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RESEARCH

Development and optimization of a photoautotrophic phycoerythrin production process

Jothyswarupha Krishna Kumar Athilakshmi[1,2] · Harinisri Aravind Raman[1,2] · Uttam Kumer Roy[1] · Dale D. McClure[1]

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Abstract

Phycoerythrin is a pink pigment produced by photosynthetic microorganisms which has potential to be used as a sustainable colorant in the food, cosmetics and textile industries. In order for this to happen it is necessary to identify suitable strains for large-scale production and develop and optimize production processes. Relatively few species of red algae have been evaluated for their biotechnological potential and to address this gap this work examined five different species of red algae and three cyanobacteria to identify the most suitable candidates for phycoerythrin production. Results from initial screening experiments found a wide range of specific phycoerythrin contents (0.75–28 mg g^{-1}). The four most promising species (*Porphyridium purpureum, Rhodella violacea, Timspurckia oligopyrenoides* and *Flintiella sanguinaria*) were grown in 3-L photobioreactors; of the species examined it was found that *P. purpureum* and *R. violacea* had the highest growth rate (approximately 0.6 day^{-1}). As part of the scale-up process it was found that ensuring sufficient nitrate was available in the medium was key in maintaining a high specific phycoerythrin content, with maximum values being 170 ± 34 mg g^{-1} for *P. purpureum* and 120 ± 60 mg g^{-1} for *R. violacea*. Increasing the applied light intensity led to increases in the cell density (to a maximum of 3.46 ± 0.36 g L^{-1} for *P. purpureum*), while the specific phycoerythrin content was generally constant. Applying these insights led to a final volumetric phycoerythrin concentration of 360 ± 37 mg L^{-1} for *P. purpureum* this being a 50-fold improvement over the original values. Results from this work provide valuable data about the growth and phycoerythrin productivity of diverse species of red algae as well as providing valuable insights into how the phycoerythrin production can be optimized.

Keywords Microalgae · Phycobilins · Phycoerythrin · Rhodophyta · Photobioreactor

Introduction

There is increasing interest in the use of natural and sustainable colors in a range of industries including food, cosmetics and textiles (Spolaore et al. 2006; Eriksen 2008; Sekar and Chandramohan 2008; Borowitzka 2013; Carmona et al. 2022; Mutaf-Kılıc et al. 2023). Photosynthetic microorganisms (i.e., microalgae and cyanobacteria) are a particularly promising source of pigments as these organisms produce a range of colored compounds (e.g., phycobiliproteins and carotenoids), use carbon dioxide as a feedstock and can be cultivated without the need for arable land (thereby avoiding competition with food production). However, a major challenge using photosynthetic microorganisms is the production of sufficient quantities at an economic price point. Such considerations are particularly important in industries like foods and textiles where it is necessary to produce relatively large quantities at relatively low cost (Gaignard et al. 2019). It is crucial to identify strains that are suited for large-scale production, develop and optimise production processes and integrate these with suitable downstream processing techniques in order to achieve this purpose.

Phycobiliproteins are a group of water-soluble pigments found in cyanobacteria, Rhodophyta (red algae) and Cryptophyta (Borowitzka 2013; Mercier et al. 2022). Three major categories of phycobiliproteins exist; they are phycoerythrin,

✉ Dale D. McClure
dale.mcclure@brunel.ac.uk

[1] Department of Chemical Engineering, College of Engineering, Design and Physical Sciences, Brunel University, London, Uxbridge UB8 3PH, UK

[2] Department of Biotechnology, School of Bioengineering, Faculty of Engineering and Technology, SRM Institute of Science and Technology (SRM IST), Chengalpattu District, Kattankulathur, Tamil Nadu 603203, India

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phycocyanin and allophycocyanin (MacColl 1998). Phycoerythrin, phycocyanin and allophycocyanin have absorption maxima at approximately 540–570, 610–620 and 650–655 nm, respectively, wavelengths where chlorophylls and carotenoid pigments do not absorb significantly (Eriksen 2008; Carmona et al. 2022). This reflects their role in nature, which is to harvest light and transfer it to the photosystems (MacColl 1998). Phycobiliproteins may also play a secondary role as nitrogen storage compounds, it has been observed in cyanobacteria that the concentration of phycocyanin decreased when the organisms were starved of nitrogen (Allen and Smith 1969; Yamanaka and Glazer 1980). Due to their color and optical properties, phycobiliproteins have applications as fluorescent probes in biotechnology and as colors in a range of applications (Spolaore et al. 2006; Eriksen 2008; Sekar and Chandramohan 2008; Carmona et al. 2022; Mutaf-Kılıc et al. 2023). In addition to their use as dyes there is also some evidence that phycobiliproteins may have health benefits, for example, phycocyanin and phycoerythrin have antioxidant properties (Romay et al. 1998; Bhat and Madyastha 2000; Soni et al. 2010; Gargouch et al. 2018), the potential health benefits of phycoerythrin have been reviewed in detail elsewhere (Gaignard et al. 2019; Tan et al. 2023). Phycocyanin is currently produced commercially, primarily using open pond cultures of *Arthrospira platensis* (Eriksen 2008), while smaller quantities of phycoerythrin are produced for use as fluorescent probes in biotechnology (Glazer 1994). The aim of this work is to identify species which could be used in the large-scale production of phycoerythrin.

Table 1 summarises work examining phycoerythrin production. The majority of the work has been done at flask-scale, and significant variation in the phycoerythrin content has been observed; with reported values ranging between 5–10 mg g^{-1} (Narindri Rara Winayu et al. 2022) and 475 mg g^{-1} (Khattar et al. 2015). Cyanobacteria generally had a higher total concentration of phycobiliproteins, while the fraction of phycoerythrin was typically higher in species of red algae. It was also interesting to note that relatively few species of red algae (primarily species of *Porphyridium*) have been evaluated for phycoerythrin production. Mercier et al. (2022) examined the concentration of phycoerythrin in ten different species of cryptophytes, with concentrations varying between 0.67 and 7.88 mg g^{-1}. In their work they reported a phycobiliprotein concentration of 0.7 mg g^{-1} for *Rhodomonas salina* CCMP 757 cultivated in flasks, whereas Latsos et al. (2021) reported 70 mg g^{-1} for *R. salina* CCMP 1319 cultivated in a flat-panel photobioreactor. While the difference between these values may be due to the different strain used it is much more likely that it is due to the differences in culture conditions. Hence, there is a clear need to evaluate species at a scale and reactor configuration representative of large-scale production. In order to maximize the production of phycoerythrin it is important to understand the effect of the cultivation conditions and use this knowledge to optimize the process. For photosynthetic microorganisms key factors include the amount of light the cells are exposed to and the availability of nutrients (i.e., carbon and nitrogen). It has been found that low light intensities led to higher specific phycobilin concentrations for a range of cyanobacteria (Gris et al. 2017; Arashiro et al. 2020; Schipper et al. 2020) as well as the red alga *Porphyridium purpureum* (Arashiro et al. 2020). Changes in light intensity have also been observed to change the ratio of the different phycobiliproteins in *Chroococcidiopsis* sp. (Montero-Lobato et al. 2020), similarly the wavelength of the incident light has also been shown to change the ratio of different phycobiliproteins (Bennett and Bogorad 1973; Tandeau de Marsac 1977; Mishra et al. 2012). The light intensity obviously influences the organism's growth; in general, higher light intensities result in more growth and higher cell densities. This results in increased self-shading, which may limit the effective light intensity. To maximise process productivity, it is vital to understand how varying light intensity impacts the phycoerythrin content.

