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REVIEW ARTICLE

Biological and technical aspects on valorization of red microalgae genera *Porphyridium*

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Abstract
Red microalgae genera *Porphyridium* are photoautotrophic microalgae with potential application as a natural source of high-value chemicals, including sulfated exopolysaccharides (EPs), phycobiliproteins (PBPs), and long-chain polyunsaturated fatty acids (LC-PUFAs) such as α-linolenic (ALA), arachidonic (ARA), and eicosapentaenoic acid (EPA). Their good tolerances toward dynamic environmental changes lead them to be attractive for sustainable large-scale production of biomass and biochemicals. Discussion on the biological nature and culture strategies of *Porphyridium* are helpful for improving more effective and efficient cultivation system, which are presented in this review. The typical biological characteristics and the main targeted biochemicals of *Porphyridium* like polysaccharides, PUFAs, and PBPs are firstly presented. Some crucial parameters such as temperature, pH, salinity, and light intensity are then discussed for their effect on cell growth and biochemical yield. Recent technology development in large-scale cultivation is also evaluated in the final, which compared the established large-scale culture and recent novel cultures. It is expected that this review could provide new insight for development strategy in optimizing and accelerating more sustainable as well as economically viable industrial biotechnology based on red microalga genera *Porphyridium*.

Keywords Red microalgae · Porphyridium · Sulfated exopolysaccharides · Phycobiliproteins · Arachidonic acid · Eicosapentaenoic acid

1 Introduction

Microalgae have been regarded as a promising natural source for biochemical production such as proteins [1], fatty acids [2], carbohydrates [3], and pigments [4]. As a photosynthetic microorganism, they can use solar energy and capture CO_2 to synthesize those multiple organic molecules efficiently. In nature, their photosynthetic efficiency was reported to be 3%. Meanwhile, it reached 10% in the optimum growth condition, which is 2–10-folds higher than C3/C4 plants [5, 6]. In case of CO_2 uptaking, 1 kg of microalgae biomass has the potency to uptake 1.83 kg of CO_2 [5]. Furthermore, marine microalgae class of Bacillariophyceae, also known as diatoms, have ability to use the dissolved silicate in sea water to build their siliceous cell wall with hierarchical porous architecture [7]. As such, microalgae offer multifunctional advantages, either as chemical/material producer or biological CO_2 sequestration agent.

To date, no more than 10 genera of microalgae have been exploited at an industrial scale, which are *Chlorella*, *Nannochloropsis*, *Isochrysis*, *Muriellopsis*, *Odontella*, *Dunaliella*, *Haematococcus*, *Rhodella*, and *Porphyridium* [3]. All of them are usually cultivated to produce high-value microalgal products including high-nutritious biomass or valuable biomolecules with the application for food/feed production, pharmaceutical, and cosmeceutical products. Techno-economic analysis suggested that the total production cost of their large-scale cultivations is still economically viable when the processes are developed for the production of that

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kind of products rather than to produce microalgal-based biofuels [3, 5, 8].

Among those exploited microalgae, red microalgae genera *Porphyridium* and *Rhodella* have attracted much interest of researchers currently owing to their potential nutritional values and biochemicals market [3, 9]. Particularly, the former microalgae are being intensively explored because they could produce multiple valuable compounds such as extracellular polysaccharides (EPs), phycobiliproteins (PBPs) pigments, protein, and long chain poly-unsaturated fatty acids (LC-PUFAs). EPs are one of the main targeted compounds because of their potential biological activities as antioxidant, antibacterial, antivirals, and anticancer [9–11]. Meanwhile, PBPs are highly fluorescent pigments with the application as natural cosmetic dyes or as fluorescent probes. In red microalgae *Porphyridium*, the main targets of their PBPs molecules are allophycocyanin and phycoerythrin [12]. Moreover, *Porphyridium* could accumulate a relatively high amount of carbohydrates (23–52% d.w.) and/or protein (15–47% d.w.) [13–16]. Furthermore, an acute and subchronic safety study of a feed fortified with *P. purpureum* biomass in albino Wistar rats confirmed no changes in physiology (LD_{50} 5.0 g kg^{-1} body weight), e.g., body weight gain, relative organ weights, histopathology, and hematological as well as serum biochemical indices, and even decreased serum cholesterol as well as triglyceride levels [17]. This result indicates that *Porphyridium* biomass is safe and has high nutritional value.

Cultivation of red microalgae genera *Porphyridium* has been studied in either laboratory or pilot scale. Many information is available from the species of *P. cruentum* [18, 19], *P. purpureum* [14, 20], *P. sordidum* [21, 22], and *P. aerugineum* [23, 24], including the growth characteristics and biochemical profiles. Large-scale cultivation has also been conducted by several biotechnological industries in France (Greensea, AlgoSource, Phyco-Biotech, Isua Biotechnologie & Compagnie), Israel (Frutarom, Asta Technologies, Yemoja), Italy (Micoperi Blue Growth), and USA (Solazyme), commonly using *P. cruentum* [3, 25–27]. For instance, Frutarom develops outdoor cultivation of *Porphyridium* sp. using vertical polyethylene bags based on Arad's work in the Negev Desert area, Israel. The process is directed to produce EPs for its commercial product Alguard™ [28, 29]. Their closed-culture system provides two-fold higher biomass and polysaccharides productivity with minimum contamination compared with the open-pond system [30]. The closed outdoor cultivation of *P. cruentum* is also carried out by Greensea and Algosource for extracting EPs or PBPs, which are used as the main ingredient of their cosmeceutical product Silidine® and Porphyderm®, respectively [3].

As typical photosynthetic microorganisms, the growth characteristics and biochemical profile of Porphyridium family are varied depending on the species [22] and their cultivation techniques [20]. Naturally, each species has different internal biological characters as resulted from their genetic adaptation towards the living habitats. As a result, their cellular metabolism processes are distinct for each species, which give variety of the cell growth and the types as well as the yields of their biomolecules. Meanwhile, the selection of cultivation system is generally considering several aspects. These are including the typical characters of the algae, the geographic condition of the farm location, the targeted of final products, and the economic feasibility of the processes (capital and operational cost). Comparative data on the biological and technical information is helpful to provide more insight for deeply understanding and accelerating the more sustainable and economically viable strategy on the valorization of red microalgae *Porphyridium* in the future. As such, recent updates on these two aspects of the development of *Porphyridium* cultivation are necessary and being the main part of discussion in this review. First, the characteristics of biological and biochemicals of *Porphyridium* are presented. The later topic is limited on the discussion of the main targeted components for commercial product, i.e., polysaccharides, polyunsaturated fatty acids, and phycobiliproteins. Furthermore, general factors on affecting the cultivation productivity are presented. In the final, updating two general cultivation systems, i.e., open ponds and photobioreactors, is taken up.

2 Biological and chemical characteristics of *Porphyridium*

2.1 Morpshology and physiology

Several species of *Porphyridium* have been reported to date and some of them have synonyms. In general, red microalga genus *Porphyridium* is composed of four species, namely, *P. purpureum* (also registered as *P. cruentum* and *P. marinum*), *P. aerugineum*, *P. sordidum*, and *P. wittrockii* [31–33]. Their cells have spherical shapes with an average size of 6 μm (Fig. 1) and nuclear membrane covered by ribosomes. Sometimes, they embed each other to form aggregates by a water-soluble gelatinous polysaccharide layer. This layer also encapsulates the cells and plays a role as a cell wall [34–36] because these microalgae lack microfibrillar component cell walls [28, 37]. During the logarithmic phase, the layer is thinnest and becomes thickest at the stationary phase. By the time, it dissolves and enriches the liquid medium, leading the medium to become viscous at the end of culture. As such, this carbohydrate layer is called exopolysaccharides (EPs).

Porphyridium's cells are generally brownish red in color because they contain B-phycoerythrin and chlorophyll. However, some strains are different because of other

colorant pigments as a major component instead of phycoerythrin. The amount of these proteinaceous pigments could also be affected by their culture conditions. As such, manipulating the culture parameters would give variety in terms of the types and the yields of their pigments and this is the eminence of pigment production from microalgal than other photosynthetic organisms. Detailed discussion on the culture parameters' effect on *Porphyridium*'s pigments would be presented in the next section.

