant marine cyanobacterium. Science 276, 1221–1229.
Carvalho, C.M., Francisco, F.R., Almeida, K., Sato, S., Converti, A., 2004. Cultivation of *Arthrospira (Spirulina) platensis* (cyanophyceae) by Fed batch addition of ammonium chloride at exponentially increasing feeding rates. J. Phycol. 40, 589–597.
Cecchin, M., Ben Fatto, S., Griggio, F., Mori, A., Azzaniga, S., Vitulo, N., Delledonne, M., Ballottari, M., 2018. Molecular basis of autotrophic vs mixotrophic growth in *Chlorella sorokiniana*. Sci. Rep. 8, 6465.
Chaloub, R.M., Motta, N.M.S., de Araujo, S.P., de Aguiar, P.F., da Silva, A.F., 2015. Combined effects of irradiance, temperature and nitrate concentration on phycoerythrin content in the microalga *Rhodomonas sp.* (Cryptophyceae). Algal Res. 8, 89–94.
Chao, L., Bowen, C.C., 1971. Purification and properties of glycogen isolated from a blue-green alga, *Nostoc muscorum*. J. Bacteriol. 105, 331–338.
Chen, F., Zhang, Y., 1997. High cell density mixotrophic culture of *Spirulina platensis* on glucose for phycocyanin production using a fed-batch system. Enzym. Microb. Technol. 20, 221–224.
Chen, F., Zhang, Y., Guo, S., 1996. Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. Biotechnol. Lett. 18, 603–608.
Chen, B., You, W., Huang, J., Yu, Y., Chen, W., 2010. Isolation and antioxidant property of the extracellular polysaccharide from *Rhodella reticulate*. World J. Microbiol. Biotechnol. 26, 833–840.
Chojnacka, K., Noworyta, A., 2004. Evaluation of *Spirulina* sp. growth in photo-autotrophic, heterotrophic and mixotrophic cultures. Enzym. Microb. Technol. 3, 461–465.
Cian, R.E., Drago, S.R., de Medina, F.S., Martinez-Augustin, O., 2015. Proteins and carbohydrates from red seaweeds: evidence for beneficial effects on gut function and microbiota. Mar. Drugs 13, 5358–5383.
Cohen, E., Arad, S.M., 1989. A closed system for outdoor cultivation of *Porphyridium*. Biomass 18, 59–67.
Cohen, Z., Cohen, S., 1991. Preparation of eicosapentaenoic acid (EPA) concentrate from *Porphyridium cruentum*. J. Am. Oil Chem. Soc. 68, 16–19.
Cohen, Z., Vonshak, A., Richmond, A., 1988. Effect of environmental conditions on fatty acid composition of the red alga *Porphyridium cruentum*: correlation to growth rate. J. Phycol. 24, 328–332.
Cohen, Z., Norman, H.A., Heimer, Y.M., 1995. Microalgae as a source of omega-3 fatty acids. World Rev. Nutr. 77, 1–31.
Colman, B., Gehl, K.A., 1983. Physiological characteristics of photosynthesis in *Porphyridium cruentum*: evidence for bicarbonate transport in a unicellular red alga. J. Phycol. 19, 216–219.
Colman, B., Huertas, I.E., Bhatti, S., Dason, J.S., 2002. The diversity of inorganic carbon acquisition mechanisms in eukaryotic algae. Funct. Plant Biol. 29, 261–270.
Comte, S., Guibaud, G., Baudu, M., 2006. Relations between extraction protocols for activated sludge extracellular polymeric substances (EPS) complexation properties: Part I. Comparison of the efficiency of eight EPS extraction methods. Enzym. Microb. Technol. 38, 237–245.
Converti, A., Scapazzoni, S., Lodi, A., Carvalho, J.C.M., 2006. Ammonium and urea removal by *Spirulina platensis*. J. Ind. Microbiol. Biotechnol. 33, 8–16.
Cornet, J.F., 2010. Calculation of optimal design and ideal productivities of volumetrically lightened photobioreactors using the constructal approach. Chem. Eng. Sci. 65, 985–998.
Cornet, J.F., Dussap, C.G., 2009. A Simple and reliable formula for assessment of maximum volumetric productivities in photobioreactors. Biotechnol. Prog. 25, 424–435.
Cornet, J.F., Dussap, C.G., Cluzel, P., Dubertret, G., 1992. A structured model for simulation of cultures of the cyanobacterium *Spirulina platensis* in photobioreactors: II Identification of kinetic parameters under light and mineral limitations. Biotechnol. Bioeng. 40, 826–834.
Cuellar-Bermudez, S.P., Aguilar-Hernandez, I., Cardenas-Chavez, D.L., Ornelas-Soto, N., Romero-Ogawa, M.A., Parra-Salvidar, R., 2015. Extraction and purification of high-value metabolites from microalgae: essential lipids, astaxanthin and phycobiliproteins. Microb. Biotechnol. 8, 190–209.
Cunningham, F.X., Dennenberg, R.J., Mustardy, L., Jursinic, P.A., Gantt, E., 1989. Stoichiometry of photosystem I, photosystem II, and phycobilisomes in the red alga

Porphyridium cruentum as a function of growth irradiance. Plant Physiol. 91, 1179–1187.
Cunningham, F.X., Lee, H., Gantt, E., 2007. Carotenoid biosynthesis in the primitive red alga *Cyanidioschyzon merolae*. Eukaryot. Cell 6, 533–545.
Da Silva, A.F., Lourenço, S.O., Chaloub, R.M., 2009. Effects of nitrogen starvation on the photosynthetic physiology of a tropical marine microalga *Rhodomonas sp.* (Cryptophyceae). Aquat. Bot. 91, 291–297.
Dai, G.-Z., Qiu, B.-S., Forchhammer, K., 2014. Ammonium tolerance in the cyanobacterium *Synechocystis* sp. strain PCC 6803 and the role of the psbA multigene family. Plant Cell Environ. 37, 840–851.
Dainippon InK and Chemicals Inc., Jpn. Pat., 06 (1987), pp. 691.
Darley, W.M., 1987. Biología de las Algas: Enfoque Fisiológico.
Datla, P., 2011. The Wonder Molecule Called Phycocyanin. Parry Nutraceuticals, Valensa International, pp. 1–6.
De Luca, P., Moretti, A., 1983. Floridosides in *Cyanidium caldarium*, *Cyanidioschyzon merolae* and *Galdieria sulphuraria* (Rhodophyta, Cyanidiophyceae). J. Phycol. 19, 368–369.
De-Bashan, L.E., Trejo, A., Huss, V.A.R., Hernandez, J.P., Bashan, Y., 2008. *Chlorella sorokiniana* UTEX 2805, a heat and intense, sunlight-tolerant microalga with potential for removing ammonium from wastewater. Bioresour. Technol. 99, 4980–4989.
Degrenne, B., Pruvosta, J., Christophe, G., Cornet, J.F., Cognea, G., Legrand, J., 2010. Investigation of the combined effects of acetate and photobioreactor illuminated fraction in the induction of anoxia for hydrogen production by *Chlamydomonas reinhardtii*. Int. J. Hydrog. Energy 35, 10741–10749.
Delattre, C., Pierre, G., Laroche, C., Michaud, P., 2016. Production, extraction and characterization of microalgal and cyanobacterial exopolysaccharides. Biotechnol. Adv. 34, 1159–1179.
Dere, S., Dalkiran, N., Karacaoglu, D., Yildiz, G., Dere, E., 2003. The determination of total protein, total soluble carbohydrate and pigment contents of some macroalgae collected from Gemli-Karacaali (Bursa) and Erdek-Ormanli (Balikesir) in the Sea of Marmara, Turkey. Oceanologia 45, 453–471.
Dermoun, D., Chaumont, D., Thebault, J.M., Dauta, A., 1992. Modelling of growth of *Porphyridium cruentum* in connection with two interdependent factors: Light and temperature. Bioresour. Technol. 42, 113–117.
Diaz, J., Ingall, E., Benitez-Nelson, C., Paterson, D., de Jonge, M.D., McNulty, I., Brandes, J.A., 2008. Marine polyphosphate: a key player in geologic phosphorus sequestration. Science 320, 652–655.
Dillon, H.F., Somanchi, A., Rao, K., 2006a. Methods and Compositions for Cholesterol Reduction in Mammals, US 20070167396A1.
Dillon, H.F., Somanchi, A., Rao, K., 2006b. Methods and Compositions for Cholesterol Reduction in Mammals, US 20070167398A1.
Dillon, H.F., Zaman, A., Day, A.G., Coragliotti, A., 2006c. Microalgae-Derived Compositions for Improving the Health and Appearance of Skin, US20150140051A1.
Dixon, P.S., 1973. Biology of the Rhodophyta. Oliver and Boyd, Edinburgh, Scotland.
Drath, M., Kloft, N., Batschauer, A., Marin, K., Novak, J., Forchhammer, K., 2008. Ammonia triggers photodamage of photosystem II in the cyanobacterium *Synechocystis* sp. strain PCC 6803. Plant Physiol. 147, 206–215.
Dupré, C., Guary, J.-C., Grizeau, D., 1994. Effect of photon fluence rate, nitrogen limitation and nitrogen recovery on the level of phycoerythrin in the unicellular alga, *Rhodosorus marinus* (Rhodophyceae). Physiol. Plant. 92, 521–527.
Dvir, I., Chayoth, R., Sod-Moriah, U., Shany, S., Nyska, A., Stark, A.H., Madar, Z., Arad, S.M., 2000. Soluble polysaccharide and biomass of red microalga *Porphyridium* sp. alter intestinal morphology and reduce serum cholesterol in rats. Br. J. Nutr. 84, 469–476.