Another key factor in the growth of the algae is ensuring sufficient availability of nutrients like carbon and nitrogen. Carbon is typically supplied by either adding bicarbonate to the medium, or more commonly by bubbling air or an air/CO_2 mixture through the culture. Ensuring sufficient nitrogen is present in the medium is likely to be a key factor, as nitrogen starvation has been shown to lead to degradation of phycobiliproteins in cyanobacteria (Allen and Smith 1969; Yamanaka and Glazer 1980); similarly nitrogen starvation was found to lead to a reduction in the phycoerythrin content in the red alga *P. purpureum* (Guihéneuf and Stengel 2015). The same authors showed that adding a nitrogen source to a nitrogen starved culture led to a restoration of the phycoerythrin concentration.

The aims of this work are to: i) screen different species (particularly red algae) with the aim of identifying potential new species for phycoerythrin production; ii) evaluate these species under conditions representative of an industrial-scale process and iii) identify growth conditions that maximize the phycoerythrin productivity.

Materials and methods

Algae and culture conditions

All the species used in this work were purchased from the Culture Collection of Algae and Protozoa and a complete list of the species along with the media used is provided in Table 2. Cultures were maintained in flasks in an airconditioned room (approximately 20 °C) with light provided by a white LED pad for 12 h per day at an intensity of approximately 30–60 µmol photons m^{-2} s^{-1}. Cultures were passaged every four to six weeks.

Table 1 Comparison of literature work examining the production of phycoerythrin using cyanobacteria and red algae

Species	Growth medium	Growth conditions	PBR design	Biomass concentration ($g\ L^{-1}$)	Biomass productivity ($g\ L^{-1}\ day^{-1}$)	Total phycobilin concentration ($mg\ g^{-1}$)	Phycoerythrin concentration ($mg\ g^{-1}$)	Reference
Anabaena fertilissima	Modified Chu- 10 medium	28 ±2 ℃ 45 µmol photons $m^{-2}\ s^{-1}$ 14:10 light:dark	250 mL Erlenmayer flasks	0.34	0.04	696 ±35	475 ±24	(Khattar et al. 2015)
Anabaena sp. ATCC 33047	Medium developed by Moreno et al. (Moreno et al. 1995)	30 ℃ minimum temperature pH controlled at 8.5 Natural light	Open ponds, 1 m^2 and maximum 0.3 m depth with paddlewheel for mixing	0.11–0.23	0.08	123–189	110–120	(Moreno et al. 2003)
Anabaena sp. BTA 903	BG- 11 with 0.3 M glycine and 5 mM sodium glutamate	28 ℃ 6000 lx, 14:10 light:dark cycle	500 mL Erlenmayer flasks (100 mL working volume)	1.17	0.039	107.88	49.96	(Nath et al. 2023)
Arthrospira platensis	Modified Zarrouk medium	22 ℃ 65, 150 and 230 µmol photons $m^{-2}\ s^{-1}$ 14:10 light:dark cycle	Erlenmayer flasks, 600 mL volume 3 L min^{-1} air flow	2.3	0.13	303	30	(Arashiro et al. 2020)
Chroococcidiopsis sp.	BBM medium	70 µmol photons $m^{-2}\ s^{-1}$	Erlenmayer flasks	3	0.21	200	47	(Montero-Lobato et al. 2020)
Nostoc sp.	BG- 11 medium with 0.75, 1.5 and 2.25 g L^{-1} $NaNO_3$	22 ℃ 65, 150 and 230 µmol photons $m^{-2}\ s^{-1}$ 14:10 light:dark cycle	Erlenmayer flasks, 600 mL volume 3 L min^{-1} air flow	2.2	0.11	199	7	(Arashiro et al. 2020)
Nostoc sp.	BG- 11_0 medium	30 ℃ 100 µmol photons $m^{-2}\ s^{-1}$	1.2 L flat plat PBR 0.2 vvm air	2.14	0.27	130	60	(Johnson et al. 2014)
Nostoc sp. PCC 9202	Arnon medium	34–36 ℃ 175 µmol photons $m^{-2}\ s^{-1}$	1 L glass airlift reactors				150	(Reis et al. 1998)
Thermosynechococcus sp. CL- 1	Modified Fitzgerald medium	50 ℃ 200 µmol photons $m^{-2}\ s^{-1}$	Flat panel PBR, 0.5 vvm aeration	2.1	0.7	98.1 ±6.7	5–10	(Narindri Rara Winayu et al. 2022)
Tolypothrix sp. NQAIF319	Nitrogen free BG-11 medium	30 and 100 230 µmol photons $m^{-2}\ s^{-1}$	2 L cultures with bubbling	0.67–2.8	0.024–0.1	67	32 ±5	(Velu et al. 2015)
Porphryridium cruentum UTEX 161	Hemericks medium	20 ℃ Sunlight, 280–1460 µmol photons $m^{-2}\ s^{-1}$	220 L PBR	0.5–3.1		7–36	14–29	(Rebolloso Fuentes et al. 2000)
Porphyridium marinum CCAP 1380/10	Pm medium	70–150 µmol photons $m^{-2}\ s^{-1}$, 110 rpm, 20 °C	2 L Erlyenmayer flasks	2			40	(Gargouch et al. 2018)

Table 1 (continued)

Species	Growth medium	Growth conditions	PBR design	Biomass concentration ($g\ L^{-1}$)	Biomass productivity ($g\ L^{-1}\ day^{-1}$)	Total phycobilin concentration ($mg\ g^{-1}$)	Phycoerythrin concentration ($mg\ g^{-1}$)	Reference
Porphyridium purpureum	Modified artificial seawater medium	22 ℃ 65, 150 and 230 µmol photons $m^{-2}\ s^{-1}$ 14:10 light:dark cycle	Erlenmayer flasks, 600 mL volume 3 L min^{-1} air flow	3.0	0.15	93.2	70.6	(Arashiro et al. 2020)
Porphyridium purpureum	*f*/2-RSE medium	20 ℃ 30, 100, 200 230 µmol photons $m^{-2}\ s^{-1}$	Erlenmayer flasks	3.4	0.33	18	15	(Guihéneuf and Stengel 2015)
Porphyridium purpureum	ASW medium	25 ℃ 20 µmol photons $m^{-2}\ s^{-1}$	250 mL Erlemayer flasks	3.4–9.4		48	33	(Kathiresan et al. 2007)
Porphyridium purpureum	'Red algae medium'	26–28 °C 5–200 W m^{-2} irradiance	Flat-panel PBR, 50 mm path length, 2.5 L volume 0.5 vvm air with 2–3% CO_2	0.55–3.1	0.06–0.75		30–110	(Borovkov et al. 2023)
Porphyridium purpureum SCS- 02	ASW medium with 3.5, 5.9 and 17.6 mM KNO_3	25 °C 350 µmol photons $m^{-2}\ s^{-1}$	60 mm diameter tubular reactors, 1.2 L volume Bubbled with 1% CO_2 in air	Up to 5.54	0.35	5–100	2–82	(Li et al. 2019)
Rhodomonas salina CCMP1319	*f*/2 medium	22℃ 300 µmol photons $m^{-2}\ s^{-1}$	Flat-panel PBR, 14 mm depth, 0.4 L volume Continuous culture	1.30 ± 0.04	1.17 ($\mu = 0.88\ day^{-1}$)	n.d	70	(Latsos et al. 2021)

Table 2 Summary of species used in this work

Division	Species	Collection number	Medium used for maintenance
Rhodophyta	*Erythrolobus* sp.	CCAP 1393/4	*f*/2
Rhodophyta	*Flintiella sanguinaria*	CCAP 1371/1	80:20 freshwater cyanobacteria medium: *f*/2
Rhodophyta	*Porphyridium purpureum*	CCAP 1380/3	*f*/2
Rhodophyta	*Rhodella violacea*	CCAP 1388/5	*f*/2
Rhodophyta	*Timspurckia oligopyrenoides*	CCAP 1393/2	*f*/2
Cyanophyta	*Anabaena cylindrica*	CCAP 1403/2B	Freshwater cyanobacteria medium
Cyanophyta	*Aphanothece elabens*	CCAP 1413/1	*f*/2
Cyanophyta	*Synechococcus* sp. (PCC 7002)	CCAP 1400/1	Marine cyanobacteria medium

Screening experiments were performed using 100 mL of medium in 250 mL flasks. Cultures were grown in an Incu-Shake Compact SQ- 4040 incubator (SciQuip, UK) at 25 °C with continuous illumination from a white LED light pad at an intensity of approximately 70 µmol photons m^{-2} s^{-1}. Cultures of *P. purpureum, R. violacea* and *T. oligopyrenoides* were harvested after 10 days, while cultures of *A. cylindrica, A. elabens* and *Synechococcus* sp. PCC 7002 were harvested after 14 days. *Flintiella sanguinaria* cultures were harvested after 21 days, and cultures of *Erythrolobus* sp. were harvested after 26 days. The different cultivation durations were used to ensure sufficient biomass was present for extraction and analysis. At the end of the growth period the cultures were freeze dried for analysis.