In the *Porphyridium* family, *P. purpureum* is the most frequently studied and has been intensively cultured in a large scale. This marine microalga possesses a water-soluble pigment B-phycoerythrin and chlorophyll *a* with a cell size of 6–12 µm [32, 38]. The saline culture mediums are typically used to grow it such as the enriched sea water ("f" medium) developed by Guillard [39]. It is an adaptable microalga toward environmental changes. For instance, *P. purpureum* cultivated in "f" medium was reported to be highly tolerant to grow at the salinity of 12–32% (μ_{max} of 0.48–0.53 day^{-1}) [32]. Moreover, it could maintain a rapid growth rate in a deficient nutrient media [25]. In artificial seawater (ASW) medium, *P. purpureum* was found to be insensitive towards a high concentration of antibiotics hygromycin and kanamycin (1000 µg mL^{-1}) [40]. This microalga could live at 4–35 °C; however, the cultivation at lower than 35 °C is preferable [33]. It should be noted that saline media is strictly required for growing *P. purpureum*. Low salinity could significantly suppress the growth (μ_{max} of 0.20–0.30 day^{-1} at 2–8% salinity) and changes its cell morphology. Moreover, *P. purpureum* does not survive in freshwater [32, 41].

Meanwhile, *P. aerugineum* is a freshwater red microalga, having a bright blue-green-colored chloroplast with a cell size of 4–13 µm [31, 35]. The blue-green color is resulted from the presence of C-phycocyanin and chlorophyll *a* with no trace of phycoerythrin in the cells. Pekarkova et al. informed that increasing nutrients and light intensity could increase phycocyanin content and induce the growth of *P. aerugineum* (μ_{max} 0.11–0.13 h^{-1}) [35]. The optimum condition for culturing *P. aerugineum* was suggested to be around 28 °C with photo irradiances of 70–100 W m^{-2} PhAR and media containing a trace amount of vitamin B_{12}.

P. sordidum has an olive-green color because this microalga's chloroplast contains a phycobilin mixture of C-phycocyanin, B-phycoerythrin, and a trace of allophycocyanin. The cell has a size of 9–11 µm and is well grown in the saline medium [22], although it was firstly isolated from the freshwater environment [42, 43]. However, Medina-Cabrera et al. observed that *P. sordidum* has slower growth rates than *P. purpureum*, e.g., μ_{max} 0.17 and 0.22 day^{-1}, 4 and 3 days doubling time, respectively [22]. This is resulted due to its lower nutrient (nitrate and phosphate) uptake capability compared with the later species.

2.2 Chemical components

As a photoautotrophic microorganism, the chemical components of *Porphyridium* are dependent on many factors, including species, cultivation techniques, and environmental culture conditions (Table 1). In general, they contain a large fraction of carbohydrates, either in the form of intracellular or EPs. In some conditions, they could significantly accumulate proteins as well. The most attractive component is PBPs which have high market value in the cosmeceutical and pharmaceutical industries. Furthermore, although *Porphyridium* family is not a lipid producer, their fatty acids could contain a significant fraction of arachidonic acid (ARA) and eicosapentaenoic acid (EPA) (Table 2). In this section, the discussion is limited on the presentation of those three attractive compounds isolated from red macroalgae genera *Porphyridium*.

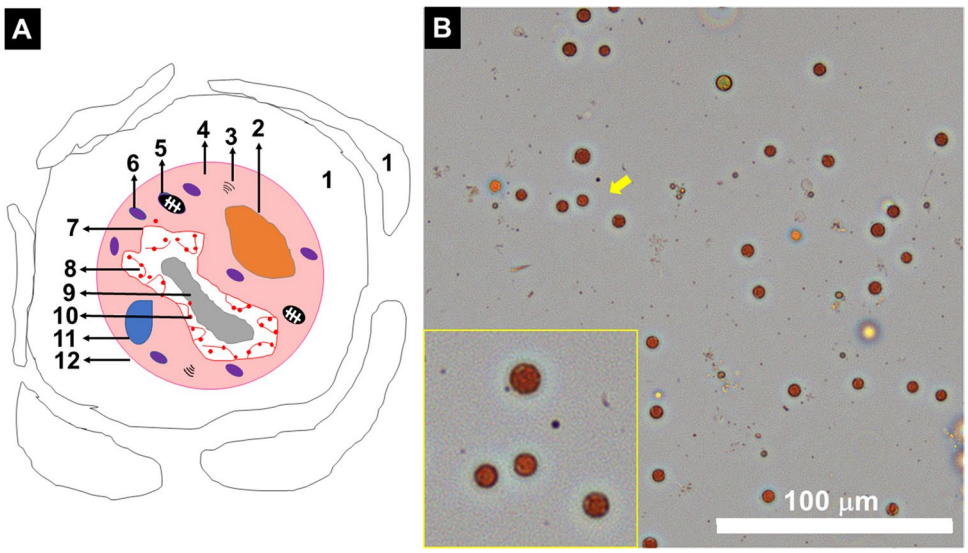

Fig. 1 **A** The general schematic cell structure of red microalga genera *Porphyridium*: 1 EPs, 2 nucleus, 3 Golgi, 4 cytosol, 5 mitochondrion, 6 intracellular polysaccharide, 7 chloroplast, 8 thylakoid, 9 pyrenoid, 10 phycobilisome, 11 vacuole, 12 plasmic membrane [3] **B** The cell image of red microalgae *Porphyridium* spp. obtained with Biotek cytation Imaging Reader. The inset figure shows an enlarge image of the selected cells (yellow arrow)

2.2.1 Polysaccharides

Porphyridium contains two types of carbohydrates, which are floridean starch (floridean glycogen) and sulfated polysaccharides [36]. The first one is a storage polysaccharide built up of D-glucopyranose residues linked by α-(1 → 4) glycosidic chains [44, 45] and found in the cellular component (cytosol) [46]. The structure is similar to starch with lesser amylose content (< 10%) and lower crystallinity A-type (16–37%) [47]. Nevertheless, each species might have a variety of glucan structures and granule size [48]. Size exclusion chromatography with iodine staining revealed two fractions of α-polyglucans in *Porphyridium*'s floridean starch corresponding to (1) semi-amylopectin-types with a mass of ~ 2.0×10^6 Da (similar to certain cyanobacteria) and (2) amylose-types with a mass of 1–2×10^5 Da [48]. Meanwhile, sulfated polysaccharides could be found as intracellular (bounded to the cytoplasmic membrane) and extracellular as EPs [28]. They account for a major component of the cells (Table 1) and are a heteropolymer as similar as sulfated galactans (phycocolloids) of red macroalgae (seaweeds) like agar and carrageenan.

EPs of Porphyridium are non-toxic with an approximate molecular weight of 1–7×10^6 Da [23, 49–51]. The assessment on Vero cells did not observe proliferation at 500 µg mL^{-1}. EPs are synthesized in the Golgi apparatus [52] and their main sugars are composed of xylose, galactose, and glucose in addition with the presence of glucuronic acid (in the form of 3-*O*-(α-D-glucopyranosyl uronic acid)-L-galactose) and sulfate groups (Table 3). A study on the electrophoresis of EPs isolated from *P. aerugineum* observed sulfate-poor and sulfate-rich fraction, in which the sulfate is present in the form of glucose-6-sulfate, galactose-3-sulfate, and galactose-6-sulfate [53]. Besides, the intensive study observed protein linkage in the range of 0.11–0.55 µmol mg^{-1} [54]. All these features make this polysaccharide to bring a negative charge (polar) and acidic properties. Moreover, EPs have thermally reversible gel character [36].

EPs isolated from *Porphyridium* are soluble in water, yielding a viscous solution at a relatively low concentration (0.25%). The aqueous solution of EPs has a pH in the range of 5.5 [23]. The viscosity of the EPs solution was higher compared with κ-carrageenan, xanthan gum (~ 32–55, 8 and 45 cp, respectively) [53] and λ-carrageenan [36]. Its concentrated solution (1–2 g L^{-1}) is stable at a wide range of pH (2–9), temperatures (20–150 °C), salinities [28, 55], and towards enzymatic degradation by hyaluronidase and carbohydrolases [56]. The high stability of EPs has been shown by no different physico-chemical characteristic changes of its solution after being stored for 90 days [50] and no viscosity changes with the addition of Na^+ and K^+ [53] as well as NaCl or $CaCl_2$ (1 M) [23]. As such, EPs serve as a natural protector of *Porphyridium* cells against extreme environmental changes and microbial predators such as bacteria, fungi, and viruses [28, 57].

The physico-chemical characteristics of EPs isolated from *Porphyridium* offer many potential applications for biotechnological industries (Table 4). Their good stability under environmental changes and against hyaluronidase-degrading enzymes makes them to be attractive for biolubricant applications. EPs and their acid-hydrolysate fractions have been found to show antioxidant activity, even better than carrageenan. A 0.1% w/v of EPs solution was active against *Escherichia coli* and *Bacillus subtilis* (~ 70% and ~ 35% inhibition, respectively) [57]. Besides the sulfated groups [11], the presence of functionalized sugars such as uronic acid and other bounded chemicals (e.g., trace metals, proteins) have been considered to be responsible for their activity [23, 27, 36, 54]. It is plausible because the lack of microfibrillar cell wall leads *Porphyridium* to biosynthesize EPs with possessing function as a protective layer.