Ellner, P.D., Steers, E., 1955. Urea as a carbon source for *Chlorella* and *Scenedesmus*. Arch. Biochem. Biophys. 59, 534–535.
El-Sheekh, M.M., Bedaiwy, M.Y., Osman, M.E., Ismail, M.M., 2012. Mixotrophic and heterotrophic growth of some microalgae using extract of fungal-treated wheat bran. Int. J. Recycl. Org. Waste Agric. 1, 12.
Eteshola, E., Karpasas, M., Arad, S., Gottlieb, M., 1998. Red microalga exopolysaccharides: 2. Study of the rheology, morphology and thermal gelation of aqueous preparations. Acta Polym. 49, 549–556.
Evans, L.V., Maureen, E., 1974. Studies on the synthesis and composition of extracellular mucilage in the unicellular red alga *Rhodella*. J. Cell Sci. 16, 1–21.
Fȧbregas, J., García, D., Lamela, T., Otero, A., 1999a. Mixotrophic production of phycoerythrin and exopolysaccharide by the microalga *P.cruentum*. Cryptogam. Algol. 20, 89–94.
Fȧbregas, J., Garcıa, D., Fernandez-Alonso, M., Rocha, A.I., Gomez-Puertas, P., Escribano, J.M., Otero, A., Coll, J., 1999b. In vitro inhibition of the replication of haemorrhagic septicaemia virus (VHSV) and African swine fever virus (ASFV) by extracts from marine microalgae. Antivir. Res. 44, 67–73.
Flechtner, V.R., 2007. North American microbiotic soil, crust communities: diversity despite challenge. In: Seckbach, J. (Ed.), Algae and Cyanobacteria in Extreme Environments. Springer, Dordrecht, The Netherlands, pp. 539–551.
Fleck-Schneider, P., Lehr, F., Posten, C., 2007. Modelling of growth and product formation of *Porphyridium purpureum*. J. Biotechnol. 132, 134–141.
Freshwater, D.W., Fredericq, S., Butler, B.S., Hommersand, M.H., Chase, M.W., 1994. A gene phylogeny of the red algae (*Rhodophyta*) based on plastid rbcL. Proc. Natl. Acad. Sci. U. S. A. 91, 7281–7285.
Frølund, B., Palmgren, R., Keiding, K., Nielsen, P.H., 1996. Extraction of extracellular polymers from activated sludge using a cation exchange resin. Water Res. 30, 1749–1758.
Fuentes, M.M.R., Sȧnchez, J.L.G., Sevilla, J.M.F., Fernȧndez, F.G.A., Pérez, J.A.S., Grima, E.M., 1999. Outdoor continuous culture of *Porphyridium cruentum* in a tubular photobioreactor: quantitative analysis of the daily cyclic variation of culture parameters. J. Biotechnol. 70, 271–288.
Fuentes, M.M.R., Fernandez, G.G.A., Pérez, J.A.S., Guerrero, J.L.G., 2000. Biomass nutrient profiles of the microalga *Porphyridium cruentum*. Food Chem. 70, 345–353.
Fuentes-Grünewald, C., Baylissa, C., Zanain, M., Pooley, C., Scolamacchia, M., Silkinaa, A., 2015. Evaluation of batch and semi-continuous culture of *Porphyridium purpureum* in a photobioreactor in high latitudes using Fourier Transform Infrared spectroscopy for monitoring biomass composition and metabolites production. Bioresour. Technol. 189, 357–363.
Fuggi, A., 1989. Competition between nitrate and nitrite as a tool to study regulation by ammonium of nitrate metabolism in the microalga *Cyanidium caldarium*. Plant Physiol. Biochem. 27, 563–568.
Fuggi, A., 1990. Uptake of inorganic nitrogen compounds in an acidophilic alga. In: Ullrich, W.R., Rigano, C., Fuggi, A., Aparicio, P.J. (Eds.), Aparicio Inorganic Nitrogen in Plants and Micro-Organisms. Springer-Verlag, Berlin, pp. 66–72.
Fuggi, A., 1993. Uptake and assimilation of nitrite in the acidophilic red alga *Cyanidium caldarium* Geitler. New Phytol. 125, 351–360.
Fukuda, S.Y., Yamamoto, R., Iwamoto, K., Minoda, A., 2018. Cellular accumulation of cesium in the unicellular red alga *Galdieria sulphuraria* under mixotrophic conditions. J. Appl. Phycol. 1–5.
Gabrielson, P.W., Garbary, D.J., Scagel, R.F., 1985. The nature of the ancestral red alga: inferences from a cladistic analysis. Biosystems 18, 335–346.
Gaignard, C., Macao, V., Gardarin, C., Picton, L., Rihouey, C., Michaud, P., Laroche, C., 2018. The red microalga *Flintiella sanguinaria* as a new exopolysaccharide producer. J. Appl. Phycol. 1–12.
Galvan, A., Fernández, E., 2001. Eukaryotic nitrate and nitrite transporters. Cell. Mol. Life Sci. 58, 225–233.
Gantt, E., Conti, S.F., 1965. The ultrastructure of *Porphyridium cruentum*. J. Cell Biol. 26, 365–381.
Garbary, D.J., Gabrielson, P.W., 1990. Taxonomy and evolution. In: Cole, K.M., Sheath, R.G. (Eds.), Biology of the Red Algae. Cambridge University Press, New York, pp. 477–498.
Gardian, Z., Bumber, L., Schrofel, A., Herbstova, M., Nebesarova, J., Vacha, F., 2007. Organisation of photosystem I and photosystem II in red alga *Cyanidium caldarium*: Encounter of cyanobacterial and higher plant concept. Biochim. Biophys. Acta Bioenerg. 1767, 725–731.
Geresh, S., Arad, S., 1991. The extracellular polysaccharides of the red microalgae: Chemistry and rheology. Bioresour. Technol. 38, 195–201.
Geresh, S., Dubinsky, O., Arad, S.M., Christiaen, D., 1990. Structure of a 3-O-(α-D-glucopyranosyluronic acid)-L-galactopyranose, an aldobiouronic acid isolated from the polysaccharides of various unicellular red algae. Carbohydr. Res. 208, 301–305.
Geresh, S., Lupescu, N., Arad, S.M., 1992. Fractionation and partial characterization of the sulphated polysaccharide of *Porphyridium*. Phytochemistry 31, 4181–4186.
Geresh, S., Arad, S., Shefer, A., 1997. Chemically crosslinked polysaccharide of the red microalga *Rhodella reticulate* an ion exchanger for toxic metal ions. J. Carbohydr. Chem. 16, 703–708.
Geresh, S., Mamontov, A., Weinstein, J., 2002. Sulfation of extracellular polysaccharides of the red microalgae: preparation, characterization and properties. J. Biochem. Biophys. Methods 50, 179–187.
Geresh, S., Arad, S.M., Levy-Ontman, O., Zhang, W., 2009. Isolation and characterization of poly- and oligosaccharides from the red microalga *Porphyridium sp*. Carbohydr. Res. 344, 343–349.
Gigova, L.G., Ivanova, N.J., 2015. Microalgae respond differently to nitrogen availability during culturing. J. Biosci. 40, 365–374.
Ginzberg, A., Cohen, M., Sod-Moriah, U.A., Shany, S., Rosenshtrauch, A., Arad, S.M., 2000. Chickens fed with biomass of the red microalga *Porphyridium* sp. have reduced blood cholesterol level and modified fatty acid composition in egg yolk. J. Appl. Phycol. 12, 325–330.
Giordano, M., Beardall, J., Raven, J.A., 2005a. CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. Annu. Rev. Plant Biol. 56, 99–131.
Giordano, M., Norici, A., Hell, R., 2005b. Sulfur and phytoplankton: acquisition, metabolism and impact on the environment. New Phytol. 166, 371–382.
Givens, D.I., Gibbs, R.A., 2006. Very long chain n-3 polyunsaturated fatty acids in the food chain in the UK and the potential of animal-derived foods to increase intake. Nutr. Bull. 31, 104–110.
Glazer, A.N., 1994. Phycobiliproteins – a family of valuable, widely used fluorophores. J. Appl. Phycol. 6, 105–112.
Glazer, A.N., Hixson, C.S., 1977. Subunit structure and chromophore composition of rhodophytan phycoerythrins, porphyridium cruentum B-phycoerythrin and b-phycoerythrin. J. Biol. Chem. 252, 32–42.
Glazer, A.N., Stryer, L., 1984. Phycofluor probes. Trends Biochem. Sci. 9, 423–427.
Gloaguen, V., Ruiz, G., Morvan, H., Mouradi-Givernaud, A., Maes, E., Krausz, P., Strecker, G., 2004. The extracellular polysaccharide of *Porphyridium* sp.: an NMR study of lithium-resistant oligosaccharidic fragments. Carbohydr. Res. 339, 97–103.
Gobler, C.J., Berry, D.L., Dyhrman, S.T., Wilhelm, S.W., Salamov, A., Lobanov, A.V., Zhang, Y., Collier, J.L., Wurch, L.L., Kustka, A.B., Dill, A.D., Shah, M., VerBerkmoes, N.C., Kuo, A., Terry, A., Pangilinan, J., Lindquist, E.A., Lucas, S., Paulsen, I.T., Hattenrath-Lehmann, T.K., Talmage, S.C., Walker, E.A., Koch, F., Burson, A.M., Marcoval, M.A., Tang, Y.Z., LeCleir, G.R., Coyne, K.J., Berg, G.M., Bertrand, E.M., Saito, M.A., Gladyshev, V.N., Grigoriev, I.V., 2011. Niche of harmful alga *Aureococcus anophagefferens* revealed through ecogenomics. Proc. Natl. Acad. Sci. U. S. A. 108, 4352–4357.