Photobioreactor cultures were undertaken using a custom-built set-up as shown in Fig. 1. The PBRs were constructed from clear acrylic tubing, with an inner diameter of 104 mm, and a thickness of 3 mm. A sampling valve was located 180 mm above the base of the column. The working volume of the system was 3 L, corresponding to a liquid height of 350 mm without any aeration. The temperature of the PBRs was controlled by circulating water through a U-shaped cooling coil fabricated from 6.35 mm diameter stainless steel tubing. Air and carbon dioxide were introduced via a L-shaped stainless steel sparger fabricated from 6.35 mm diameter tubing. The sparger had one row with 10 × 1 mm diameter holes, with a 5 mm distance between the hole centres. RM series rotameters (Dwyer Instruments, USA) were used to measure the flow-rates of air and carbon dioxide.

Lighting was supplied by white (6500 K colour temperature) LED strips (Intelligent LED solutions, UK). The lights were positioned either behind the reactor, or behind the reactor and to either side (as shown in Fig. 1). The lights were connected to Osram (Munich, Germany) OT- 40 or OT- 60 LED drivers such that the light intensity could be varied; for the PBRs with a single light (behind the PBR) the maximum intensity was 450 µmol photons m^{-2} s^{-1}, while for the system with three lights (one at either side and one behind the PBR) the maximum intensity was 1200 µmol photons m^{-2} s^{-1}. Light intensities were measured using a LI-250 light-meter (LI-COR Biosciences, USA) equipped with a LI-193 spherical sensor. The light intensity in the PBRs was measured with the sensor being located approximately on the column centerline, 180 mm above the base of the column. Measurements were made with the column filled with 3 L of deionized water.

The *f*/2 medium used in this work to maintain the cultures and perform the screening experiments contained $NaNO_3$ (880 µM), K_2HPO_4 (36 µM), $FeCl_3$ (12 µM), Na_2SiO_3 (140 µM), $MnCl_2$ (940 nM), $ZnCl_2$ (85 nM), $CoCl_2$ (39 nM), $CuSO_4$ (38 nM), Na_2MoO_4 (37 nM), thiamine hydrochloride (300 nM), biotin (2 nM) and vitamin B_{12} (0.4 nM) and marine salts (35 g L^{-1}). The freshwater cyanobacteria medium used in this work to maintain the cultures and perform the screening experiments contained $NaNO_3$ (10 mM), $MgSO_4$ (4 mM), $CaCl_2$ (1.3 mM), K_2HPO_4 (1 mM), H_3BO_3 (0.1 mM) $NaHCO_3$ (0.2 mM), $FeCl_3$ (20 µM), $MnCl_2$ (1.8 µM), $ZnCl_2$ (0.77 µM), $CoCl_2$ (0.18 µM), $CuSO_4$ (0.33 µM), Na_2MoO_4 (0.12 µM), thiamine hydrochloride (0.6 µM), biotin (4 nM) and vitamin B_{12} (0.8 nM). The marine cyanobacteria medium had the same composition, with the addition of 0.34 M NaCl (20 g L^{-1}).

$NaHCO_3$, K_2HPO_4 and H_3BO_3 were from Fisher Scientific (UK). NaCl, Na_2SiO_3, $NaNO_3$, $CoCl_2$, $ZnCl_2$ and $MnCl_2$ were from Scientific Laboratory Supplies (UK). $MgSO_4$, $FeCl_3$, $CaCl_2$ and Na_2MoO_4 were from Sigma Aldrich (UK). $CuSO_4$ was from Honeywell Fluka (USA). Thiamine hydrochloride, biotin and vitamin B_{12} were from Cayman Chemicals (USA). Marine salts (Classic sea salt) were from Tropic Marin (Hünenberg, Switzerland).

Initial cultures in the PBRs used *f* medium with the omission of sodium silicate. The final nutrient concentrations were $NaNO_3$ (1.76 mM), K_2HPO_4 (72 µM), $FeCl_3$ (24 µM), $MnCl_2$ (1.88 µM), $ZnCl_2$ (190 nM), $CoCl_2$ (78 nM), $CuSO_4$ (76 nM), Na_2MoO_4 (74 nM), thiamine hydrochloride (600 nM), biotin (4 nM) and vitamin B_{12} (0.8 nM) and marine salts (35 g L^{-1}). Cultivations were also performed with 5*f* medium where the concentrations of all components except the marine salts and vitamins were increased fivefold; the vitamin concentration was doubled and the

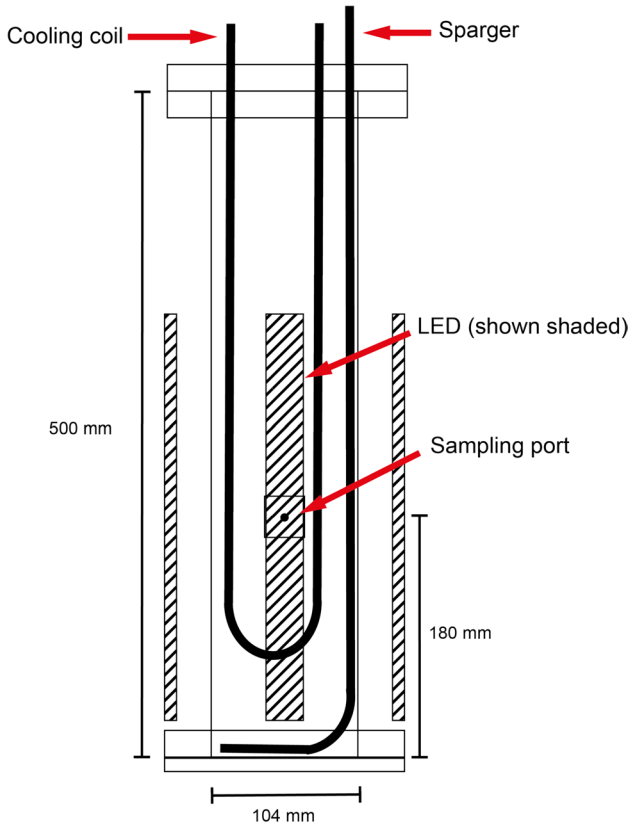


Fig. 1 Schematic showing the design of the bubble column photobioreactors used in this work