2.2.2 Polyunsaturated fatty acids (PUFAs)

Compared with other groups of microalgae, the *Porphyridium* family has relatively low lipid content (5–14% d.w.) [15, 33, 58]. Nevertheless, their lipid is very interesting because it comprises abundant long-chain polyunsaturated fatty acids (LC-PUFAs) such as arachidonic (ARA, C20:4, ω-6) and eicosapentaenoic (EPA, C20:5, ω-3) acid (Table 2). This is resulted due to the lack of stearoyl acyl-carrier-protein desaturase enzyme in the plastid stroma of *Porphyridium* cells, which makes their lipid biosynthesis produce palmitic (C16:0) and stearic (C18:0) as the precursors of those two PUFAs [33, 58].

The main fatty acids observed in most of *Porphyridium* family include palmitic, palmitoleic, stearic, oleic, linoleic, ARA, and EPA. ARA can act as an immune-suppressant and a natural antifreeze [59]. Meanwhile, EPA is well-known for its hypotriglycemic effect in the prevention/treatment of hypercholesterolemia [60] and maintaining inflammatory systems [20]. Moreover, it is important in treating several clinical diseases (e.g., atherosclerosis, diabetes, brain disorders, cancer, rheumatoid, arthritis, psoriasis, Alzheimer, age-related macular degeneration), wound healing, modulatory vascular resistance, and infant development [61, 62].

The lipid content and fatty acid composition of red microalgae genera *Porphyridium* are varied depending on many factors. Low light intensity and temperature (~ 100 µmol m^{-2} s^{-1}, 25 °C) are beneficial for inducing the lipid production. However, this irradiance level often gives lower total fatty acids (TFAs) in the lipids, particularly

Table 1 Biochemical characteristic of red microalgae, the genera of *Porphyridium*

Ent		Culture Condition				Treatment	Bio-mass (g L^{-1})	μ (day^{-1})	Proximate (%-d.w.)[a]	EPS (g L^{-1})	Ref
		Culture System	Media	T[b] (°C)	IL[c]						
1	*P. purpureum*	Batch (1.6 L tube glass); 10 days; 1.5–2.0 L min^{-1} air rate	f/2-RSE (0.1 g L^{-1} $NaNO_3$)	20	Continuous day light (100 μmol m^{-2} s^{-1})	Centrifugation (4000 rpm, 5 min)	1.9	n.r	Lip. = 1.9 Carb. = 40	n.r	[15]
2			f/2-RSE (1.0 g L^{-1} $NaNO_3$)				3.4	n.r	Lip. = 2.2 Carb. = 50	n.r	
3		Batch (10 L photobioreactor); 15 days; 0.5–1.0 L min^{-1} air supply rate	M6 (brackish), pH 8.5	23	16:8 light:dark (21.6 μmol m^{-2} s^{-1}, cool white light)	Centrifugation (4500 rpm, 15 min); oven dried (60 °C)	0.7	0.14	Moist. = 8.5 Ash = 18.6 Lip. = 1.7 Prot. = 15.1 Carb. = 63.7*	0.7	[14]
4		Batch (16 L polycarbonate tube); 14 days	f/2 (< 0.1 g L^{-1} $NaNO_3$)	20	(100 μmol m^{-2} s^{-1}, white light)	Centrifugation (4000 rpm, 15 min; oven dried 95 °C	0.9	0.20	Lip. = 23.6 Prot. = 24.6 Carb. = ~ 4	n.r	[95]
5		Batch (400 L polycarbonate tube); 14 days					1.2	0.14	Lip. = 17.8 Prot. = 21.9 Carb. = ~ 12	n.r	
6		Batch (0.25 L tube glass); 16 days	ASW (0.4 g L-1 KNO_3)	25	24:0 light:dark period (350 μmol m^{-2} s^{-1}, fluorescent lights)	Centrifugation (8000 rpm, 10 min); lyophilization	1.5	n.r	Lip. = ~ 10 Prot. = ~ 10 Carb. = ~ 52		[16]
7			ASW (0.6 g L-1 KNO_3)				3.6	n.r	Lip. = ~ 10 Prot. = ~ 10 Carb. = ~ 50		
8			ASW (1.8 g L-1 KNO_3)				5.5	n.r	Lip. = ~ 10 Prot. = ~ 18 Carb. = ~ 40		
9	*P. cruentum*	Batch (0.1 L tube glass); 7 days	F	20	(200 μmol m^{-2} s^{-1}, white fluorescent lights)	Membrane filtration (GF/C, ~ 1 μm pore size); oven dried 60 °C	n.r	0.80	Lip. = ~ 10 Prot. = ~ 10 Carb. = ~ 25	n.r	
10		Batch	Vonshak	25	16:8 light:dark period (100 μmol m^{-2} s^{-1})	Centrifugation (4500 rpm, 10 min, 4 °C); lyophilization	n.r	n.r	Lip. = 6.8 Prot. = 16.0 Carb. = 48.5	n.r	[108]
11		Continuous (200 L tubular; 10 h dilution rate); 0.7 mol min^{-1} air rate; 9 days residence time	Hemrick	20	sunlight	Centrifugation (3500 rpm, 5 min); lyophilization	3.2	n.r	Moist. = 3.2 Ash = 16.9 Lip. = 7.6 Prot. = 30.4 Carb. = 37.5	n.r	[13]

ASW artificial sea water, *n.r.* not reported, *Moist.* moistures, *Lip.* lipids, *Prot.* proteins, *Crb.* carbohydrates adry weight cultivation temperature illumination light, EPS = exopolysaccharides denoted as nitrogen-free extract and calculated by the differences of proximates (ash, lipid, crude fiber, crude protein), medium composition (in mg L^{-1}):

f/2 = $NaNO_3$ (75), $NaH_2PO_4{\cdot}2H_2O$ (5), Na_2EDTA (4.4), $FeCl_3{\cdot}6H_2O$ (3.2), $CuSO_4{\cdot}5H_2O$ (0.01), $ZnSO_4{\cdot}7H_2O$ (0.02), $CoCl_2{\cdot}6H_2O$ (0.01), $MnCl_2{\cdot}4H_2O$ (0.18), $Na_2MoO_4{\cdot}2H_2O$ (0.01), Vitamin B1 (0.1 mg), Vitamin B7 (0.5×10^{-3}) and Vitamin B12 (0.5×10^{-3}) diluted in 1 L of seawater sterile [109]

■f/2-RSE = f/2 medium developed by Guillard was diluted in 1 L of ReefSalt (34 g L^{-1}, H_2Ocean, Pro+, UK) complemented with $NaH_2PO_4{\cdot}2H_2O$ (5.65), $NaNO_3$ (100), $Na_2EDTA{\cdot}2H_2O$ (4.16), $FeCl_3{\cdot}6H_2O$ (3.15), $CuSO_4{\cdot}5H_2O$ (9.8), $ZnSO_4{\cdot}7H_2O$ (22), $CoCl_2{\cdot}6H_2O$ (10), $MnCl_2{\cdot}4H_2O$ (0.18), $Na_2MoO_4{\cdot}2H_2O$ (6.3), vitamin B1 (0.1), vitamin B_{12} (0.5×10^{-3}), vitamin B7 (0.5×10^{-3}) [109, 110]

■Artificial sea water (ASW) = NaCl (27×10^3), $MgSO_4{\cdot}7H_2O$ (6.6×10^3), $MgCl_2{\cdot}6H_2O$ (5.6×10^3), $CaCl_2{\cdot}2H_2O$ (1.5×10^3), $NaHCO_3$ (40), $ZnCl_2$ (40), H_3BO_3 (600), $CoCl_2{\cdot}6H_2O$ (15), $CuCl_2{\cdot}2H_2O$ (40), $MnCl_2{\cdot}4H_2O$ (400), $Na_2MoO_4{\cdot}2H_2O$ (510), $FeCl_3{\cdot}4H_2O$ (2.4), Na_2EDTA (4.4), pH 7.5, thiamine-HCl (0.1), vitamin B12 (trace), vitamin B7 (trace) [85]