Goddijn, O.J.M., van Dun, K., 1999. Trehalose metabolism in plants. Trends Plant Sci. 4, 315–319.
Gomez-Garcia, M.R., Losada, M., Serrano, A., 2003. Concurrent transcriptional activation of ppA and ppX genes by phosphate deprivation in the cyanobacterium *Synechocystis* sp. strain PCC 6803. Biochem. Biophys. Res. Commun. 302, 601–609.

Götz, R., Gnann, A., Zimmermann, F.K., 1999. Deletion of the carbonic anhydrase-like gene *NCE103* of the yeast *Saccharomyces cerevisiae* causes an oxygen-sensitive growth defect. Yeast 15, 855–864.
Graziani, G., Schiavo, S., Nicolai, M.A., Buono, S., Fogliano, V., Pinto, G., Pollio, A., 2013. Microalgae as human food: chemical and nutritional characteristics of the thermos-acidophilic microalga *Galdiera sulphuraria*. Food Funct. 4, 144–152.
Gross, W., 1999. Revision of comparative traits for the acido- and thermophilic red algae *Cyanidium* and *Galdieria*. In: Seckbach, J. (Ed.), Enigmatic Microorganisms and Life in Extreme Environments. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 437–446.
Gross, W., Schnarrenberger, C., 1995. Heterotrophic growth of two strains of the acido-thermophilic red alga *Galdieria sulphuraria*. Plant Cell Physiol. 36, 633–638.
Guihéneuf, F., Stengel, D.B., 2015. Towards the biorefinery concept: Interaction of light, temperature and nitrogen for optimizing the co-production of high-value compounds in *Porphyridium purpureum*. Algal Res. 10, 152–163.
Guil-Guerrero, J., Navarro-Juárez, R., López-Martnez, J.C., Campra-Madrid, P., Rebolloso-Fuentes, M.M., 2004. Functional properties of the biomass of three microalgal species. J. Food Eng. 65, 511–517.
Guiry, M.D., 2012. How many species of algae are there? J. Phycol. 48, 1057–1063.
Guschina, I.A., Harwood, J.L., 2006. Lipids and lipid metabolism in eukaryotic algae. Prog. Lipid Res. 45, 160–186.
Guzman-Murillo, M.A., Ascencio, F., 2000. Anti-adhesive activity of sulphated exopolysaccharides of microalgae on attachment of red sore disease-associated bacteria and *Helicobacter pylori* to tissue cultured cells. Lett. Appl. Microbiol. 30, 473–478.
Handayani, N.A., Ariyanti, D., Hadiyanto, H., 2011. Potential Production of Polyunsaturated Fatty Acids from Microalgae. Int. J. Sci. Eng. 2, 13–16.
Harun, R., Singh, M., Forde, G.M., Danquah, M.K., 2010. Bioprocess engineering of microalgae to produce a variety of consumer products. Renew. Sust. Energ. Rev. 14, 1037–1047.
Hasui, M., Matsuda, M., Okutani, K., Shigeta, S., 1995. In vitro antiviral activities of sulfated polysaccharides from a marine microalga (*Cochlodinium polykrikoides*) against human immunodeficiency virus and other enveloped viruses. Int. J. Biol. Macromol. 17, 293–297.
Heimann, K., Huerlimann, R., 2015. Microalagal classification: major classes and genera of commercial microalgal species. In: Kim, S.K. (Ed.), Handboock of Marine Microalgae: Biotechnology Advances. Elsevier Inc., London, pp. 25–41.
Henriques, M., Silva, A., Rocha, J.M., 2007. Extraction and quantification of pigments from a marine microalga: A simple and reproducible method. In: Méndez-Vilas, A. (Ed.), Communicating Current Research and Educational Topics and Trends in Applied Microbiology, pp. 586–593.
Hewett-Emmett, D., Tashian, R.E., 1996. Functional diversity, conservation, and convergence in the evolution of the α-, β-, and γ-carbonic anhydrase gene families. Mol. Phylogenet. Evol. 5, 50–77.
Hirabaru, C., Izumo, A., Fujiwara, S., Tadokoro, Y., Shimonaga, T., Konishi, M., Yoshida, M., Fujita, N., Nakamura, Y., Yoshida, M., Kuroiwa, T., Tsuzuki, M., 2010. The primitive rhodophyte Cyanidioschyzon merolae contains a semiamylopectin-type, but not an amylose-type, alpha-glucan. Plant Cell Physiol. 51, 682–693.
Hirabayashi, S., Prillutsky, A., Sadamatsu, H., 1999. Method of Culturing Algae Capable of Producing Phototrophic Pigments, Highly Unsaturated Fatty Acids, or Polysaccharides at High Concentration, US6579714B1.
Ho, S.H., Chen, C.Y., Chang, J.S., 2013. Effect of light intensity and nitrogen starvation on CO_2 fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N. Bioresour. Technol. 113, 244–252.
Hu, Q., 2005. Environmental effects on cell composition. In: Richmond, A. (Ed.), Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Blackwell Publishing, Oxford, pp. 83–93.
Hu, J., Nagarajan, D., Zhang, Q., Chang, J.S., Lee, D.J., 2018. Heterotrophic cultivation of microalgae for pigment production: A review. Biotechnol. Adv. 36, 54–67.
Huang, J., Li, Y., Wan, M., Yan, Y., Feng, F., Qu, X., Wang, J., Shen, G., Li, W., Fan, J., Wang, W., 2014. Novel flat-plate photobioreactors for microalgae cultivation with special mixers to promote mixing along the light gradient. Bioresour. Technol. 159, 8–16.
Huleihel, M., Ishanu, V., Tal, J., Arad, S.M., 2001. Antiviral effect of red microalgal polysaccharides on Herpes simplex and Varicella zoster viruses. J. Appl. Phycol. 13, 127–134.
Huleihel, M., Ishanu, V., Tal, J., Arad, S., 2002. Activity of *Porphyridium* sp. polysaccharide against *herpes simplex* viruses *in vitro* and *in vivo*. J. Biochem. Biophys. Methods 50, 189–200.
Ibanez-Gonzáleza, J.M., Mazzuca-Sobczuka, T., Redondo-Mirandaa, R.M., Molina-Grimaa, E., Cooney, C.L., 2016. A novel vortex flow reactor for the purification of B-phycoerythrin from *Porphyridium cruentum*. Chem. Eng. Res. Des. 111, 24–33.
Iijima, N., Shimamatsu, H., 1982. Anti-tumor agent and method of treatment therewith, US Patent Pending.
Iqbal, M., Zafar, S.I., Stepan-Sarkissian, G., Fowler, M.W., 1993. Indoor mass cultivation of red alga *Porphyridium cruentum* in different types of bioreactors: effect of scale-up and vessel shape. J. Ferment. Bioeng. 75, 76–78.
Ivanova, J.G., Kabaivanova, L.V., Petro, P.D., Yankova, S.N., 2015. Optimization strategies for improved growth, polysaccharide production and storage of the red microalga *Rhodella reticulate*. Bulg. Chem. Commun. 47, 167–174.
Jain, K., Krause, K., Grewe, F., Nelson, G.F., Weber, A.P.M., Christensen, A.C., Mower, J.P., 2015. Extreme features of the *Galdieria sulphuraria* organellar genomes: a consequence of polyextremophily? Genome Biol. Evol. 7, 367–380.
de Jesus Raposo, M.F., de Morais, R.M.S.C., de Morais, A.M.M.B., 2013. Bioactivity and applications of sulphated polysaccharides from marine microalgae. Mar. Drugs 11, 233–252.
de Jesus Raposo, M.F., Morais, A.M.M.B., Morais, R.M.S.C., 2014. Influence of sulphate on the composition and antibacterial and antiviral properties of the exopolysaccharide from *Porphyridium cruentum*. Life Sci. 101, 56–63.
Jiao, G., Yu, G., Zhang, Y., Ewart, H.S., 2011. Chemical structures and bioactives of sulphated polysaccharides from marine algae. Mar. Drugs 9, 196–223.
Jones, R.F., Speer, H.L., Kury, W., 1963. Studies on the growth of the red Alg *Porphyridium cruentum*. Physiol. Plant. 16, 636–643.
Jubeau, S., Marchal, L., Pruvost, J., Jaouen, P., Legrand, J., Fleurence, J., 2013. High pressure disruption: a two-step treatment for selective extraction of intracellular components from the microalga *Porphyridium cruentum*. J. Appl. Phycol. 25, 983–989.
Kathiresan, S., Sarada, R., Bhattacharya, S., Ravishanka, G.A., 2007. Culture media optimization for growth and phycoerythrin production from *Porphyridium purpureum*. Biotechnol. Bioeng. 96, 456–463.
Kavitha, M.D., Kathiresan, S., Bhattacharya, S., Sarada, R., 2016. Culture media optimization of *Porphyridium purpureum*: production potential of biomass, total lipids, Arachidonic and eicosapentaenoic acid. J. Food Sci. Technol. 53, 2270–2278.
Kim, M., Yim, J.H., Kim, S.Y., Kim, H.S., Lee, W.G., Kim, S.J., Kang, P.S., Lee, C.K., 2012. In vitro inhibition of influenza A virus infection by marine microalga-derived sulfated polysaccharide p-KG03. Antivir. Res. 93, 253–259.