concentration of the marine salts was fixed. Final concentrations in the medium were $NaNO_3$ (8.8 mM), K_2HPO_4 (360 µM), $FeCl_3$ (120 µM), $MnCl_2$ (9.4 µM), $ZnCl_2$ (950 nM), $CoCl_2$ (390 nM), $CuSO_4$ (380 nM), Na_2MoO_4 (370 nM), thiamine hydrochloride (1.2 µM), biotin (8 nM) and vitamin B_{12} (1.6 nM) and marine salts (35 g L^{-1}). Finally, experiments were performed with 20*f* medium, here concentrations of all components except for the vitamins and marine salts were increased by a factor of 20 relative to *f* medium. The vitamins concentration was increased by a factor of 10 and the concentration of marine salts was fixed. Final nutrient concentrations were $NaNO_3$ (35.2 mM), K_2HPO_4 (1.44 mM), $FeCl_3$ (480 µM), $MnCl_2$ (37.6 µM), $ZnCl_2$ (3.8 µM), $CoCl_2$ (1.56 µM), $CuSO_4$ (1.52 µM), Na_2MoO_4 (1.48 µM), thiamine hydrochloride (6 µM), biotin (40 nM) and vitamin B_{12} (8 nM) and marine salts (35 g L^{-1}). Cultivations of *F. sanguinaria* were performed using medium based on that developed by Gaignard et al. (2018) which consisted of NaCl (89 mM) $NaNO_3$ (20 mM), K_2HPO_4 (5 mM), $FeCl_3$ (300 µM), $MgSO_4$ (60 mM), $CaCl_2$ (14 mM), H_3BO_3 (2 mM), $MnCl_2$ (9.4 µM), $ZnCl_2$ (950 nM), $CoCl_2$ (390 nM), $CuSO_4$ (380 nM), Na_2MoO_4 (370 nM), thiamine hydrochloride (1.2 µM), biotin (8 nM) and vitamin B_{12} (1.6 nM).

Analytical methods

Growth was quantified by measuring the optical density of the cultures at a wavelength of 600 nm using a Jenway 6305 spectrophotometer (Cole-Parmer, UK). Where necessary, samples were diluted such that the measured OD was < 1.

Nitrate concentration in the medium was measured using a Shimadzu UV-1800 spectrophotometer at wavelengths of 220 nm and 275 nm (American Public Health Association 2012). Prior to analysis, the aliquot was centrifuged to remove any solids (5 min, 14,800 × *g*) and was diluted such that the absorbance was < 1.

Dry cell weight measurements were also used to quantify growth. Quantitative glass fiber filters (GF6, Whatman, USA) were placed on a watch glass and weighed on an analytical balance. A known volume of culture (typically 50–70 mL) was filtered, the filter papers were washed with approximately 3 volumes of 0.5 M ammonium bicarbonate as per Zhu and Lee (1997). Samples were then dried overnight in an oven at 105 °C before being cooled in a desiccator and weighed. The dry cell weight (in g L^{-1}) was determined by dividing the mass of algae by the known culture volume.

To prepare the samples for extraction they were firstly centrifuged (5 min, ~ 16,200 RCF). The medium was removed and ~ 50 mL deionized water was added and the sample was resuspended. After washing the sample was centrifuged again and the supernatant was removed. This process was performed a total of three times for species grown in marine media to ensure total removal of the salts. Samples were then freeze-dried overnight. A known mass of the freeze-dried algae (typically 5–10 mg) was added to a bead-beater tube with 0.5 mm high-impact zirconium beads. 1 mL of phosphate buffered saline solution (PBS – containing 0.137 M NaCl, 0.01 M phosphate buffer, 2.7 mM KCl, pH 7.4, from Sigma Aldrich) was added and the samples were shaken for 15 min at 30 Hz using a VWR Star-Beater. Samples were then incubated at 4–5 °C overnight. After incubation the samples were centrifuged (5 min, ~ 16,200 RCF) and the absorbance of the supernatant was measured at 562, 615 and 652 nm. Where necessary, samples were diluted using PBS such that the absorbance was < 1. The supernatant was aspirated from the samples and 1 mL fresh PBS was added. Samples were then shaken for 15 min at 30 Hz, before being centrifuged (5 min, ~ 16,200 RCF). The absorbance of the supernatant was measured again. After two extractions the supernatant was typically clear or pale colored, indicating the phycobilins had been fully extracted. In some cases where the 5*f* or 20*f* medium was used additional extraction steps (up to 10) were required to recover all of the phycoerythrin.

The concentration of phycobilins was quantified as per Bennett and Bogorad (1973), this was converted to the

mass of phycobilin per gram of dry algae. Supplementary Fig. 1 shows spectra of the extracts from the PBR cultures.

Statistical analysis and experimental design

Screening experiments were performed in triplicate, with the reported values being the mean of the three replicates; error bars denote one standard deviation about the mean.

The scale-up experiments were performed in the 3-L PBRs using *f* medium at a light intensity of approximately 140 µmol photons m^{-2} s^{-1}, the illumination was constant (i.e., 24 h per day). Air was introduced at a flow rate of 3 L per minute to mix the cultures. Carbon dioxide was introduced at a flow rate of 30 mL per minute from day 8 onwards. Cultures were inoculated from flasks containing approximately 250 mL which were grown for 7–10 days at a light intensity of 60 µmol photons m^{-2} $s.^{-1}$

Cultures were run in batch mode for 14 days, with samples for the dry cell weight and specific phycoerythrin content being taken every second day from day 6 onwards. Measurements of the optical density and nitrate concentration were made daily (though samples were not necessarily taken every day). Four cultures were performed for *P. purpureum* and *R. violacea* and three for *T. oligopyrenoides*. Values for all replicates are shown for the optical density and nitrate measurements; reported values for the dry cell weight and specific phycoerythrin concentration are the average, with error bars denoting one standard deviation about the mean.

Experiments were also performed in the 3-L PBRs using 5*f* medium and a light intensity of approximately 450 µmol photons m^{-2} s^{-1} using *P. purpureum* and *R. violacea*. The same sampling approach was used for the first set of scale-up experiments. Cultivations were done in triplicate for *R. violacea* and five cultures were performed for *P. purpureum.* Additional cultures were performed using *P. purpureum* with higher nutrient concentrations (i.e. 20*f* medium) and light intensities, these were performed in triplicate. *Flintiella sanguinaria* was grown using modified FS medium (Gaignard et al. 2018) using a light intensity of 70 µmol photons m^{-2} s^{-1} for days 0–10 and 140 µmol photons m^{-2} s^{-1} from day 11 onwards. Cultures were performed in triplicate.

Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test using GraphPad Prism version 9.5.1 for Windows (GraphPad Software, USA). Specific growth rates were calculated by plotting the natural logarithm of the optical density at 600 nm as a function of time for days 0–6 (to avoid any issues with nutrient limitation or self-shading). Values for the different experimental conditions were pooled, and the reported error values indicate the 95% confidence intervals of the regression. Unless stated otherwise reported values in the text are given as mean ± standard deviation.

Results

Results from the flask screening are shown in Fig. 2. One-way ANOVA ($F_{7,16} = 31.79$, $p < 0.0001$, Tukey's test, $p < 0.05$) showed that *A. cylindrica* had a significantly higher total phycobilin content than all other species examined, a significant difference was also observed between *A. elabens* and *F. sanguinaria*. One-way ANOVA ($F_{7,16} = 9.42$, $p = 0.0001$, Tukey's test, $p < 0.05$) showed no significant difference in specific phycoerythrin content between cyanobacteria, *P. purpureum* and *F. sanguinaria* had significantly higher phycoerythrin contents than all of the cyanobacteria, and *F. sanguinaria* was the only species of red algae to have significantly different phycoerythrin content to other red algae.