Table 2 Fatty acids composition of red microalgae of *Porphyridium*

		P. purpureum				*P. cruentum*			*P. aerugineum*
Culture medium		n.r	f/2	ASW	KOCK	Brody-Emerson	ASW	L1	Ramus
Cultivation time	days	n.r	23	21	14–18	n.r	16	30	n.r
Lipid	%-d.w	9–14	n.r	7–8	3–5	4–8	n.r	< 2	6–10
Fatty acids		**Relative Composition (%-mol)***							
■Myristic	C14:0	n.d	0.1	4–6	n.r	n.d	0.8	n.d	n.d
■Myristoleic	C14:1	n.d	2.8	n.d	n.r	n.d	n.d	n.d	n.d
■Palmitic	C16:0	21–26	37.9	1–2	17–36	33.2	28.4	30.5	46.3
■Palmitoleic	C16:1	1–2	1.4	29–30	n.r	0.5	0.5	1	0.8
■Margaric	C17:0	n.d	n.d	n.d	n.r	n.d	n.d	n.d	n.d
■Stearic	C18:0	< 2	13.7	2	1–4	1.9	2.8	2.5	1
■Oleic	C18:1	< 1	4.4	6–8	n.r	4.7	3.3	3.2	1.3
■Linoleic	C18:2	4–21	8.1	< 1	8–19	18.9	13.4	21	5.9
■α-Linolenic	C18:3, ω-3	n.d	n.d	< 1	n.r	n.d	n.d	n.d	n.d
■γ-Linolenic	C18:3, ω-6	< 2	8.6	n.d	n.r	0.5	n.d	n.d	0.2
■Octadecatetraenoic	C18:4, ω-3	n.d	n.d	n.d	n.r	n.d	n.d	n.d	n.d
■Eicosenoic	C20:1, ω-3	n.d	n.d	n.d	n.r	n.d	1.5	n.d	n.d
■Eicosadienoic	C20:2, ω-6	n.d	n.d	12–17	n.r	0.4	n.d	0.8	0.5
■Eicosatrienoic	C20:3, ω-3	< 1	n.d	< 2	2–5	n.d	0.5	n.d	3.7
■ARA	C20:4, ω-6	6–33	5.8	15–16	9–19	30.4	35	23.9	13.4
■EPA	C20:5, ω-3	17–63	6.7	19–24	4–9	5.7	13.8	9.5	23.6
■Docosapentaenoic	C22:5, ω-3	n.d	10.5	n.d	n.r	n.d	n.d	n.d	n.d
■Docosahexaenoic	C22:6, ω-3	n.d	n.d	n.d	n.r	n.d	n.d	n.d	n.d
■Others		n.d	n.d	n.d	3–7	n.d	n.d	7.6	1.3
References		[33]	[61]	[17]	[71]	[111]	[85]	[62]	[111]

d.w. dried weight; *n.d.* not detected; *n.r.* not reported; *based on total fatty acids Medium composition (in mg L^{-1}

Brody-Emerson = KNO_3 (1.2×10^3), KCl (16×10^3), NaCl (12.5×10^3), $MgSO_4{\cdot}7H_2O$ (2.5×10^3), K_2HPO_4 (0.4×10^3), $Ca(NO_3)_2{\cdot}4H_2O$ (200×10^3), KI (49.9), KBr (500), $FeSO_4$ (3), H_3BO_3 (3.1), $MnSO_4$ (1.9), $ZnSO_4$ (0.2), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.6), $CuSO_4$ (0.1), $Al_2(SO_4)_3K_2SO_4{\cdot}2H_2O$ (0.5), $Cd(NO_3)_2{\cdot}4H_2O$ (0.1), $Co(NO_3)_2{\cdot}6H_2O$ (0.1), $NiCl_2{\cdot}6H_2O$ (0.1), $Cr(NO_3)_3{\cdot}7H_2O$ (< 0.1), $V_2O_4(SO_3)_3{\cdot}16H_2O$ (< 0.1), Na2WO4·16H_2O (< 0.1), distilled water [112]

■Artificial sea water (ASW) = NaCl (27×10^3), $MgSO_4{\cdot}7H_2O$ (6.6×10^3), $MgCl_2{\cdot}6H_2O$ (5.6×10^3), $CaCl_2{\cdot}2H_2O$ (1.5×10^3), $NaHCO_3$ (40), $ZnCl_2$ (40), H_3BO_3 (600), $CoCl_2{\cdot}6H_2O$ (15), $CuCl_2{\cdot}2H_2O$ (40), $MnCl_2{\cdot}4H_2O$ (400), $Na_2MoO_4{\cdot}2H_2O$ (510), $FeCl_3{\cdot}4H_2O$ (2.4), Na_2EDTA (4.4), pH 7.5, thiamine-HCl (0.1), vitamin B12 (trace), vitamin B7 (trace) [85]

■L1 = $NaNO_3$ (75×10^3), $NaH_2PO_4{\cdot}H_2O$ (5×10^3), $Na_2SiO_3{\cdot}9H_2O$ (30×10^3), $MnCl_2{\cdot}4H_2O$ (0.2), $ZnSO_4{\cdot}7H_2O$ (< 0.1), $CoCl_2{\cdot}6H_2O$ (< 0.1), $CuSO_4{\cdot}5H_2O$ (< 0.1), $Na_2MoO_4{\cdot}2H_2O$ (< 0.1), H_2SeO_3 (< 0.1), $NiSO_4{\cdot}6H_2O$ (< 0.1), Na_3VO_4 (< 0.1), K_2CrO_4 (< 0.1) [113]

■Ramus = $NaNO_3$ (442), KCl (30), $CaCl_2{\cdot}2H_2O$ (36.6), $FeCl_3{\cdot}6H_2O$ (1.9), $MgSO_4{\cdot}7H_2O$ (100), Na_2 glycerophosphate·5H_2O (90), Tricine buffer (986), pH trace metal mix (10 mL), vitamin B_{12} (3.5×10^{-3}), distilled water, pH 7.6 [24]

■KOCK = $MgSO_4{\cdot}7H_2O$ (400), KH_3PO_4 (50), KNO_3 (1500), Fe(III) Citrate (5), basal medium (20 mL), pH 8.1 [114]

PUFAs, compared with the use of intense light higher than that value [58]. Furthermore, the nutrition in culture medium has a significant impact on the lipid content and its composition. It should be noted that the total accumulation of lipids and/or fatty acids are also dependent on the biomass productivity of culture. As such, manipulating the environmental conditions by controlling an intense light and moderate temperature is mainly applied to obtain a maximal ratio of unsaturated fatty acids like ARA and EPA [58]. The detailed information on these would be discussed in the cultivation parameter section.

2.2.3 Phycobiliproteins (PBPs)

Based on their light absorption spectrum, there are four classes of PBPs, which are phycoerythrin (pink-red, λ_{max} = 490–570 nm), phycocyanin (blue, λ_{max} = 610–625 nm), allophycocyanin (blue-green, λ_{max} = 650–660 nm), and phycoerythrocyanin (blue-pink, 560–600 nm) (Fig. 2). EFSA approves phycocyanin and allophycocyanin (EU Regulation No. 1333/2008, 231/2012) and the US FDA (21CFR73.1530) as coloring foodstuff. Herein, the ratio of the absorbance maximum of each PBPs and the

Table 3 Chemical composition of polysaccharides isolated from red microalgae genera *Porphyridium* [9, 23, 53, 115]

Species	Molar Ratio*				Glucuronic acid (%)	Sulfate (%)
	Xyl	**Gluc**	**Galact**	**Other components**		
Porphyridium sp.	2.5	1.0	1.8	Methyl hexoses (4.8), Mannose (1.1), rhamnose (1.0)	8–10	8
P. aerugineum	1.5–1.7	1.0	0.9–1.1	Methyl hexoses (0.9–1.1)	4–10	< 3
P. cruentum	1.1–2.4	1.0	1.4–2.1	Amino acids (1–2%)	5–8	9–21
P. purpureum	1.3	1.0	1.3	n.r	~4	20–23
P. marinum	2.3	1.0	1.2	Fucose (0.1), arabinose (0.1), arabinose (0.2), uronic acid (1.1)	5	9

*Compared with glucose as the main sugars of polysaccharides

Xyl xylose, *Gluc* glucose, *Galact* galactose, *n.r.* not reported

Table 4 Some potential characters of EPs and derivatives isolated from red microalgae genera of *Porphyridium*

Materials; Species	Applications	Eminences	Ref
EPs; *Porphyridium* sp.	Biolubricant	■Similar magnitude of viscosity and elasticity with hyaluronic acid at low concentration (2%) ■Relatively stable (17–29% reduction) with the temperature changes from 25–70 °C than hyaluronic acid (51–64% reduction) ■Superior stability against hyaluronidase degrading enzyme than hyaluronic acid (0 and 70% viscosity reduction, respectively)	[49]
■EPs; *Porphyridium* sp.; *EPs, P. aerugineum*	Antioxidant	Relatively strong antioxidant activity on linoleic acid auto-oxidation (ferrous oxidation assay) than λ-carrageenan and cellulose (~47, < 1, and 7% inhibition at 2 mg mL^{-1})	[11]
EPs; *P. cruentum* EPs; *P. purpureum*	Antioxidant (Alguard®)	Comparable hydroxyl-radical-scavenging activity with ascorbic acid (IC50 of 0.6–0.7 and 0.3 mg mL^{-1}, respectively)	[27, 116]
■EPs; *Porphyridium* sp.; ■Zn-EPs; *Porphyridium* sp.	antibacterial	■EPs inhibited *E. coli* and *B. subtilis* in the range of 36% and 72%, respectively, at 0.1% w/v ■Zn-modified EPS inhibited 74–100% of *E. coli* and *B. subtilis* at 0.1% w/v	[57]
EPs; *Porphyridium* sp.; *P. cruentum*	Antiviral	■Active against *Herpes simplex*-infection HSV-1 (EC_{50} 1–56 μg mL^{-1}), HSV-2 (EC_{50} 5–20 μg mL^{-1}), vaccinia virus (EC_{50} 12–20 μg mL^{-1}) ■No proliferation on Vero cells at 500 μg mL ■No cytotoxic on HEL cells at 100 μg mL	[10, 117]
EPs-derived oligosaccharide; *P. purpureum*, *P. cruentum*	Vascular toning (Silidine®)	■Improves vascular tone ■Inhibits rosacea and skin redness ■Corrects heavy leg syndrome	[118]
EPs; *P. purpureum*	Melanin booster (Epsinil®)	■Increasing melanin synthesis and transport to the skin surface ■Improving the skin moisturization and softness	[118]

absorbance at 280 nm (a total amount of proteins in the product) could be used as one of the standard methods to evaluate their purity grade; i.e., food (> 0.7), cosmetic (> 1.5), reactive (> 3.9), and analytical (4.0) [12].