Kim, K.M., Park, J.H., Bhattacharya, D., Yoon, H.S., 2014. Applications of next-generation sequencing to unraveling the evolutionary history of algae. Int. J. Syst. Evol. Microbiol. 64, 333–345.
Kim, H.M., Oh, C.H., Bae, H.J., 2017. Comparison of red microalgae (*Porphyridium cruentum*) culture conditions for bioethanol production. Bioresour. Technol. 233, 44–50.
Kraan, S., 2012. Algal polysaccharides, novel applications and outlook. In: Chang, C.F. (Ed.), Carbohydrates - Comprehensive Studies on Glycobiology and Glycotechnology. Intech Chapter 22.
Kraft, G.T., Woelkerling, W.J., 1990. Rhodophyta-systematics and biology. In: Clayton, M.N., King, R.J. (Eds.), Biology of Marine Plants. Longman Cheshire, Melbourne, pp. 41–85.
Lamers, P.P., Janssen, M., De Vos, R.C.H., Bino, R.J., Wijffels, R.H., 2012. Carotenoid and fatty acid metabolism in nitrogen-starved *Dunaliella salina*, a unicellular green microalga. J. Biotechnol. 162, 21–27.
Larkum, A.W., 2016. Photosynthesis and light harvesting in algae. In: Borowitzka, M.A., Beardall, J., Raven, J.A. (Eds.), The Physiology of Microalgae. Springer International Publishing, Switzerland, pp. 67–87.
Lee, Y.K., Tan, H.M., 1988. Effect of temperature, light intensity and dilution rate on the cellular composition red alga *Porphyridium cruentum* in light-limited chemostat cultures. MIRCEN J. Appl. Microbiol. Biotechnol. 4, 231–237.
Lee, J., Ghosh, S., Sair, M.H., 2017. Comparative genomic analyses of transport proteins encoded within the red algae *Chondrus crispus*, *Galdieria sulphuraria* and *Cyanidioschyzon merolae*. J. Phycol. 53, 503–521.
Lehninger, A.L., Nelson, D.L., Cox, M.M., 2005. Lehninger Principles of Biochemistry, 4th ed. W.H. Freeman and company, New York.
Leupold, M., Hindersin, S., Gust, G., Kerner, M., Hanelt, D., 2013. Influence of mixing and shear stress on *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Chlamydomonas reinhardtii*. J. Appl. Phycol. 25, 485–495.
Levy, I., Gantt, E., 1988. Light acclimation in *Porphyridium purpureum* (rhodophyta): growth, photosynthesis, and phycobilisomes. J. Phycol. 24, 452–458.
Levy, L., Gantt, E., 1990. Development of photosynthetic activity in *Porphyridium purpureum* (Rhodophyta) following nitrogen starvation. J. Phycol. 26, 62–68.
Lewis, L.A., Flechtner, V.R., 2002. Green algae (chlorophyta) of desert microbiotic crusts: Diversity of North American taxa. Taxon 51, 443–451.
Li, Q.Y., Gao, X.S., Sun, Y., Zhang, Q.Q., Song, R.T., Xu, Z.K., 2006. Isolation and characterization of a sodium-dependent phosphate transporter gene in *Dunaliella viridis*. Biochem. Biophys. Res. Commun. 340, 95–104.
Li, S.Y., Lellouche, J.P., Shabtai, Y., Arad, S., 2001. Fixed carbon partitioning in the red microalga *Porphyridium* sp. (Rhodophyta). J. Phycol. 37, 289–297.
Li, Y., Han, D., Sommerfeld, M., Hu, Q., 2011a. Photosynthetic carbon partitioning and lipid production in the oleaginous microalga *Pseudochlorococcum* sp. (Chlorophyceae) under nitrogen-limited conditions. Bioresour. Technol. 102, 123–129.
Li, H., Li, Z., Xiong, S., Zhang, H., Li, N., Zhou, S., Liu, Y., Huang, Z., 2011b. Pilot-scale isolation of bioactive extracellular polymeric substances from cell-free media of mass microalgal cultures using tangential-flow ultrafiltration. Process Biochem. 46, 1104–1109.
Li, S.H., Xia, B.B., Zhang, C., Cao, J., Bai, L.H., 2012. Cloning and characterization of a phosphate transporter gene in *Dunaliella salina*. J. Basic Microbiol. 52, 429–436.
Liang, Y., Sarkany, N., Cui, Y., 2009. Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. Biotechnol. Lett. 31, 1043–1049.
Liqin, S., Wang, C., Lei, S., 2008. Effects of light regime on extracellular polysaccharide production by *Porphyridium cruentum* cultured in flat plate photobioreactors. In: Int. Conf. Bioinf. Biomed. Eng. 2nd, pp. 1488–1491.
Liu, H., Fang, H.P., 2002. Extraction of extracellular polymeric substances (EPS) of sludges. J. Biotechnol. 95, 249–256.
Liu, J., Huanga, J., Suna, Z., Zhong, Y., Jiang, Y., Chen, F., 2011. Differential lipid and fatty acid profiles of photoautotrophic and heterotrophic *Chlorella zofingiensis*: Assessment of algal oils for biodiesel production. Bioresour. Technol. 102, 106–110.
Liu, L., Pohnert, G., Wei, D., 2016. Extracellular metabolites from industrial microalgae and their biotechnological potential. Mar. Drugs 14, 191.
López, C.V.G., García, M.C.C., Fernández, F.G.A., Bustos, C.S., Chisti, Y., Sevilla, J.M.F., 2010. Protein measurements of microalgal and cyanobacterial biomass. Bioresour. Technol. 101, 7587–7591.
Lourenço, S.O., Barbarino, E., Mancini-Filho, J., Schinke, K.P., Aidar, E., 2002. Effects of different nitrogen sources on the growth and biochemical profile of 10 marine microalgae in batch culture: an evaluation for aquaculture. Phycologia 41, 158–168.

Lû, J., Sheahan, C., Fu, P., 2011. Metabolic engineering of algae for fourth generation biofuels production. Energy Environ. Sci. 4, 2451–2466.
Luo, H.P., Al-Dahhan, M.H., 2011. Airlift column photobioreactors for *Porphyridium* sp. culturing: part I. Effects of hydrodynamics and reactor geometry. Biotechnol. Bioeng. 109, 932–941.
Lupescu, N., Arad, S.M., Geresh, S., Bernstein, M.A., Glazer, R., 1991. Structure of some sulfated sugars isolated after acid hydrolysis of the extracellular polysaccharide of *Porphyridium sp*. a unicellular red alga. Carbohydr. Res. 210, 349–352.
Lutzu, G.A., Zhang, L., Zhang, Z., Liu, T., 2017. Feasibility of attached cultivation for polysaccharides production by *Porphyridium cruentum*. Bioprocess Biosyst. Eng. 40, 73–83.
Marcati, A., Ursu, A.V., Laroche, C., Soanen, N., Marchal, L., Jubeau, S., Djelveh, G., Michaud, P., 2014. Extraction and fractionation of polysaccharides and B-phycoerythrin from the microalga *Porphyridium cruentum* by membrane technology. Algal Res. 5, 258–263.
Markou, G., Muylaert, K., 2016. Effect of light intensity on the degree of ammonia toxicity on PSII activity of *Arthrospira platensis* and *Chlorella vulgaris*. Bioresour. Technol. 216, 453–461.
Marquardt, J., 1998. Effects of carotenoid-depletion on the photosynthetic apparatus of a *Galdieria sulphuraria* (Rhodophyta) strain that retains its photosynthetic apparatus in the dark. J. Plant Physiol. 152, 372–380.
Marquardt, J., Rehm, A.M., 1995. *Porphyridium purpureum* (Rhodophyta) from red and green light: characterization of photosystem I and determination of in situ fluorescence spectra of the photosystems. J. Photochem. Photobiol. B 30, 49–56.
Marquez, F.J., Sasaki, K., Kakizono, T., Nishio, N., Nagai, S., 1993. Growth characteristics of *Spirulina platensis* in mixotrophic and heterotrophic conditions. J. Ferment. Bioeng. 76, 408–410.
Martinez, M.J.A., Del Olmo, L.M.B., Benito, P.B., 2005. Antiviral activities of polysaccharides from natural sources. Stud. Nat. Prod. Chem. 30, 393–418.
Masojídek, J., Torzillo, G., Koblížek, M., 2013. Photosynthesis in microalgae. In: Richmond, A., Hu, Q. (Eds.), Handbook of Microalgal Culture: Applied Phycology and Biotechnology, 2nd ed. .
Mateo, P., Douterelo, I., Berrendero, E., Perona, E., 2006. Physiological differences between two species of cyanobacteria in relation to phosphorus limitation. J. Phycol. 42, 61–66.
Matsui, M.S., Muizzuddin, N., Arad, S., Marenus, K., 2003. Sulfated polysaccharides from red microalgae have antiinflammatory properties *in vitro* and *in vivo*. Appl. Biochem. Biotechnol. 104, 13–22.
Matsuzaki, M., Misumi, O., Shin-I, T., Maruyama, S., Takahara, M., Miyagishima, S.Y., Mori, T., Nishida, K., Yagisawa, F., Nishida, K., Yoshida, Y., Nishimura, Y., Nakao, S., Kobayashi, T., Momoyama, Y., Higashiyama, T., Minoda, A., Sano, M., Nomoto, H., Oishi, K., Hayashi, H., Ohta, F., Nishizaka, S., Haga, S., Miura, S., Morishita, T., Kabeya, Y., Terasawa, K., Suzuki, Y., Ishii, Y., Asakawa, S., Takano, H., Ohta, N., Kuroiwa, H., Tanaka, K., Shimizu, N., Sugano, S., Sato, N., Nozaki, H., Ogasawara, N., Kohara, Y., Kuroiwa, T., 2004. Genome sequence of the ultrasmall unicellular red alga *Cyanidioschyzon merolae* 10D. Nature 428, 653–657.