Figure 3 shows results from the 3 L PBRs for *P. purpureum*, *R. violacea* and *T. oligopyrenoides*. Cultivation of *F. sanguinaria* in the PBRs using the same media as used in the screening was not particularly successful. Use of a modified version of the FS medium developed by Gaignard et al. (2018) led to improved growth, however it was found the alga grew in clumps which made obtaining representative measurements challenging (see Supplementary Fig. 3). Growth rates of *P. purpureum* (0.61 ± 0.13 day^{-1}, $R^2 = 0.85$) and *R. violacea* (0.57 ± 0.16 day^{-1}, $R^2 = 0.77$) were comparable, whereas the growth rate of *T. oligopyrenoides* (0.35 ± 0.13, $R^2 = 0.72$) was significantly slower ($F_{2,49} = 3.87$, $p = 0.0275$). For both *P. purpureum* and *R. violacea* the nitrate in the medium was depleted after seven days, while this occurred after day 12 for *T. oligopyrenoides*. A significant decrease in specific phycoerythrin content was observed between day 6 and days 12 and 14 for *P. purpureum* (one-way ANOVA, $F_{4,14} = 4.46$, $p = 0.0157$, Tukey's test, $p < 0.05$) and day 8 and day 12 for *R. violacea* (one-way ANOVA, $F_{4,14} = 3.61$, $p = 0.0318$, Tukey's test, $p < 0.05$). For each species no significant change in the volumetric phycoerythrin content with time was found.

Figure 4 shows results from cultivations performed with *P. purpureum* and *R. violacea* using 5*f* medium and a light intensity of 450 µmol photons m^{-2} s^{-1}. Growth between the species was comparable. Calculated specific growth rates were 0.62 ± 0.06 day^{-1} ($R^2 = 0.96$) for *P. purpureum* and 0.65 ± 0.10 day^{-1} ($R^2 = 0.94$) for *R. violacea*. These values did not differ significantly from each other and the values calculated with *f* medium and a light intensity of 140 µmol photons m^{-2} s^{-1} ($F_{3,72} = 0.273$, $p = 0.845$). No significant difference in the specific phycoerythrin content as a function of time was observed for either species, with the volumetric phycoerythrin concentration being significantly different between day 6 and days 12 and 14 for *P. purpureum* (one-way ANOVA, $F_{4,19} = 4.51$, $p = 0.0099$, Tukey's test, $p < 0.05$). Comparison between the

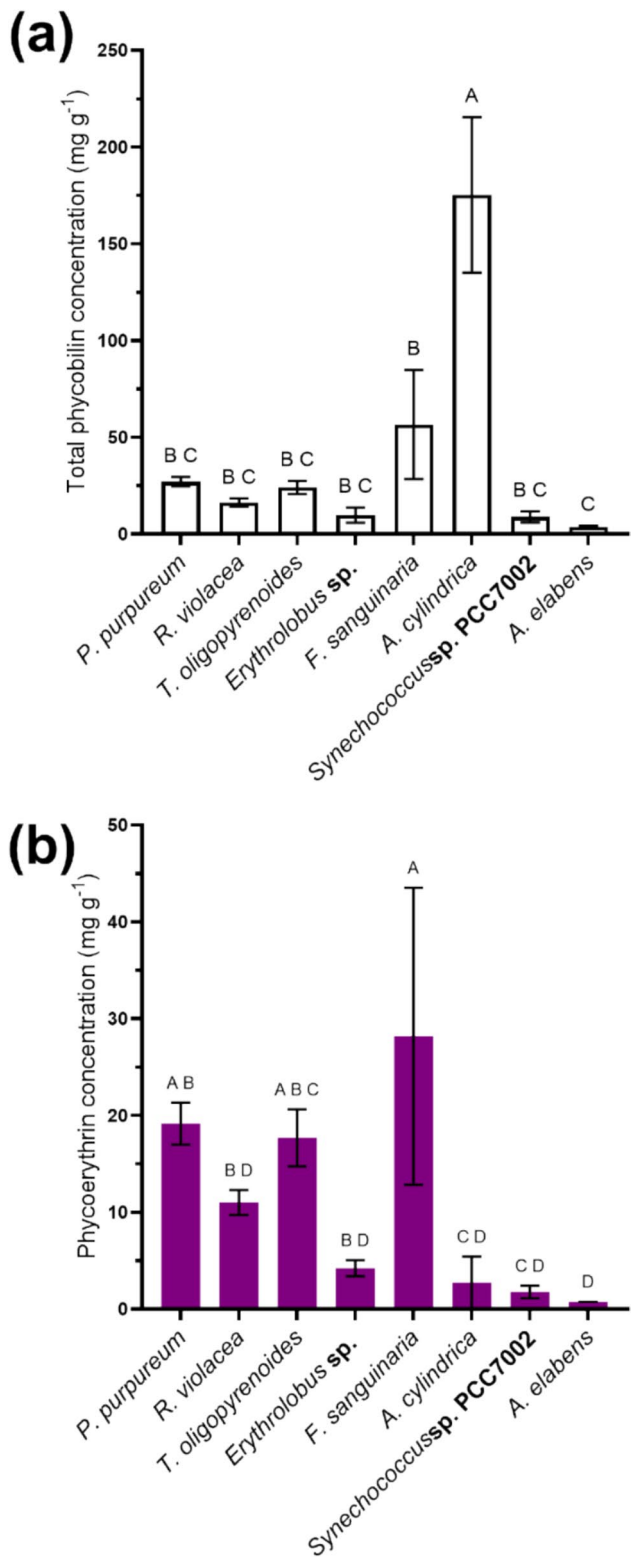


Fig. 2 Results from flask screening experiments. (**a**) the total phycobilin concentration as well as (**b**) the specific phycoerythrin concentration. Reported results are the average of three replicates, with error bars denoting one standard deviation about the mean. Results annotated with different letters are significantly ($p < 0.05$) different

volumetric phycoerythrin concentration on day 14 showed a significant increase for both species compared to experiments with *f* medium and 140 µmol photons m^{-2} s^{-1} (one-way ANOVA, $F_{3,12} = 14.04$, $p = 0.0003$, Tukey's test, $p < 0.05$). No significant difference was observed between species at the same experimental conditions.

Figure 5 shows the results for *P. purpureum* cultured using 20*f* medium and increased light intensity throughout the batch. Unlike previous experiments the nitrate was not depleted throughout the course of the experiment. The calculated growth rate was 0.37 ± 0.16 day^{-1} ($R^2 = 0.66$), this was significantly less than that for the previous experiments ($F_{2,54} = 6.32$, $p = 0.0034$). One-way ANOVA ($F_{10,22} = 4.49$, $p = 0.0016$, Tukey's test, $p < 0.05$) showed some significant differences between the specific phycoerythrin content on different days (i.e., between day 6 and 18, day 8 and days 12, 18 and 27 and day 18 and 22).

Discussion

Significant variation in the total phycobilin content was observed in the screening experiments performed in flasks, with concentrations ranging from 3.7 ± 0.6 mg g^{-1} for *A. elabens* to 175 ± 40 mg g^{-1} for *A. cylindrica*. Phycoerythrin was between 2–20% of the total phycobiliproteins for the cyanobacteria examined; while it was 40–70% of the phycobiliproteins for the red algae (a detailed breakdown of the composition from the flask screening is provided in Supplementary Fig. 2). Of the species examined *F. sanguinaria* had the highest phycoerythrin content (28 ± 15 mg g^{-1}), followed by the other species of red algae, with the concentration in the cyanobacteria being relatively low (0.8–2.7 mg g^{-1}). It was also observed that all of the species of red algae tended to form films and clumps when grown, with this tendency becoming more pronounced the longer the algae were cultured. This trend was particularly notable for *F. sanguinaria* which tended to grow in a film which was strongly attached to the base of the flask. Such behavior may complicate scale-up, as species which clump and prefer attached growth may not grow well in suspension culture.