In recent years, PBPs have attracted much interests owing to their spectral, fluorescent, and colorant properties [63]. Apart from their natural colorant applications, they are also currently used in the therapeutic sciences; e.g., antiviral, antibacterial, anti-inflammatory, immunemodulatory, anticancer, antiaging, antioxidant; and detection kits [64, 65]. PBPs are stable and water-soluble molecules composed of proteins and noncyclic tetrapyrroles (viz., phycobilins) acting as a chromophore, covalently linked by cysteine [66]. In *P. purpureum*, about 5% of PBPs could be obtained under optimum condition, in which, mostly it composes of phycoerythrin (70%), R-phycocyanin (20%), and allophycocyanin (10%) [67]. Herein, phycoerythrin is the main economically valuable compound of PBPs isolated from *Porphyridium* because it composes for more than 70–80% [15].

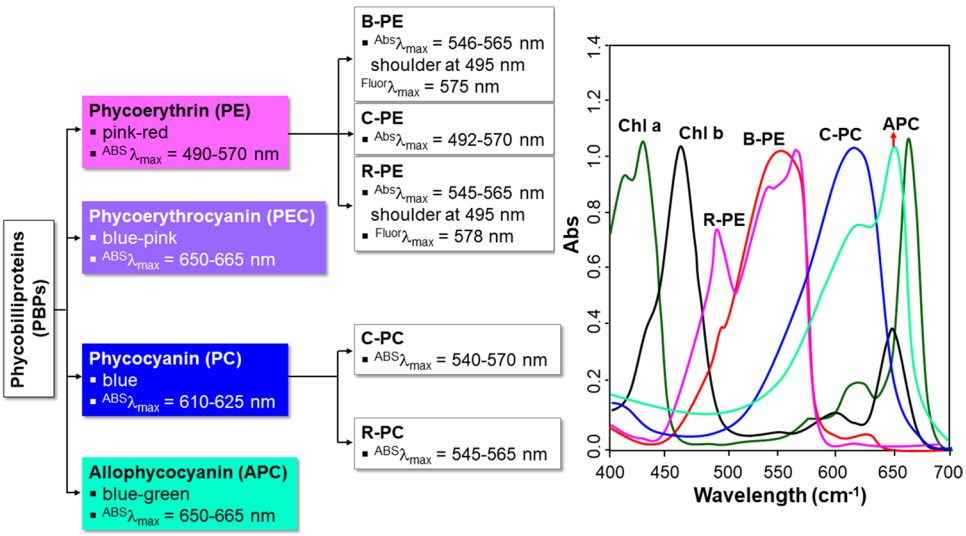

Fig. 2 Diversity of phycobiliproteins in nature [64, 66, 105] and the UV–Vis spectrum of some phycobiliproteins (adapted from [106])

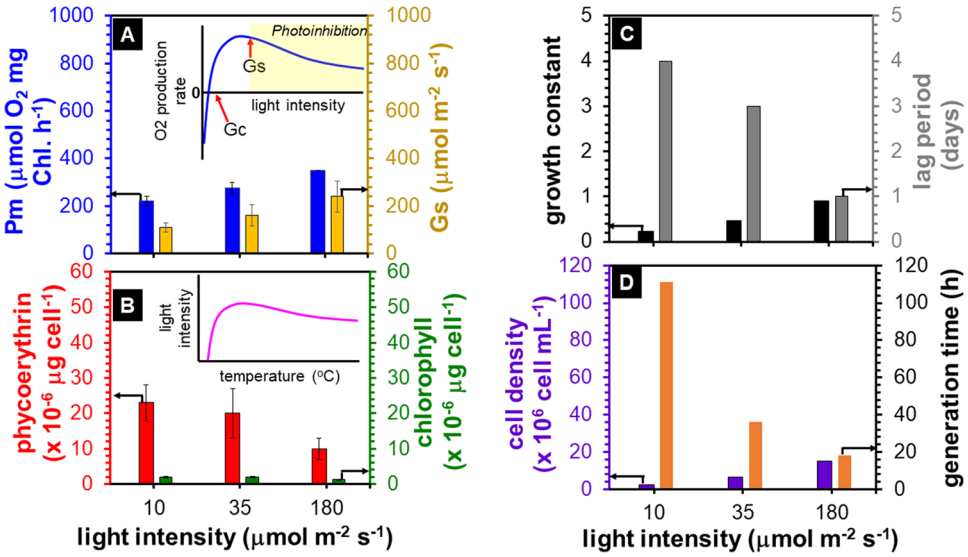

Fig. 3 Effect of light irradiance on growth cultures of *P. purpureum* [38] and *P. cruentum* [69]: **A** maximal oxygen production rate (Pm) and saturated light intensity (Gs), **B** phycoerythrin and chlorophyll content per cells, **C** growth constant and lag period, **D** cell density and generation time. Inset image on figure (**A**) exhibits typical curve of photosynthetic activity of red microalgae *Porphyridium* as a function of light intensity; Gc and Gs are the abbreviations of compensate and saturated light intensity [3, 68, 72, 74]

3 Cultivation of *Porphyridium*

As common photoautotrophic microalgae, *Porphyridium* require good condition of several culture parameters for supporting their growth and metabolism. Light is necessary to catalyze their photosynthetic reaction;, however, it could also be negative on the cells at certain levels. Performing the large culture system has also challenges because of the dynamic changes of physical parameters to be controlled such as temperature and pH in the culture, especially for the outdoor cultivation. In addition, the nutrients have important effect on the cell's growth and the biosynthesis metabolism of molecules. In this section, some critical parameters in the cultures of *Porphyridium* is presented and discussed at first. Furthermore, the discussion is focused on the growth characteristics and biochemical productivity of *Porphyridium* cultivated under open- and closed-culture systems. The comparative information on the established large-scale culture system and recent development of these culture systems are delivered.

3.1 Abiotic culture parameters

3.1.1 Light

Porphyridium requires light for catalyzing their cellular photosynthesis reaction. Compared with the use of low irradiance, the use of intense light would significantly increase cellular photosynthetic activity, resulting in a fast growth rate, a short lag phase, high growth constant, and more abundant biomass (Fig. 3). The enhancement of photosynthetic cellular activity could be indicated experimentally by (1) a linear correlation between light intensity and oxygen

Table 5 Photoinhibition of some species of red microalgae genera *Porphyridium*

Entry	Species	Culture conditions		Light intensity (μmol m^{-2} s^{-1})			Refs
		System	Medium	T (°C)	IL source	Photo- inhibition	
1	*P. purpureum*	Batch, 5% CO_2, agitation, daylight lamp (continuous irradiation)	Jones ASW	18	10	90–110	[38]
2					35	115–160	
3					180	175–240	
4	*P. cruentum*	Batch (250 mL), 5% CO_2, halogen lamp	Jones	25	6–280	60–111*	[68]
5	*P. cruentum*	Batch (250 mL), 100 rpm agitation, 12/12 photoperiod	Hemrick	25	5–1100	~400	[74]
6	*P. cruentum*	Batch (500 mL), white fluorescent tube	ASW	25	30–90	70	[73]
7	*P. cruentum*	Batch (15 L), 25 L min^{-1}, 1% CO_2, air flow, cool-white lamps	OM I	25	20–150	80	[70]
8	*P. marinum*	Batch (5 L), 200 rpm agitation, continuous light (halogen lamp)	Provasoli	25	0–1600	360	[72]
9	*P. purpureum*	Batch, 1.5–2 L min^{-1} air, continuous light (cool daylight fluorescent)	f/2	20	100	90	[15]

T temperature, *t* cultivation time, *IL* illumination light, *ASW* artificial sea water; *calculated based on specific light absorption rate and chlorophyll content

evolution of the culture [38, 68] and (2) the changes of cells morphology, e.g., short cell generation time, increasing cell volume and starch granules, and reduction of thylakoid membrane area [69]. As a result, the use of intense light would also benefit polysaccharide formation as the main photosynthetic product [4]. The increasing polysaccharide content in *P. purpureum* and *P. cruentum* have been observed under the light irradiances of 30 to 100 μmol m^{-2} s^{-1} [15, 70]. However, raising the intensity above 150 μmol m^{-2} s^{-1} tends to slightly decrease polysaccharides [15, 70]. Moreover, it could significantly reduce total lipids [58, 62], TFAs, LC-PUFAs (ARA, EPA) [71] as well as pigments composition in *Porphyridium* cells.