Mazard, S., Wilson, W.H., Scanlan, D.J., 2012. Dissecting the physiological response to phosphorus stress in marine *Synechococcus* isolates (cyanophyceae). J. Phycol. 48, 94–105.
McCracken, D.A., Cain, J.R., 1981. Amylose in floridean starch. New Phytol. 88, 67–71.
Merchuk, J.C., Gluz, M., Mukmenev, I., 2000. Comparison of photobioreactors for cultivation of the red microalga Porphyridium sp. J. Chem. Technol. Biotechnol. 75, 1119–1126.
Meseck, S.L., Alix, J.H., Wikfors, G.H., 2005. Photoperiod and light intensity effects on growth and utilization of nutrients by the aquaculture feed microalga, *Tetraselmis chui* (PLY429). Aquaculture 246, 393–404.
Miao, X., Wu, Q., 2004. High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella protothecoides*. J. Biotechnol. 110, 85–93.
Michaud, P., Laroche, C., Villay, A., Roussel, M., Diogon, M., Elalaoui, H., Delbac, F., 2012. Sulphated Polysaccharide Composition, US20150328253A1.
Mihova, S.G., Georgiev, D.I., Minkov, K.M., 1996. Phycobiliproteins in *Rhodella reticulate* and photoregulatory effects on their content. J. Biotechnol. 48, 251–257.
Mihova, S.G., Minkova, K.M., Petkov, G.D., Georgiev, D.I., 1997. Thermal and photoregulation of the lipid and fatty acid content in the cells of *Porphyridium sordidum*. In: Comptes rendus de l'académie bulgare des sciences: Sciences mathématiques et naturelles. vol. 50. pp. 111–114.
Miller, S.R., Castenholtz, R.W., 2001. Ecological Physiology of *Synechococcus*sp. Strain SH-94-5, a Naturally Occurring Cyanobacterium Deficient in Nitrate Assimilation. Appl. Environ. Microbiol. 67, 3002–3009.
Minhas, A.K., Hodgson, P., Barrow, C.J., Adholeya, A., 2016. A review on the assessment of stress conditions for simultaneous production of microalgal lipids and carotenoids. Front. Microbiol. 7, 1–19.
Minkova, K., Michailov, Y., Toncheva-Panova, T., Houbavenska, N., 1996. Antiviral activity of *Porphyridium cruentum* polysaccharide. Pharmazie 51, 194.
Misumi, O., Yoshida, Y., Nishida, K., Fujiwara, T., Sakajiri, T., Hirooka, S., Nishimura, Y., Kuroiwa, T., 2008. Genome analysis and its significance in four unicellular algae *Cyanidioschyzon merolae*, *Ostreococcus tauri*, *Chlamynomodas reinhardtii* and *Thalassiosira pseudonana*. J. Plant Res. 121, 3–17.
Mitsuhashi, S., Miyachi, S., 1996. Amino acid sequence homology between N- and C-terminal halves of a carbonic anhydrase in *Porphyridium purpureum*, as deduced from the cloned cDNA. J. Biol. Chem. 271, 28703–28709.
Mitsuhashi, S., Mizushima, T., Yamashita, E., Yamamoto, M., Kumasaka, T., Moriyama, H., Ueki, T., Miyachi, S., Tsukihara, T., 2000. X-ray structure of β-carbonic anhydrase from the red alga, *Porphyridium purpureum*, reveals a novel catalytic site for CO_2 hydration. J. Biol. Chem. 275, 552–5526.
Miyachi, S., Kanai, R., Mihara, S., Miyachi, S., Aoki, S., 1964. Metabolic roles of inorganic polyphosphates in *Chlorella* cells. Biochim. Biophys. Acta Gen. Subj. 93, 625–634.
Morales-Sànchez, D., Tinoco, R., Kyndt, J., Martinez, A., 2013. Heterotrophic growth of *Neochloris oleoabundans* using glucose as a carbon source. Biotechnol. Biofuels 6, 1–12.
Morales-Sànchez, D., Tinoco-Valencia, R., Caro-Bermudez, M.A., Martinez, A., 2014. Culturing *Neochloris oleoabundans* microalga in a nitrogen-limited, heterotrophic fed-batch system to enhance lipid and carbohydrate accumulation. Algal Res. 5, 61–69.
Mori, N., Moriyama, T., Toyoshima, M., Sato, N., 2016. Construction of global acyl lipid metabolic map by comparative genomics and subcellular localization analysis in the red alga *Cyanidioschyzon merolae*. Front. Plant Sci. 7, 958.
Moriyama, T., Mori, N., Sato, N., 2015. Activation of oxidative carbon metabolism by utritional enrichment by photosynthesis and exogenous organic compounds in the red alga *Cyanidioschyzon merolae*: evidence for heterotrophic growth. Springerplus 4, 559.
Moroney, J.V., Ruby, A., Ynalvez, R.A., 2007. Proposed carbon dioxide concentrating mechanism in *Chlamydomonas reinhardtii*. Eukaryot. Cell 6, 1251–1259.
Morot-Gaudry, J.F., 1997. Assimilation de l'azote chez les plantes: aspects physiologique, biochimique et moléculaire. INRA, pp. 1–422.
Muller-Feuga, A., Le Guédesa, R., Pruvost, J., 2003. Benefits and limitations of modeling for optimization of *Porphyridium cruentum* cultures in an annular photobioreactor. J. Biotechnol. 103, 153–163.
Muravenko, O.V., Selyakh, I.O., Kononenko, N.V., Stadnichuk, I.N., 2001. Chromosome numbers and nuclear DNA contents in the red microalgae *Cyanidium caldarium* and three *Galdieria* species. Eur. J. Phycol. 36, 227–232.
Nagashima, H., Fukuda, I., 1983. Floridosides in unicellular hot-spring algae. Phytochemistry 22, 1949–1951.
Naval, J., Antia, B., Berland, R., Bonin, D.J., Maestrini, S.Y., 1977. Effects of urea concentration in supporting growth of certain marine microplanktonic algae. Phycologia 16, 105–111.
Naylor, A.W., 1970. Phylogenetic aspects of nitrogen metabolism in the algae. Ann. N. Y. Acad. Sci. 175, 511–523.
Nigam, N.S., Sing, A., 2011. Production of liquid biofuels from renewable resources. Prog. Energy Combust. Sci. 37, 52–68.
Nimrod, A., Greenman, B., 1986. Heavy Metal Salts of Hyaluronic Acid and Their Use as Antimicrobial Agents, US4746504A.
Nishikawa, K., Machida, H., Yamakoshi, Y., Ohtomo, R., Saito, K., Saito, M., Tominaga, N., 2006. Polyphosphate metabolism in an acidophilic alga *Chlamydomonas acidophila* KT-1 (Chlorophyta) under phosphate stress. Plant Sci. 170, 307–313.
Nishikawa, K., Tominaga, N., Uchino, T., Oikawa, A., Tokunaga, H., 2009. Polyphosphate contributes to Cd tolerance in *Chlamydomonas acidophila* KT-1. In: Hagen, K.N. (Ed.), Algae: Nutrition, Pollution Control and Energy Sources. Nova Science Publishers, pp. 13–21.
Norton, T.A., Melkonian, M., Anderson, R.A., 1996. Algal biodiversity. Phycologia 35, 308–326.
Oesterhelt, C., Schnarrenberger, C., Gross, W., 1999. Characterization of a sugar/polyol-uptake system in the red alga *Galdieria sulphuraria*. Eur. J. Phycol. 34, 271–277.
Oh, S.H., Han, J.G., Kim, Y., Ha, J.H., Kim, S.S., Jeong, M.H., Jeong, H.S., Kim, N.Y., Cho, J.S., Yoon, W.B., Lee, S.Y., Kang, D.H., Lee, H.Y., 2009. Lipid production in *Porphyridium cruentum* grown under different culture conditions. J. Biosci. Bioeng. 108, 429–434.
Orchard, E.D., Benitez-Nelson, C.R., Pellechia, P.J., Lomas, M.W., Dyhrman, S.T., 2010. Polyphosphate in *Trichodesmium* from the lowphosphorus Sargasso Sea. Limnol. Oceanogr. 55, 2161–2169.
Otha, N., Sato, N., Kuroiwa, T., 1998. Structure and organization of the mitochondrial genome of the unicellular red alga *Cyanidioschyzon merolae* deduced from the complete nucleotide sequence. Nucleic Acids Res. 26, 5190–5198.
Otha, N., Matsuzaki, M., Misumi, O., Miyagishima, S.Y., Nozaki, H., Tanaka, K., Shin-I, T., Kohara, Y., Kuroiwa, T., 2003. Complete sequence and analysis of the plastid genome of the unicellular red alga *Cyanidioschyzon merolae*. DNA Res. 30, 67–77.
Oyala, P.H., Stich, T.A., Debus, R.J., Britt, R.D., 2015. Ammonia binds to the dangler manganese of the photosystem II oxygen-evolving complex. J. Am. Chem. Soc. 137, 8829–8837.
Pade, N., Linka, N., Ruth, W., Weber, A.P.M., Hagemann, M., 2015. Floridoside and isofloridoside are synthesized by trehalose 6-phosphate synthase-like enzymes in the red alga *Galdieria sulphuraria*. New Phytol. 205, 1227–1238.