This was found to be the case, as growth of *F. sanguinaria* in the PBRs proved to be challenging. Reformulating the medium by swapping from the 80:20 (v:v) mixture of freshwater cyanobacteria medium and *f*/2 medium to a modified form of FS medium (Gaignard et al. 2018) led to improved growth, however the cells tended to grow in large clumps, with a significant portion of the biomass attached to the walls of the PBR (see Supplementary Fig. 3(c)). This behavior also made obtaining representative samples of the culture challenging. The specific phycoerythrin content for *F. sanguinaria* reached a maximum of 71 ± 5 mg g^{-1} on day 14, comparable with *P. purpureum* and *R. violacea*. These

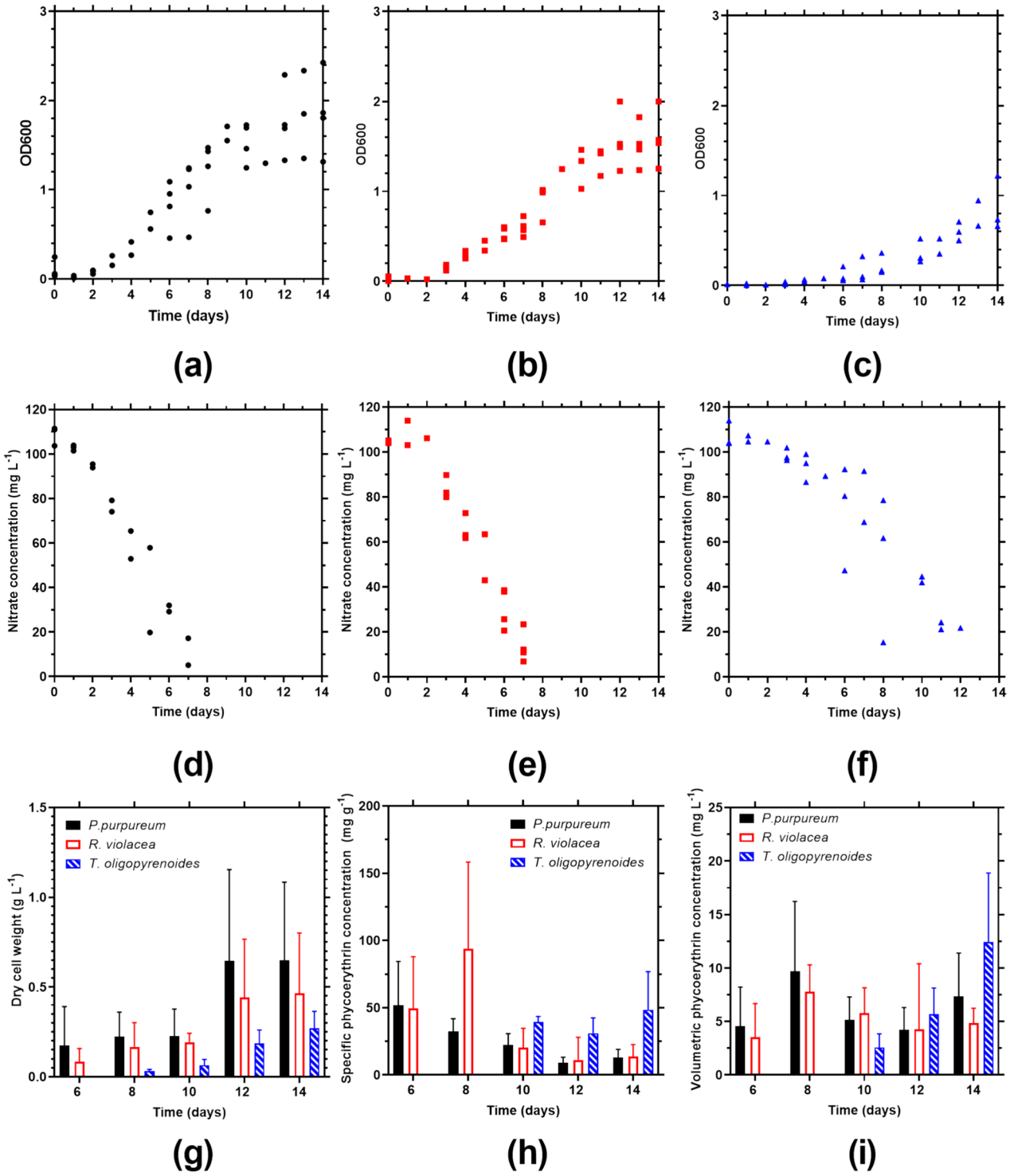


Fig. 3 Results from scale-up experiments in 3-L PBRs using *f* medium and a light intensity of 140 µmol photons m^{-2} s^{-1}. Panels (**a**)-(**c**) show the optical density at 600 nm; panels (**d**)-(**f**) show the nitrate concentrations. Panel (**g**) is a comparison of the dry cell weights; (**h**) is a comparison of the specific phycoerythrin concentration and panel (**i**) shows the volumetric phycoerythrin concentration. Results from all replicates are shown in panels (**a**)-(**f**), while panels (**g**)-(**i**) show the mean values with error bars denoting one standard deviation. Experiments were performed in triplicate for *T. oligopyrenoides* and quadruplicate for *P. purpureum* and *R. violacea*