Although light is essential, there is a threshold value (a saturated point, Gs) where a light inhibits microalgae cell's growth, viz., photoinhibition (inset Fig. 3A). At this point, cellular photosynthetic activity is starting to decrease slowly [38, 72] and cell growth becomes slow [73, 74]. The excess high light exposure could give negative impacts on a cell such as chloroplast destruction, inactivating enzymes involved in CO_2 fixation [25, 70], significant reduction of pigments (chlorophylls, PBPs, carotenoids) [75], lowering thylakoid membrane area, and decreasing three major light-harvesting complexes per cell, i.e., photosynthesis I, photosynthesis II, and phycobilisomes [38, 69, 76]. PBP pigment reduction leads cell's color to change from reddish to more lighter or yellowish [36] This saturated point is varied depending on the species and culture conditions (Table 5). One of important factors is temperature, which significantly affects the parameter coefficients of light intensity on cellular photosynthetic activity (Fig. 3B). Especially, light-temperature interaction is significantly correlated and interacted (p value < 0.0001) under non-limited nutrient conditions [15].

A low light irradiance is essential for the cells to initially synthesize proteins, including PBP pigments. In the cultivation of *Porphyridium* sp., it could be indicated by the color changes of cells from a dark red into yellowish accompanied by a distinct level of protein per cell under light increment [36]. Also, low light is necessary for lipid accumulation. For instance, *P. purpureum* cultivated in ASW medium under 70 μmol m^{-2} s^{-1} light irradiance at 25 °C contained 15% of lipid while it was decreased to 12% after irradiated over 280 μmol m^{-2} s^{-1} [58]. Nevertheless, the first one gave no significant cells growth of *P. purpureum* and lower TFAs in the lipids (23% and 30%, respectively), suggesting that sufficient light is required to promote cellular photosynthetic activity.

In general, the light intensity around 50–100 μmol m^{-2} s^{-1} is recommended for minimizing photoinhibition and obtaining optimal growth of *Porphyridium* [15]. Since intense light is also required for cell growth and determining cellular photosynthetic products, controlling light intensity should be strictly considered. A recent report presented an increase of *P. purpureum* biomass productivity by ~ 52% after regulating light irradiance incrementally from 65 to 540 μmol m^{-2} s^{-1} [77]. Meanwhile, a drastic biomass reduction was obtained when the culture was irradiated with high light regulation (65 to 1160 μmol m^{-2} s^{-1}). Interestingly, a recent study found that different colors of LED light could also affect the growth characteristics and biochemical components of *P. cruentum* cultivated in column photobioreactor. Herein, green LED light enhanced cell growth (2.9 g L^{-1}), EPS (255.6 mg L^{-1}), and B-phycoerythrin (51.6 mg g^{-1} biomass), while white wavelength promoted total fatty acids

(7.5%) and PUFAs (48.2 mg g^{-1} biomass) [78]. Applying two-stage cultivation, green wavelength for the first period and white wavelength for the second time, could simultaneously obtain culture with high biomass productivity (~3 g L^{-1}); significant content of macromolecules, e.g., carbohydrates (~25%), proteins (~30%), TFAs (~40%); and high-value compounds, e.g., EPS (315,2 mg L^{-1}), B-phycoerythrin (52 mg g^{-1} biomass), and PUFAs (139,7 mg L^{-1}).

3.1.2 Temperature and pH

The maximal growth rate of *Porphyridium* is generally obtained at 20–30 °C [72]. Cells are not grown at 10 °C, while they grow slow at ≤ 20 or > 35 °C and require longer lag times for adaptation [62, 79, 80]. In the cultivation of *P. purpureum* using ASW medium, [81] reported that the culture maintained at 25–30 °C produced the highest biomass productivity (9–11 g L^{-1}), total fatty acids (32–38 mg g^{-1}), unsaturated fatty acids (18–20 mg g^{-1}), EPA (2–3 mg g^{-1}), and ARA (7–10 mg g^{-1}). In other research, increasing temperature from 25 to 35 °C under 280 µmol m^{-2} s^{-1} decreased the lipid content (~15 to ~10%) and EPA in TFAs (30–40 to 10–20 mg L^{-1}) while the contents of TFAs in lipid fraction (30–55 mg g^{-1}) as well as ARA in TFAs were increased (70–80 to 120–200 mg L^{-1}) [58].

Meanwhile, the optimum pH for red microalga *Porphyridium* cultivation ranges between 6.0 and 8.5. In the weak acidic state (pH ~ 5.0), the *Porphyridium* sp. cells grown in ASW medium were significantly inhibited and they were died at pH 3.5 [79]. The cell density enhancement of *P. purpureum* in the stationary phase at pH 6.0 to 8.0 was observed by [41], in which it grew faster in the initial stage of the logarithmic phase at pH 7. However, they mentioned the shrinkage pigment bodies at pH 6 and 7 but it was more vigorous at pH 8. The highest content of polysaccharides (10%), phycoerythrin (13%), and total lipids (10%) were also in line with the pH order of 6 < 7 < 8. In other research, the EPA per cell of *P. purpureum* remained unchanged but the ARA production decreased by 33% with increasing pH from 5 to 8 [82]. It should be noted that the basic pH of the solution (> 9) flocculated cells of *Porphyridium* sp. and *P. cruentum* [80].

3.1.3 Nutrient

In a recent report, *P. purpureum* showed different growth in KOCK, ASW, and "f/2" medium [83], implying that the nutrient composition in media affects *Porphyridium* cell growth. One of the important nutrients is nitrogenous components such as nitrates, which were reported to have statistically significant effect (p value < 0.05) [20, 84]. Under different nitrate concentrations in ASW and KOCK medium, for example, *P. purpureum* cultivated in a batch photobioreactor did not show any different lag phase. However, the culture with a high initial nitrate concentration had the fastest log phase and yielded the highest biomass [16, 41]. A sufficient nitrate was also reported to be a benefit for the growth of *P. cruentum* in "f" medium [34] and could improve 2–threefold of biomass yield in "f/2" medium [85]. In another report, the use of ammonium or nitrate as nitrogen sources was reported to be insignificantly different, but their interaction with light should be considered [34]. Moreover, neither nitrogen concentration nor source between nitrate and urea significantly affected the biomass production and biochemical components of *P. purpureum* in Jones medium under high nitrogen level (14–30 mM) [86].

In some microalgae species, nitrogen sources of the culture medium has been well known to benefit for lipid accumulation [87]. In other words, this nutrient could have impact on the biosynthesis of biomolecules in microalgae. Li et al. observed that nitrogen-replete cultures are beneficial for PBPs production (volumetric productivity and content), especially phycoerythrin, from *P. purpureum* cultivated in ASW medium because of their impact on enhancing proteins metabolism [16]. These could be simply indicated by the intense cell's color appearances related to PBP major pigments. For example, *P. purpureum* cells had intense brownish red and yellowish when the cultures were carried out under nitrogen-sufficient (12–17 mM nitrate) and nitrogen-deficient (1–3 mM nitrate) medium, respectively [16, 83]. In contrast, a nitrogen-limited medium is required to promote carbohydrate biosynthesis [16] including EPs [72] and starch [45, 88]. Meanwhile, this condition significantly reduced pigments (~0.4–1.3-fold reduction) such as phycoerythrin [15, 45] and chlorophylls (~80 and 99% reduction, respectively) [89, 90], lowering the photosynthetic activity of cells and thus minimizing cells' growth rate. In addition, the total lipid is not significantly affected by nitrogen (p value < 0.1) but it affects the LC-PUFA proportion such as a-linolenic, ARA, and EPA [16, 20]. Herein, nitrogen stress has been found to promote the a-linolenic as well as ARA content and production and proportion in total fatty acids [85]. Nevertheless, some reports mentioned about twofold enhancement of neutral lipid triglycerides in the cultivation of *P. cruentum* [45] and *P. purpureum* [91] under severe nitrate starvation (< 2 mM nitrate ions). Since *Porphyridium* has good adaptability towards the environmental changes, the cells could be regenerated after 24-h adaptation in a normal nitrate medium [90].