Pan, Q., Chen, M., Li, J., Wu, Y., Zhen, C., Liang, B., 2013. Antitumor function and mechanism of phycoerythrin from *Porphyra haitanensis*. Biol. Res. 46, 87–95.
Patel, A.K., Laroche, C., Marcati, A., Ursu, A.V., Jubeau, S., Marchal, L., Petit, E., Djelveh, G., Michaud, P., 2013. Separation and fractionation of exopolysaccharides from *Porphyridium cruentum*. Bioresour. Technol. 145, 345–350.
Patron, N.J., Keeling, P.J., 2005. Common evolutionary origin of starch biosynthetic enzymes in green and red algae. J. Phycol. 41, 1131–1141.
Pérez, N.M., Ames, W.M., Nilsson, H., Lohmiller, T., Pantazis, D.A., Rapatskiy, L., Nowaczyk, M.M., Neese, F., Boussac, A., Messinger, J., Lubitz, W., Cox, N., 2013. Ammonia binding to the oxygen-evolving complex of photosystem II identifies the solvent-exchangeable oxygen bridge (μ-oxo) of the manganese tetramer. Proc. Natl. Acad. Sci. U. S. A. 110, 15561–15566.
Perez-Garcia, O., Bashan, Y., 2015. Microalgal heterotrophic and mixotrophic culturing for bio-refining: From metabolic routes to techno-economics. In: Prokop, A., Bajpai, R.K., Zappi, M.E. (Eds.), Algal Biorefineries, Volume 2: Products and Refinery Design. Springer International Publishing, Switzerland, pp. 61–131.
Perez-Garcia, O., Escalante, F.M.E., de-Bashan, L.E., Bashan, Y., 2011. Heterotrophic cultures of microalgae: metabolism and potential products. Water Res. 45, 11–36.
Pierre, G., Delattre, C., Laroche, C., Michaud, P., 2014. Galactans and its applications. In: Ramawat, K.G., Mérillon, J.-M. (Eds.), Polysaccharides: Bioactivity and Biotechnology. Springer International Publishing, Switzerland, pp. 753–794.

Pignolet, O., Jubeau, S., Vaca-Garcia, C., Michaud, P., 2013. Highly valuable microalgae: biochemical and topological aspects. J. Ind. Microbiol. Biotechnol. 40, 781–796.
Poddar, N., Sen, R., Martin, G.J.O., 2018. Glycerol and nitrate utilization by marine microalgae *Nannochloropsis salina* and *Chlorella sp.* And associated bacteria during mixotrophic and heterotrophic growth. Algal Res. 33, 298–309.
Portis, A.R., Parry, M.A.J., 2007. Discoveries in Rubisco (Ribulose 1,5-bisphosphate carboxylase/oxygenase): a historical perspective. Photosynth. Res. 94, 121–143.
Potter, A., Thibaut, S., Ribaut, C., 2011. Use of Sulphated Polysaccharides as Antidandruff Agent, WO2013093307A1.
Potter, A., Ghibaudo, M., Baltenneck, C., 2013. Association of Sulfated Polysaccharides and c-Glycoside and the Uses Thereof, WO2014174188A1.
Pruvost, J., Cornet, J.F., Goetz, V., Legrand, J., 2012. Theoretical investigation of biomass productivities achievable in solar rectangular photobioreactors for the cyanobacterium *Arthrospira platensis*. Biotechnol. Prog. 28, 699–714.
Pulz, O., Gross, W., 2004. Valuable products from biotechnology of microalgae. Appl. Microbiol. Biotechnol. 65, 635–648.
Pushkareva, E., Johansen, J.R., Elster, J., 2016. A review of the ecology, ecophysiology and biodiversity of microalgae in Arctic soil crusts. Polar Biol. 39, 2227–2240.
Ragan, M.A., Bird, C.J., Rice, E.L., Gutell, R.R., Murphy, C.A., Singh, R.K., 1994. A molecular phylogeny of the marine red algae (Rhodophyta) based on the nuclear small-unit rRNA gene. Proc. Natl. Acad. Sci. U. S. A. 91, 7276–7280.
Rahaoui, A., 1999. Écophysiologie de *Rhodella violacea* (Rhodophyta): production et propriétés structurales des exopolysaccharides et de l'amidon floridéen. PhD thesis. Université des Sciences et Technologies de Lille, pp. 1–130.
Raines, C.A., 2011. Increasing Photosynthetic Carbon Assimilation in C3 Plants to Improve Crop Yield: Current and Future Strategies. Plant Physiol. 155, 36–42.
Rajvanshi, S., Sharma, M.P., 2012. Microalguae: a potential source of biodiesel. J. Sustain. Bioenergy Syst. 2, 49–59.
Ramus, J., 1972. The production of extracellular polysaccharide by unicellular red alga *Porphyridium aerugineum*. J. Phycol. 8, 97–111.
Ramus, J., 1981. The capture and transduction of light energy. In: Lobban, C.S., Wynne, M.J. (Eds.), The Biology of Seaweeds. Blackwell Scientific Publications, Boston, pp. 458–492.
Ras, M., Steyer, J.P., Bernard, O., 2013. Temperature effect on microalgae: a crucial factor for outdoor production. Rev. Environ. Sci. Biotechnol. 12, 153–164.
Rasmussen, R.S., Morrissey, M.T., 2007. Marine biotechnology for production of food ingredients. Adv. Food Nutr. Res. 52, 237–292.
Raven, J.A., Giordano, M., 2016. Combined Nitrogen. In: Borowitzka, M., Beardall, J., Raven, J. (Eds.), The Physiology of Microalgae. Springer International Publishing, Switzerland, pp. 143–154.
Raven, J.A., Johnston, A.M., MacFarlane, J.J., 1990. Carbon metabolism. In: Cole, K.M., Sheath, R.G. (Eds.), Biology of the Red Algae. Cambridge University Press, Cambridge, UK, pp. 172–202.
Raven, J.A., Johnston, A.M., Kübler, J.E., Korb, R., McInroy, S.G., Handley, L.L., Scrimgeour, C.M., Walker, D.I., Beardall, J., Vanderklift, M., Fredrikson, S., Dunton, K.H., 2002. Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses. Funct. Plant Biol. 29, 355–378.
Read, B.A., Kegel, J., Klute, M.J., Kuo, A., Lefebvre, S.C., Maumus, F., Mayer, C., Miller, J., Monier, A., Salamov, A., Young, J., Aguilar, M., Claverie, J.M., Frickenhaus, S., Gonzalez, K., Herman, E.K., Lin, Y.C., Napier, J., Ogata, H., Sarno, A.F., Shmutz, J., Schroeder, D., de Vargas, C., Verret, F., von Dassow, P., Valentin, K., Van de Peer, Y., Wheeler, G., Dacks, J.B., Delwiche, C.F., Dyhrman, S.T., Glockner, G., John, U., Richards, T., Worden, A.Z., Zhang, X.Y., Grigoriev, I.V., 2013. Pan genome of the phytoplankton *Emiliania underpins* its global distribution. Nature 499, 209–213.
Reeb, V., Bhattacharya, D., 2010. The thermo-acidophilic cyanidiophyceae (cyanidiales). In: Chapman, D. (Ed.), Seckbach, J. Red Algae in the Genomic Age, Dordrecht, pp. 409–426.
Richa, K.V.K., Kesheri, M., Singh, G., Sinha, R.P., 2011. Biotechnological potentials of phycobiliproteins. Int. J. Pharma. Biosci. 2, 446–454.
Richardson, K., Bearddall, J., Raven, J.A., 1983. Adaptation of unicellular algae to irradiance: an analysis of strategies. New Phytol. 93, 157–191.
Richmond, A., 2004. Handboock of Microalgal Culture: Biotechnology and Applied Phycology. Blackwell Sciences Ltd., India.
Rigano, C., 1971. Studies on nitrate reductase from *Cyanidium caldarium*. Arch. Mikrobiol. 76, 265–276.
Rigano, C., Violante, U., 1972. Effect of heat treatment on the activity in vitro of nitrate reductase from *Cyanidium caldarium*. Biochim. Biophys. Acta Bioenerg. 256, 524–532.
Rigano, C., Fuggi, A., Martino, D., Rigano, V., Aliotta, G., 1976. Studies on utilization of 2-ketoglutarate, glutamate and other amino acids by the unicellular alga *Cyanidium caldarium*. Arch. Microbiol. 107, 133–138.
Rigano, C., Aliotta, G., Rigano, V.D., Fuggi, A., Vona, V., 1977. Heterotrophic growth patterns in the unicellular alga *Cyanidium caldarium*: a possible role for threonine dehydrase. Arch. Microbiol. 113, 191–196.
Ritz, M., Thomas, J.C., Spilar, A., Etienne, A.L., 2000. Kinetics of photoacclimatation in response to a shift to high light irradiance of the red algae *Rhodella violacea* adapted to low irradiance. Plant Physiol. 123, 1415–1425.
Romans, K.M., Carpenter, E.J., Bergman, B., 1994. Buoyancy regulation in the colonial diazotrophic cyanobacterium *Trichodesmium tenue*: ultrastructure and storage of carbohydrate, polyphosphate, and nitrogen. J. Phycol. 30, 935–942.
Roussel, M., Villay, A., Delbac, F., Michaud, P., Laroche, C., Roriz, D., El Alaoui, H., Diogon, M., 2015. Antimicrosporidian activity of sulphated polysaccharides from algae and their potential to control honeybee nosemosis. Carbohydr. Polym. 133, 213–220.