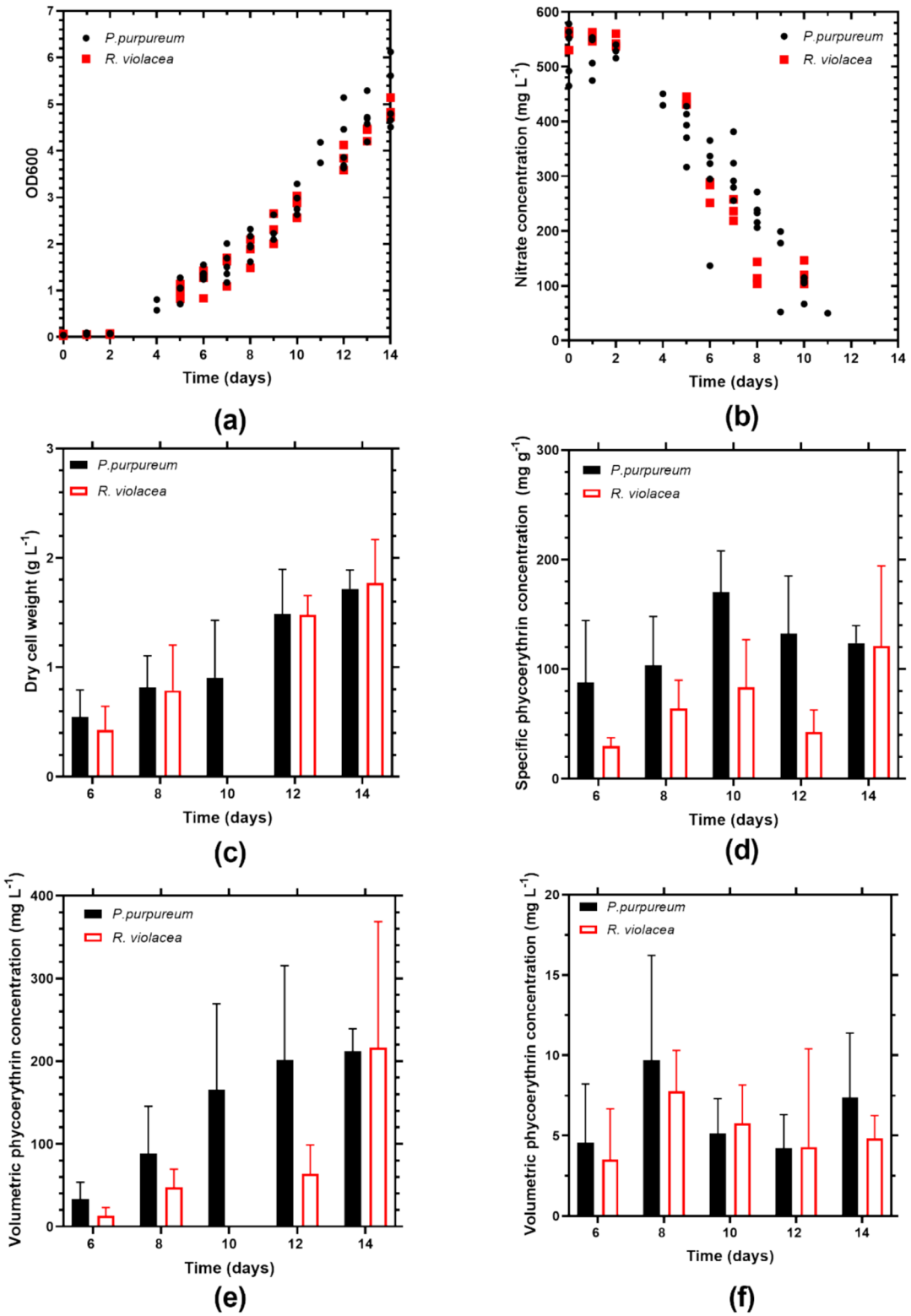


Fig. 4 Results from experiments in PBRs using increased lighting (450 µmol photons m^{-2} s^{-1}) and 5*f* medium. Panel (**a**) shows the optical density at 600 nm, panel (**b**) shows the nitrate concentration, panel (**c**) shows the dry cell weight, panel (**d**) shows the specific phycoerythrin concentration, panel (**e**) shows the volumetric phycoerythrin concentration and panel (**f**) shows the same data for the experiments with *f* media and 140 µmol photons m^{-2} s^{-1} lighting for comparison (note different scales on the *y*-axes). Five replicates were performed for the *P. purpureum* and three for the *R. violacea*. All replicates are shown in panels (**a**) and (**b**), with results in panels (**c**)-(**f**) being the average with error bars denoting one standard deviation about the mean

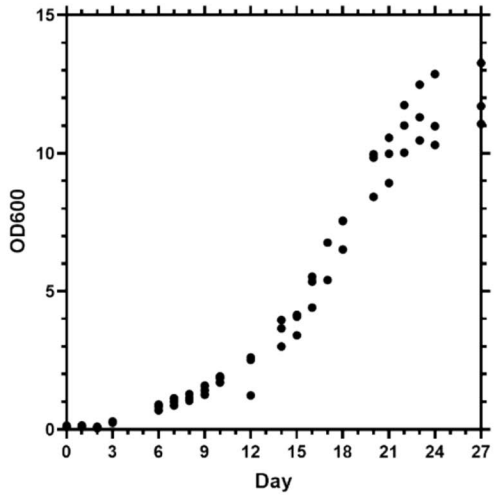
results indicate that *F. sanguinaria* has potential to be used for phycoerythrin production; however, its tendency to clump and grow on the surface of the PBR is likely to cause challenges with large-scale cultivation in flat-panel and bubble column PBRs. These results also highlight the importance of performing screening at conditions representative of large-scale PBRs in order to obtain results which can be reliably applied to large-scale systems.

Of the other species of red algae examined *P. purpureum*, *R. violacea* and *T. oligopyrenoides* were found to grow in the PBRs (*Erythrolobus* sp. was not considered due to its relatively slow growth rate in flasks). Interestingly, the dry cell weight increased between days 10 and 12 for *P. purpureum* and *R. violacea* while the optical density remained constant; this may be due to the formation of extracellular polysaccharides (Guihéneuf and Stengel 2015), it was observed that towards of the end of the batch the liquid appeared to be more viscous and it became more challenging to filter. This may also explain the relatively large variation between the dry cell weight measurements. The specific phycoerythrin concentration was highest (52 ± 28 mg g^{-1}) on day 6 for *P. purpureum* and on day 8 (94 ± 56 mg g^{-1}) for *R. violacea*. As the batch continued the specific phycoerythrin content decreased; measured values on day 14 were 13 ± 5 mg g^{-1} for *P. purpureum* and 14 ± 8 mg g^{-1} for *R. violacea.* Due to the relatively small amount of biomass present specific phycoerythrin contents could only be measured from day 10 onwards for *T. oligopyrenoides*. As shown in Fig. 3(h) the concentration was relatively consistent (31–49 mg g^{-1}) throughout the batch. Specific phycoerythrin contents reported here are consistent with values reported by others for red algae (Table 1). It was observed that for *P. purpureum* phycoerythrin made up approximately 70% of the phycobiliproteins on days 6, with this value decreased to approximately 50% on day 14. Similar behavior was observed for *R. violacea*, where phycoerythrin made up approximately 70% of the total phycobiliproteins on day 8, and approximately 50% on day 14. Less variation was observed for *T. oligopyrenoides* where phycoerythrin made up approximately 50% of the total phycobiliproteins. The observed changes in the pigment composition are thought to be related to the changes in the cellular composition as a result of nitrogen depletion in the medium; this may also explain why minimal changes were observed for *T. oligopyrenoides* as the batch was concluded before the cells became nitrogen starved. As shown in Fig. 3(i) the volumetric phycoerythrin concentration was not found to change in time, showing that increases in dry cell weight and decreases in specific phycoerythrin content effectively 'balanced out'. From a production perspective, this suggests work should focus on maintaining the specific phycoerythrin content while also increasing the dry cell weight.

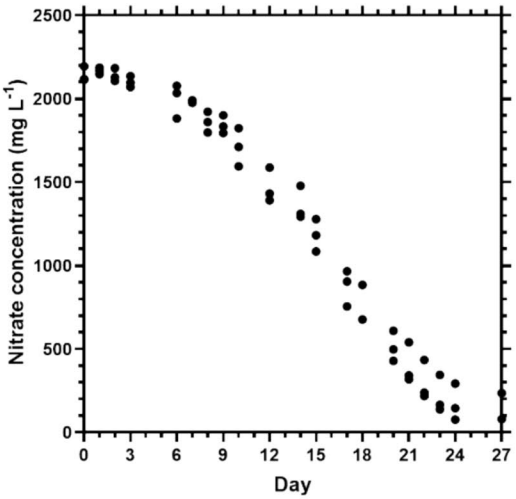
As shown in Fig. 4 increasing the light intensity and concentration of nutrients in the medium unsurprisingly led to an increase in the cell density with specific growth rates of 0.62 ± 0.06 day^{-1} for *P. purpureum* and 0.65 ± 0.10 day^{-1} for *R. violacea*. These values were similar to those obtained using *f* medium and a light intensity of 140 µmol photons m^{-2} s^{-1}. Such results suggest either that there is another factor limiting growth, or that the species is growing at the maximum rate. Maximum reported values of the specific growth rates for *Porphyridium* species are of the order 0.4–0.7 day^{-1} (García Camacho et al. 2000; Merchuk et al. 2000; You and Barnett 2004), similarly maximum dilution rates used in continuous cultures are typically 0.5 day^{-1} (Fábregas et al. 1998; Borovkov et al. 2023). These results are consistent with the idea that the measured value of the specific growth rate is close to the maximum. Calculated values of the biomass productivity are also given in Table 3; it was found that increasing the light intensity and nutrient concentration led to improved biomass productivities (120 ± 11 mg L^{-1} day^{-1} for *P. purpureum* and 130 ± 23 mg L^{-1} day^{-1} for *R. violacea*). Literature values are of the order 300–350 mg L^{-1} day^{-1} for batch systems (Guihéneuf and Stengel 2015; Li et al. 2019) and 750–1100 mg L^{-1} day^{-1} for continuous systems (Latsos et al. 2021; Borovkov et al. 2023).