Other nutrients have also been studied for their effect on the growth as well as biochemicals of *Porphyridium*. Sulfate deficiency exhibited a similar trend effect with the culture under nitrogen starvation, but its starch/volume ratio effect is more pronounced (2.7 and 1.3–1.6, respectively) [45]. Despite the importance of nitrogen in culture medium, a recent report found the key role of carbon as well. In this

context, a high C/N ratio in medium tends to decrease biomass production as well as protein and increased carbohydrate content in the end of culture time [83]. Moreover, the ratios of ARA and EPA in total fatty acids were increased. Therefore, adjusting carbon sources should also be considered which could be done by external addition of either inorganic (CO_2) or organic form (e.g., bicarbonate ions) [58]. However, the use of these two carbons should also be well controlled because of the possible changes of the pH culture solution. In another research, phosphorus limitation could enhance a-linolenic formation as well as ARA and give opposite effect on EPA [81, 85]. Decreasing P/N ratios has also been reported to yield high cell density in the stationary phase [41], confirming the importance of nitrogen for cell growth.

3.2 Open pond systems

Arad and co-workers reported that *Porphyridium* sp. and *P. aerugineum* could be easily grown in an open-pond raceway under CO_2 aeration during winter and summer. The high sunlight irradiance in summer is benefit for growing more biomass, i.e., twofold higher [30, 36]. Recently, Castro-Varela et al. reported that this system could produce comparable biochemicals per *P. purpureum*'s biomass with the photobioreactor laboratory scale, including proteins, lipids, EPA, and ARA [86] However, they reported that its biomass productivity was fourfold lower than the culture grown in the photobioreactor. Interestingly, they showed the potency of urea as an alternative low-cost nitrogen source to replace nitrate in the medium.

However, open raceway ponds face several major problems. First, water lost by evaporation should not be hindered and it might change salinity as well as viscosity resulted by EPS accumulation of medium. Consequently, the additional use of freshwater to dilute and maintain the culture suspension should be controlled. Secondly, the inhomogeneous and ineffective light irradiance resulted by limitation penetration depth at a dense cell and one side irradiation light through surface only are often occurred. As a result, the open-pond culture has always lower biomass productivity compared with the closed culture photobioreactor. Lastly, the open system is at risk of contamination by dinoflagellates such as *Gymnodinium* sp. This microorganism is a predator of *Porphyridium* cells, and thus, it might ingest *Porphyridium* cells and reduce the product quality and quantity [79]. In some cases, a dilution technique to control the cell density as applied in *Spirulina* culture has been reported to effectively suppress contamination of *Porphyridium* sp. and *P. cruentum* cultivations in a 1-m^2 pond (130-L culture suspension) [80], although it might be depending on the geographic location of the open pond.

An open flat panel glass photobioreactor offers a light distribution advantage over open ponds. The use of transparent material glass provides a large irradiance surface area and the light path is adjustable to follow geographic conditions. This configuration could produce cell concentration about tenfold higher than a conventional pond outdoor [50]. Unfortunately, microbial contamination was still appeared (3–15%) even though it was declined as the viscosity of culture solution increased with the period of time. In indoor cultivation with a light irradiance of 280 μmol m^{-2} s^{-1}, a flat panel glass photobioreactor could significantly accumulate ARA and EPA in total fatty acids (TFA) of *P. purpureum* (~ 20 and ~ 40%, respectively) [58]. Again, this open system suffers water loss by evaporation that could increase the medium's salinity when using marine species of *Porphyridium*.

In order to minimize loss of water evaporation and microbial contamination without significantly increasing the construction as well as maintenance cost, some researchers have studied a covered raceway pond in outdoor culture (Fig. 4B). Despite its effectiveness in suppressing water loss, this construction still has not effectively solved the problem. Water is still evaporated under solar illumination and condensed to form droplets on the interior surfaces of the cover. It is resulted by the fact that about 50% of the energy in solar radiation is contained in the near- and mid-infrared above 750 nm, which enables to heat up the cultures [92]. Moreover, it could possibly affect the cells' growth as previously discussed. In addition, the water droplets have been shown to reduce light transmittance direction into the culture by 37% and thus reducing maximal biomass productivity by 2–18% [93].

3.3 Photobioreactor systems

Compared with open culture, *Porphyridium* cultivation in the closed culture enables to give higher performances. Outdoor cultivation of *Porphyridium* sp. in the batch system using polyethylene sleeves (100 L working volume) was found to be superior than in the open ponds with respect to cells' growth (160% higher), biomass production (~ 18 vs ~ 8 g m^{-2}), no microbial contamination, and polysaccharide productivity (~ 6 vs ~ 2 g day^{-1}) [55, 94]. In a recent report, indoor cultivation of *P. purpureum* in the polyethylene bag (80 L working volume) under white light irradiance (100 μmol m^{-2} s^{-1}) at 20 °C resulted in 1.25 g L^{-1} biomass with the content of carbohydrates, lipids, proteins, and phycoerythrin of 8–10%, 15%, 18–21%, and 2–21 mg L^{-1}, respectively, after 14 days [95].

Efficient utilization of light and temperature dynamics are essential factors, especially for the large-scale outdoor system [96]. One of the crucial factors is the reactor light path [77] because the cells will be denser by the time of

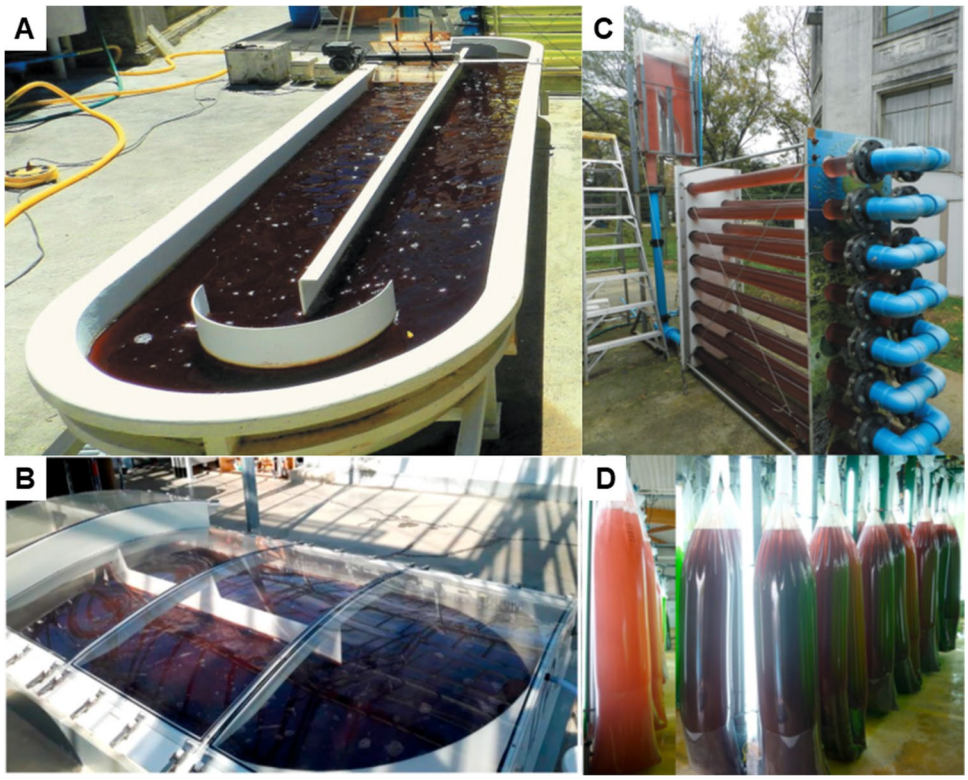

Fig. 4 Some typical cultivation systems in the cultivation of red microalga *Porphyridium*; open raceway pond (**A**), open raceway pond with cover (**B**), TBPBR (**C**), polyethylene sleeves (**D**) (reprint from [3, 86] with permissions from Taylor and Francis and Elsevier)