Rowlett, R.S., Chance, M.R., Wirt, M.D., Sidelinger, D.E., Royal, J.R., Woodroffe, M., A, Y.F., Saha, R.P., Lam, M.G., 1994. Kinetic and structural characterization of spinach carbonic anhydrase. Biochemistry 33, 13967–13976.
Roy, S., Llewellyn, C.A., Egeland, E.S., Johnsen, G., 2011. Phytoplankton Pigments: Characterization, Chemotaxonomy and Applications in Oceanography. Cambridge University Press, Cambridge.
Rubio, F.C., Camacho, F.G., Sevilla, J.M., Chisti, Y., Grima, E.M., 2003. A mechanistic model of photosynthesis in microalgae. Biotechnol. Bioeng. 81, 459–473.
Sakurai, T., Aoki, M., Ju, X., Ueda, T., Nakamura, Y., Fujiwara, S., Umemura, T., Tsuzuki, M., Minoda, A., 2016. Profiling of lipid and glycogen accumulations under different growth conditions in the sulfothermophilic red alga *Galdieria sulphuraria*. Bioresour. Technol. 200, 861–866.
Sasso, S., Pohnert, G., Lohr, M., Mittag, M., Hertweck, C., 2011. Microalgae in the post-genomic era: a blooming reservoir for new natural products. FEMS Microbiol. Rev. 36, 761–785.
Sato, N., 2009. Gclust: *trans*-kingdom classification of proteins using automatic individual threshold setting. Bioinformatics 25, 599–605.
Sato, N., Moriyama, T., 2007. Genomic and biochemical analysis of lipid biosynthesis in the unicellular rhodophyte *Cyanidioschyzon merolae*: lack of plastidic desaturation pathway results in mixed pathway of galactolipid synthesis. Eukaryot. Cell 6, 1006–1017.
Sato, N., Moriyama, T., Mori, N., Toyoshima, M., 2017. Lipid metabolism and potentials of biofuel and high added-value oil production in red algae. World J. Microbiol. Biotechnol. 33, 74.
Saunders, G.W., Hommersand, M.H., 2004. Assessing red algal suprordinal diversity and taxonomy in the context of contemporary systematic data. Am. J. Bot. 91, 1494–1507.
Schilling, S., Oesterhelt, C., 2007. Structurally reduced monosaccharide transporters in an evolutionarily conserved red alga. Biochem. J. 406, 325–331.
Schmidt, R.A., Wiebe, M.G., Eriksen, N.T., 2005. Heterotrophic high cell density fed-batch cultures of the phycocyanin producing red alga *Galdieria sulphuraria*. Biotechnol. Bioeng. 90, 77–84.
Schönknecht, G., Chen, W.H., Ternes, C.M., Barbier, G.G., Shrestha, R.P., Stanke, M., Braütigam, A., Baker, B.J., Branfield, J.F., Garavito, R.M., Carr, K., Wilkerson, C., Rensing, S.A., Gagneul, D., Dickenson, N.E., Oesterhelt, C., Lercher, M.J., Weber, A.P., 2013. Gene transfer from bacteria and archaea facilitated evolution of an extremophilic eukaryote. Science 339, 1207–1210.
Schubert, N., García-Mendoza, E., Pacheco-Ruiz, I., 2006. Carotenoid composition of marine red algae. J. Phycol. 42, 1208–1216.
Scott, J.L., Yokoyama, A., Billard, C., Fresnel, J., Hara, Y., West, K.A., West, J.A., 2008. *Neorhodella cyanea*, a new genus in the rhodellophyceae (rhodophyta). Phycologia 47, 560–572.
Scott, J., Yang, E.C., West, J.A., Yokoyama, A., Kim, H.J., de Goër, S.L., Okelly, C.J., Orlova, E., Kim, S.Y., Park, J.K., Yoon, H.S., 2011. On the genus *Rhodella*, the emended orders Dixoniellales and Rhodellales with a new order Glaucosphaerales (Rhodellophyceae, Rhodophyta). Algae 26, 277–288.
Selvaratnam, T., Pegallapati, A.K., Reddy, H., Kanapathipillai, N., Nirmalakhandan, N., Deng, S., Lammers, P.J., 2015. Algal biofuels from urban wastewaters: Maximizing biomass yield using nutrients recycled from hydrothermal processing of biomass. Bioresour. Technol. 182, 232–238.
Serive, B., Nicolau, E., Bérard, J.B., Kaas, R., Pasquet, V., Picot, L., Cadoret, J.P., 2017. Community analysis of pigment patterns from 37 microalgae strains reveals new carotenoids and porphyrins characteristic of distinct strains and taxonomic groups. PLoS One 12.
Shimonaga, T., Fujiwara, S., Kaneko, M., Izumo, A., Nihei, S., Francisco, P.B., Satoh, A., Fujita, N., Nakamura, Y., Tsuzuki, M., 2007. Variation in storage a-polyglucans of red algae: amylose and semi-amylopectin types in porphyridium and glycogen type in cyanidium. Mar. Biotechnol. 9, 192–202.
Shimonaga, T., Konishi, M., Oyama, Y., Fujiwara, S., Satoh, A., Fujita, N., Colleoni, C., Buléon, A., Putaux, J.L., Ball, S.G., Yokoyama, A., Hara, Y., Nakamura, Y., Tsuzuki, M., 2008. Variation in storage α-glucans of the porphyridiales (rhodophyta). Plant Cell Physiol. 49, 103–116.
Silva, P.C., Moe, R.L., 1997. Taxonomic classification of algae. In: Lobban, C.S., Harrison, P.J. (Eds.), Seaweed Ecology and Phycology. Cambridge University press, New York, pp. 301–308.
Singh, S., Arad, S.M., Richmond, A., 2000. Extracellular polysaccharide production in outdoor mass cultures of *Porphyridium sp.* in flat plate glass reactors. J. Appl. Phycol. 12, 269–275.
Sloth, J.K., Wiebe, M.G., Eriksen, N.T., 2006. Accumulation of phycocyanin in heterotrophic and mixotrophic cultures of the acidophilic red alga *Galdieria sulphuraria*. Enzym. Microb. Technol. 38, 168–175.
Sloth, J.K., Jensen, H.C., Pleissner, D., Eriksen, N.T., 2017. Growth and phycocyanin synthesis in the heterotrophic microalga *Galdieria sulphuraria* on substrates made of food waste from restaurants and bakeries. Bioresour. Technol. 238, 296–305.
Smith, K.S., Jakubzick, C., Whittam, T.S., Ferry, J.G., 1999. Carbonic anhydrase is an ancient enzyme widespread in prokaryotes. Proc. Natl. Acad. Sci. U. S. A. 96, 15184–15189.
So, A.K., Espie, G.S., Williams, E.B., Shively, J.M., Heinhorst, S., Cannon, G.C., 2004. A novel evolutionary lineage of carbonic anhydrase (epsilon class) is a component of the carboxysome shell. J. Bacteriol. 186, 623–630.
Soanen, N., Silva, E.D., Gardarin, C., Michaud, P., Laroche, C., 2016. Improvement of exopolysaccharide production by *Porphyridium marinum*. Bioresour. Technol. 213, 231–238.
Sobczuk, T.M., Camacho, F.G., Grima, E.M., Chisti, Y., 2006. Effects of agitation on the microalgae *Phaeodactylum tricornutum* and *Porphyridium cruentum*. Bioprocess Biosyst. Eng. 28, 243–250.
South, G.R., Whittick, A., 1987. Introduction to Phycology. Blackwell Scientific Publications, London.
Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications

of microalgae. J. Biosci. Bioeng. 101, 87–96.
Stein, R., Gross, W., Schnarrenberger, C., 1997. Characterization of a xylitol dehydrogenase and a D-arabitol dehydrogenase from the thermo- and acidophilic red alga *Galdieria sulphuraria*. Planta 202, 487–493.
Stengel, D.B., Connan, S., Popper, Z.A., 2011. Algal chemodiversity and bioactivity: sources of natural variability and implications for commercial application. Biotechnol. Adv. 29, 483–501.
Sun, L., Wang, C., Shi, Q., Ma, C., 2009. Preparation of different molecular weight polysaccharides from *Porphyridium cruentum* and their antioxidant activities. Int. J. Biol. Macromol. 45, 42–47.
Sun, L., Wang, L., Zhou, Y., 2012. Immunomodulation and antitumor activities of different-molecular-weight polysaccharides from *Porphyridium cruentum*. Carbohydr. Polym. 87, 1206–1210.
Suzuki, A., Knaff, D.B., 2005. Glutamate synthase: structural, mechanistic and regulatory properties, and role in the amino acid metabolism. Photosynth. Res. 83, 191–217.
Tajima, N., Sato, S., Maruyama, F., Furokawa, K., Otha, H., Tabata, S., Sekine, K., Moriyama, T., Sato, N., 2014. Analysis of the complete plastid genome of the unicellular red alga *Porphyridium purpureum*. J. Plant Res. 127, 389–397.
Takache, H., Christophe, G., Cornet, J.F., Pruvost, J., 2010. Experimental and theoretical assessment of maximum productivities for the micro-algae *Chlamydomonas reinhardtii* in two different geometries of photobioreactors. Biotechnol. Prog. 26, 431–440.
Takahashi, E., Ledauphin, J., Goux, D., Orvain, F., 2009. Optimizing extraction of extracellular polymeric substances (EPS) from benthic diatoms: comparison of the efficiency of six EPS extraction methods. Mar. Freshw. Res. 60, 1201–1210.