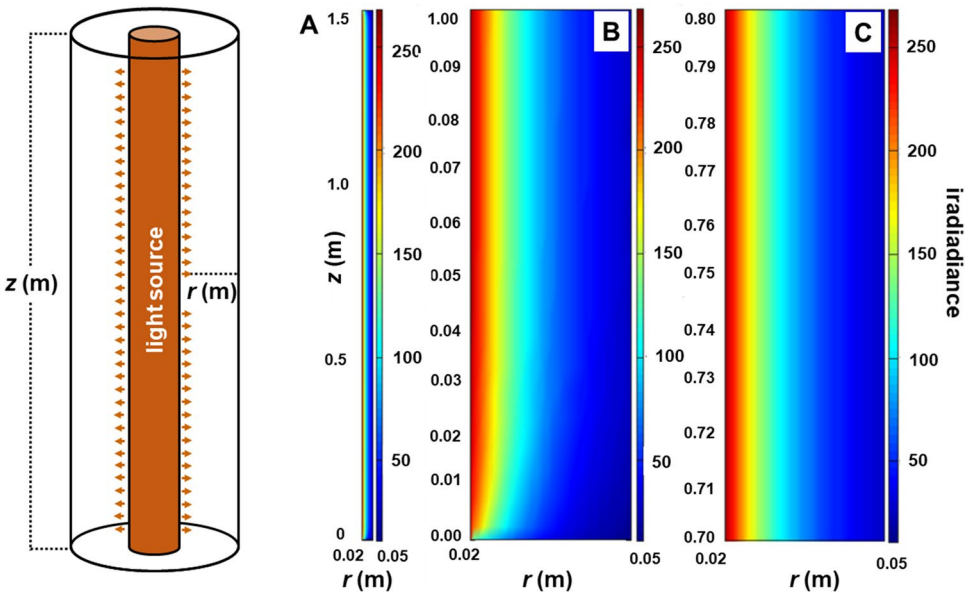

Fig. 5 Light distribution inside annular photobioreactor in the culture of *P. cruentum* (0.3 g L^{-1}) at general view (**A**), bottom (**B**), and middle tube (**C**) (permission [107])

cultivation. The light distribution is surely non-uniform inside the reactor (Fig. 5), even for a dilute culture. The cells near the light sources are exposed to high photon flux density while other cells receive less light due to the self-shading effect. This usually causes photoinhibition for cells near the wall surface, while the rest starves from light [75]. Singh et al. observed that the cell growth kinetic is inversely correlated with the light path length [50]. Moreover, they presented the diurnal changes in the culture's temperature during winter and summer responsible for the maximum volumetric cell density in the reactor. As such, a narrow light path is beneficial to give good light penetration into the culture and to result in high productivity, e.g., the sleeves with 10-cm diameter gave 3 times higher of biomass (~3 g L^{-1}) and 10-folds of total polysaccharide (~2 g L^{-1}) than that with 30-cm diameter [94]. For scaling up, maintaining

volumetric mass transfer coefficient (k_La) of CO_2 gas in the photobioreactor by increasing CO_2 gas flow rate was reported to be useful for giving similar optimum productivity of *P. purpureum* as obtained in its small scale cultivation [95]. Manipulating the fluid dynamic as in the airlift photobioreactor could improve cells distribution [97] and minimize the air and/or CO_2 gas requirement [98] because it could improve the regimen of light, temperature and liquid–gas mass transfer [99, 100].

Tubular photobioreactors (TBPBR) are the most intensively studied because the configuration provides high productivity with minimum space area and lower operational cost than other configurations such as bubble columns [101]. Fuentes-Grunewald et al. [102] reported that the cultivation of *P. purpureum* in a greenhouse during summer (11–26 °C, 8.2–893.5 µmol m^{-2} s^{-1} solar irradiance) by using an industrial scale TBPBR, e.g. 600 L culture, 100% CO_2 aerated 0.75 L h^{-1}, f/2 medium; gave cells growth in the range of 0.011–0.013 h^{-1} (1.3×10^7 cell mL^{-1} max. density) with a final biomass and EPS productivity of 0.97 g L^{-1} and 2.0 g L^{-1} (0.0266 g L^{-1} day^{-1}). The biomass productivity was similar, resulting in a bench-scale indoor culture (20 L, 19–21 °C, 150 µmol m^{-2} s^{-1} white fluorescent light, 18:6 h light:dark photoperiod, 0.04% CO_2). During autumn, [86] reported about 0.0257 g L^{-1} day^{-1} biomass production from *P. purpureum* cultivated in TBPBR with air injection and urea as nitrogen sources. In some condition, indoor cultivation is advantageous in well-control of light that could achieve higher cellular photosynthetic activity compared with the outdoor cultures which have photosynthetic conversion efficiency of only 1.3–2.4% of total solar radiation [103].

The application of a semi-continuous system offers better stability performances and could diminish contamination issues. In the study of [102], a higher cell density (> 11%) as well as EPS (~9%), shorter duplication time and stable productivity were achieved by applying partial harvestmen of culture (33% working volume). In fact, partial harvestmen of 75% of culture is recommended to maintain abiotic parameters of cultures (e.g., nutrient, dissolved gas) [55]. Another report presented a 50% enhancement of EPS isolated from *P. marinum* after conducting the culture in a semi-continuous state with 30% partial harvestmen [72]. This strategy is also beneficial to prevent limitations on cell growth due to the high viscosity of culture solutions.

4 Concluding remarks and future perspectives

Microalgae are promising biorefineries for sustainable biotechnology, converting solar energy to broad spectrum of fine chemicals and high-value natural products. They have high productivities with the potential large-scale biomass production and genetic as well as cellular engineering to boost the yield of microalgal-based products. In contrast with agricultural crops, large-scale microalgal cultivation does not face the issues on land's availability, freshwater, and arable land. The use of marine microalgae is also suitable for regions with geographical conditions of long periodic solar irradiance and coastal area.

Porphyridium contains multiple biochemicals with potential emerging market demands such as EPS, B-phycoerythrin, and LC-PUFAs like ARA and EPA. These metabolites are resulted from the photosynthetic metabolism of *Porphyridium* to support their growth during cultivation. The composition and the yield of these metabolites are diverse, depending on the genetic nature of the species and the living habitats of *Porphyridium*. Understanding the biological characteristics of *Porphyridium* family and their technical cultures is necessary to obtain a system that guarantee consistency, efficiency, good product quality, purity, safety, and suitability with the geographical location of the culture plant. *Porphyridium* have good tolerance toward wide ranges of temperature (10–35 °C) and pH (6.0–8.5). Marine species like *P. purpureum* can grow in broad salinity (12–32%). A low light irradiance is essential for *Porphyridium*'s cells to initially synthesize proteins including PBPs pigments and lipids, while intense light, at a certain level, is beneficial for cell growth and carbohydrates metabolism. To overcome, regulating irradiance incrementally and/or the use of different wavelength light are one of effective strategies for obtaining high biomass productivity with sufficient content of the targeted chemicals. Besides, controlling the ratios of nitrogen, carbon, phosphorous, and sulfur are critical, particularly for the two former nutrients, on determining the selective formation of EPS, PBPs pigments, and LC-PUFAs like ARA and EPA.

Large-scale cultivation in the pond system offers simple configuration and is suitable for culturing *Porphyridium* in the region with an annual good solar irradiance, stable air temperature, and availability of non-arable land. The main issues are related with water lost by evaporation, microbial contamination, and irregular irradiance by depth limitation. Closed systems have benefit to give more biomass productivity because the cultures are well-controlled and free of microbial contamination. Homogenous transport and distribution of solar irradiance, temperature, and liquid–gas mass transfer (dissolved oxygen/carbon dioxide) into all reactor side are crucial in order to encourage the cells for effective light energy utilization. The light path of photobioreactor influences the light as well as temperature distribution inside of culture, which also determines liquid–gas mass transfer in culture suspension. In this context, a narrow length is recommended, but the reactor configuration could also affect "cells movement" as result of the variation of fluid dynamic

of culture suspension inside reactor. These characteristics should be considered in determining design and construction on scaling up of the process. Maintaining the volumetric mass transfer coefficient (k_La) of CO_2 gas in the photobioreactor by increasing the CO_2 sparging flow rate is one of the strategies in order to give similar optimum productivity as obtained on a small scale. However, adjusting aeration at a certain value should be well-controlled since it was found to provide physical forces that could damage cells in the *P. cruentum* cultivation [99]. Noted, a study on *P. cruentum* cultivation in a tubular closed system by Gudin et al. found the cells were fragile to physical forces [104].

Porphyridium sp. is an EPs producer that excretes this sulfated polysaccharide into the medium and significantly affects the culture suspension's rheological properties. In the initial stage, when the cells' concentration is diluted, the culture is similar to water. Periodically, the medium becomes non-Newtonian and it finally impacts the viscosity of medium. The hydrodynamics of medium would be greatly changed, e.g., fluid turbulence variety in radial axial and wide range bubble gas size distribution, which determine light and temperature fluctuations and thus affect the geometry configuration of photobioreactor [75].

Batch cultures have been commonly used in large-scale cultivation. However, semicontinuous systems exhibit potential performances regarding the productivity and stability of biomass as well as targeted biochemicals. During the process, the deficiency of nutrients at the end of the lag phase would occur. The addition of fresh medium in semicontinuous would benefit to increase the nutrient availability, leading the cells to grow more. In addition, this would decrease the viscosity of the solution to some extent, reducing the possibility of cell growth limitation by viscous effect.

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Declarations

Conflict of interest The authors declare no competing interests.

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