Talyshinsky, M., Souprun, Y., Huleihel, M., 2002. Anti-viral activity of red microalgal polysaccharides against retroviruses. Cancer Cell Int. 2, 1–8.
Tannin-Spitz, T., Bergman, M., van-Moppes, D., Grossman, S., Arad, S.M., 2005. Antioxidant activity of the polysaccharide of the red microalga *Porphyridium* sp. J. Appl. Phycol. 17, 215–222.
Tcheruov, A.A., Minkova, K.M., Georgiev, D.I., Houbavenska, N.B., 1993. Method for B-phycoerythrin purification from Porphyridium cruentum. Biotechnol. Tech. 7, 853–858.
Tebbani, S., Lopes, F., Celis, G.B., 2015. Nonlinear control of continuous cultures of *Porphyridium purpureum* in a photobioreactor. Chem. Eng. Sci. 123, 207–219.
Tesson, S.V.M., Skjøth, C.A., Šanti-Temkiv, T., Löndahl, J., 2016. Airborne Microalgae: Insights, Opportunities, and Challenges. Appl. Environ. Microbiol. 82, 1978–1991.
Thepenier, C., Chaumont, D., Gudin, C., 1987. Mas culture of Porphyridium cruentum: a multiproduct strategy for the biomass valorization. In: Stadler, T., Mollion, J. (Eds.), Algal Biotechnology. Elsevier Applied Science, London and NY, pp. 413–420.
Tibbetts, S.M., Milley, J.E., Lall, S.P., 2015. Chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors. J. Appl. Phycol. 27, 1109–1119.
Turner, J., 1970. A note on the nutrition of *Rhodella*. Br. Phycol. J. 5, 15–18.
Usov, A.I., 2011. Polysaccharides of the red algae. Adv. Carbohydr. Chem. Biochem. 65, 115–217.
Van den Hoek, C., Mann, D.G., Jahns, H.M., 1995. Algae an Introduction to Phycology. Cambridge University press, Cambridge, pp. 48–102.
Van Mooy, B.A.S., Devol, A.H., 2008. Assessing nutrient limitation of *Prochlorococcus* in the North Pacific subtropical gyre by using an RNA capture method. Limnol. Oceanogr. 53, 78–88.
Vanselow, C., Weber, A.P.M., Krause, K., Fromme, P., 2009. Genetic analysis fo the Photosystem I subunits from the red alga, *Galdieria sulphuraria*. Biochim. Biophys. Acta 1787, 46–59.
Vazhappilly, R., Chen, F., 1998. Eicosapentaenoic acid and docosahexaenoic acid production potential of microalgae and their heterotrophic growth. J. Am. Oil Chem. Soc. 75, 393–397.
Vigani, M., Parisi, C., Rodriguez-Cerezo, E., Barbosa, M.J., Sijtsma, L., Ploeg, M., Enzung, C., 2015. Food and feed products from microalgae: market opportunities and challenges for the EU. Trends Food Sci. Technol. 42, 81–92.
Villay, A., Laroche, C., Roriz, D., El-Alaoui, H., Delbacb, F., Michaud, P., 2013. Optimisation of culture parameters for exopolysaccharides production by the microalga *Rhodella violacea*. Bioresour. Technol. 146, 732–735.
Vis, M.L., Sheath, R.G., 1993. Distribution and systematic of Chroodactylon and Kyliniella (Porphyridiales, Rhodophyta) from North American streams. J. Phycol. 41, 237–241.
Vonshak, A., Torzillo, G., 2004. Environmental stress physiology. In: Richmond, A. (Ed.), Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Blackwell Publishing, Oxford, pp. 57–82.
Vonshak, A., Cohen, Z., Richmond, A., 1985. The feasibility of mass cultivation of *Porphyridium*. Biomass 8, 13–25.
Walne, P.R., 1966. Experiments on the large-scale culture of the larvae of Ostrea edulis. Fishery Invest, London.
Wang, J., Yang, H., Wang, F., 2014. Mixotrophic cultivation of microalgae for biodiesel production: status and prospects. Appl. Biochem. Biotechnol. 172, 3307–3329.
Weber, A.P.M., Oesterhelt, C., Gross, W., Brautigam, A., Imboden, L.A., Krassovskaya, I., Linka, N., Truchina, J., Schneidereit, J., Voll, H., 2004. EST-analysis of the thermo-acidophilic red microalga *Galdieria sulphuraria* reveals potential for lipid A biosynthesis and unveils the pathway of carbon export from rhodoplasts. Plant Mol. Biol. 55, 17–32.
Wells, M.L., Potin, P., Craigie, J.S., Raven, J.A., Merchant, S.S., Helliwell, K.E., Smith, A.G., Camire, M.E., Braxley, S.H., 2017. Algae as nutritional and functional food sources: revisiting our understanding. J. Appl. Phycol. 29, 949–982.
West, J.A., de Goer, S.L., Zuccarello, G.C., 2014. A new species of *Bangiopsis*: *B. frankynotti* sp. nov. (Stylonematophyceae, Rhodophyta) from Australia and India and comments on genus. Algae 29, 101–109.
White, A.K., Spitz, Y., Karl, D.M., Letelier, R., 2006. Flexible elemental stoichiometry in *Trichodesmium* spp. and its ecological implications. Limnol. Oceanogr. 51, 1777–1790.
Wiethaus, J., Busch, A.W.U., Dammeyer, T., Frankenberg-Dinkel, T., 2010. Phycobiliproteins in *Prochlorococcus marinus*: Biosynthesis of pigments and their assembly into proteins. Eur. J. Cell Biol. 89, 1005–1010.
William, P.J.B., Laurens, L.M.L., 2010. Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetic & economics. Energy Environ. Sci. 3, 18–32.
Williams, S.K., Hodson, R.C., 1977. Transport of urea at low concentrations in *Chlamydomonas reinhardi*. J. Bacteriol. 130, 266–273.
Xie, T., Xia, Y., Zeng, Y., Li, X., Zhang, Y., 2017. Nitrate concentration-shift cultivation to enhance protein content of heterotrophic microalga *Chlorella vulgaris*: over-compensation strategy. Bioresour. Technol. 233, 247–255.
Yamaoka, A., Ozawa, Y., Ueno, Y., Endo, T., Morimoto, Y., Urushiyama, A., Ohmori, D., Imai, T., 2011. *Cyanidioschyzon merolae* ferredoxin: a high resolution crystal structure analysis and its thermal stability. FEBS Lett. 585, 1299–1302.
Yang, C., Hua, Q., Shimizu, K., 2000. Carbon metabolism during growth under photo-autotrophic, mixotrophic and autotrophic/dark-heterotrophic conditions. Biochem. Eng. J. 6, 87–102.
Yanhui, C., 2017. Cosmetic Composition With Functions of Repairing and Reinforcing Skin Barrier and Application Thereof, CN 107412042.
Yaron, A., Cohen, E., Arad, S.M., 1992. Stabilization of aloe vera gel by interaction with sulfated polysaccharides from red microalgae and with xanthan gum. J. Agric. Food Chem. 40, 1316–1320.
Ye, Y., Le Clech, P., Chen, V., Fane, A.G., 2005. Evolution of fouling during crossflow filtration of model EPS solutions. J. Membr. Sci. 264, 190–199.
Yokoyama, A., Scott, J.L., Zuccarello, G.C., Kajikawa, M., Hara, Y., West, J.A., 2009. *Corynoplastis japonica* gen. and sp. nov. and Dixoniellales ord. nov. (Rhodellophyceae, Rhodophyta) based on morphological and molecular evidence. Phycol. Res. 57, 278–289.
Yoon, H., Hackett, J., Pinto, G., Bhattacharya, D., 2002. The single, ancient origin of chromist plastids. Proc. Natl. Acad. Sci. U. S. A. 99, 15507–15512.
Yoon, H.S., Hackett, J.D., Ciniglia, C., Pinto, G., Bhattacharya, D., 2004. A molecular timeline for the origin of photosynthetic eukaryotes. Mol. Biol. Evol. 21, 809–818.
Yoon, H.S., Müller, K.M., Sheath, R.G., Ott, F.D., Bhattacharya, D., 2006. Defining the major lineages of red algae (Rhodophyta). J. Phycol. 42, 482–492.
You, T., Barnett, S.M., 2004. Effect of light quality on production of extracellular polysaccharides and growth rate of *Porphyridium cruentum*. Biochem. Eng. J. 19, 251–258.
Yu, S., Blennow, A., Bojko, M., Madsen, F., Olsen, C.E., Engelsen, S.B., 2002. Physico-chemical characterization of floridean starch of red algae. Starch 54, 66–74.
Zaslavskaia, L., Lippmeier, J., Shih, C., 2001. Trophic conversion of an obligate photo-autotrophic organism through metabolic engineering. Science 292, 2073–2075.
Zeriouh, O., Reinoso-Moreno, J.V., Lopez-Rosales, L., Ceron-Garcia, M.C., Sanchez-Miron, A., Garcia-Camacho, F., Molina-Grima, E., 2017. Biofouling in photobioreactors for marine microalgae. Crit. Rev. Biotechnol. 37, 1006–1023.
Zhang, Q., Wu, X., Xue, S., Wang, Z., Yan, C., 2013. Hydrodynamic characteristics and microalgae cultivation in a novel flat-plate photobioreactor. Biotechnol. Prog. 29, 127–134.
Zhu, L., 2015. Biorefinery as a promising approach to promote microalgae industry: an innovative framework. Renew. Sust. Energ. Rev. 41, 1376–1384.