Use of 5*f* medium led to the specific phycoerythrin content remaining constant through the batch (Fig. 4(d)). This may be attributed to two factors, firstly as previously discussed having additional nitrate present in the medium is likely to lead to higher specific phycoerythrin contents. Secondly, there is an increased degree of self-shading in the cultures grown with the 5*f* medium due to the higher cell density. This in turn will lead to the cells receiving less light (despite the applied light intensity being higher); to compensate for this production of pigments like phycoerythrin may be favoured (Guihéneuf and Stengel 2015; Arashiro et al. 2020; Borovkov et al. 2023). Phycoerythrin made up approximately 70% of the total phycobiliproteins for both *P. purpureum* and *R. violacea*, these results being consistent with the values obtained in the screening experiment (Supplementary Fig. 2), as well as the previous experiments using less concentrated medium.

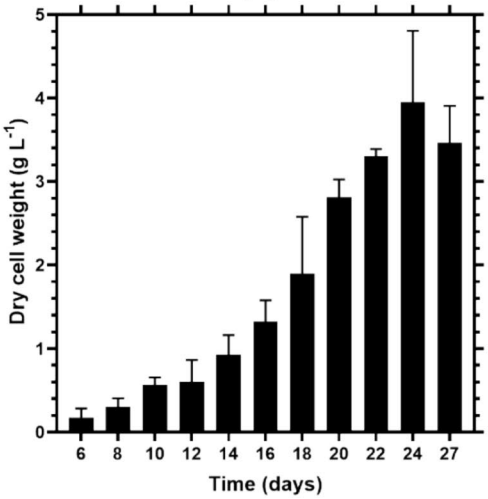
At the end of the batch the volumetric phycoerythrin concentrations were comparable for both species at 210 ± 25 mg L^{-1} for *P. purpureum* and 220 ± 120 mg L^{-1} for *R. violacea* (Table 3). For comparison the maximum volumetric concentrations for runs with *f* medium were 10 ± 4.6 mg L^{-1} for *P. purpureum* and 6.4 ± 1.0 mg L^{-1} for *R. violacea*, with both of these values occurring on day 8. Hence it is clear that increasing the nutrient concentration and light intensity was a successful strategy for improving the phycoerythrin productivity. Calculated productivity values (on day 14) were approximately 15 mg L^{-1} day^{-1} for phycoerythrin production, with these values comparable to those reported elsewhere for red algae (4–19 mg L day^{-1}) grown in batch


OD600
Day

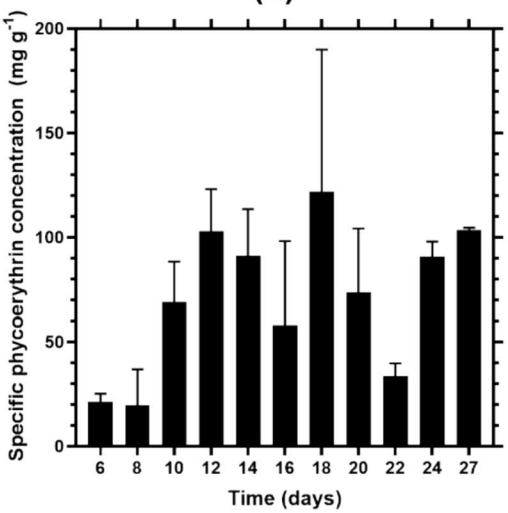
(a)


Nitrate concentration (mg L-1)
Day

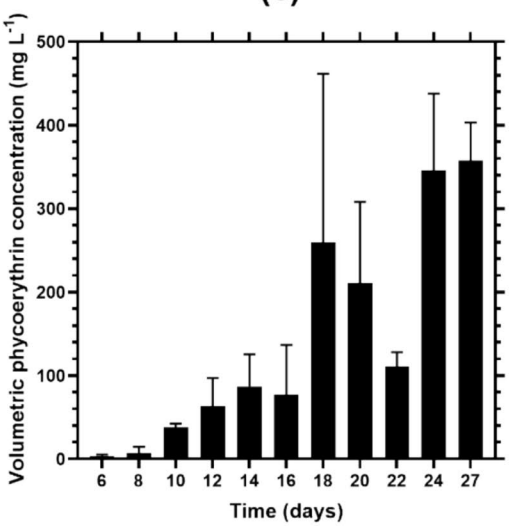
(b)


Dry cell weight (g L-1)
Time (days)

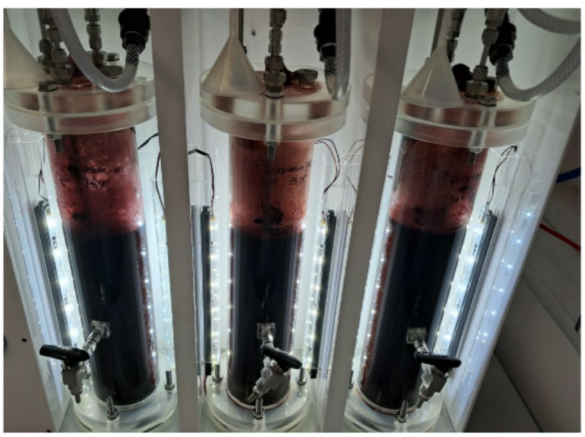
(c)


Specific phycoerythrin concentration (mg g-1)
Time (days)

(d)


Volumetric phycoerythrin concentration (mg L-1)
Time (days)

(e)



(f)

◂**Fig. 5** Results from experiments in 3-L PBRs for *P. purpureum* with increased lighting and 20*f* medium. The light intensity was 390 µmol photons m^{-2} s^{-1} for days 0–12, 650 µmol photons m^{-2} s^{-1} for days 12–14, 980 µmol photons m^{-2} s^{-1} for days 14–16 and 1200 µmol photons m^{-2} s^{-1} for day 16 onwards. Panel (**a**) shows the optical density at 600 nm, panel (**b**) shows the nitrate concentration, panel (**c**) shows the dry cell weight and panel (**d**) shows the specific phycoerythrin concentration, panel (**e**) show the volumetric phycoerythrin concentration and panel (**f**) shows a photo of the PBRs on day 27. Data from all three replicates is shown in panels (**a**) and (**b**); in panels (**c**)-(**e**) the reported results are the average of three replicates with error bars denoting one standard deviation about the mean

culture (Rebolloso Fuentes et al. 2000; Guihéneuf and Stengel 2015; Li et al. 2019), while being less than that reported for continuous culture (20–60 mg L^{-1} day^{-1}) (Fábregas et al. 1998; Latsos et al. 2021; Borovkov et al. 2023).

To determine whether or not the process could be further improved cultivations were also performed using increased nutrient concentrations and higher light intensities for *P. purpureum* (Fig. 5). In this approach the light intensity was increased with time in order to compensate for self-shading. The final dry cell weight (3.46 ± 0.36 g L^{-1}) was approximately double that of the runs with 5*f* medium (1.72 ± 0.16 g L^{-1}) and a fixed light intensity of 450 µmol photons m^{-2} s^{-1}. The biomass concentration from this work are comparable to those published for continuous culture systems (up to 3.1–3.2 g L^{-1}) (Rebolloso Fuentes et al. 2000; Borovkov et al. 2023), while being less than the value of 5.54 g L^{-1} reported by Li et al. (2019). The increased nutrient concentration and light intensity did not lead to an improvement in the biomass productivity, with the value (130 ± 13 mg L^{-1} day^{-1}) being comparable to that for the 5*f* medium (120 ± 11 mg L^{-1} day^{-1}).

Interestingly, the specific growth rate (calculated for days 0–6) for the cultivations with the enriched medium (20*f*) was lower (0.37 ± 0.16 day^{-1}), than that for the cultivations performed with lower nutrient concentrations (0.61 ± 0.13 and 0.62 ± 0.06 day^{-1}). The cause of this behavior was not clear; it may have been due to either the increased osmotic pressure due to the higher nutrient concentration or precipitation of some key nutrients. As shown in Fig. 5 the specific phycoerythrin concentration was approximately constant throughout the batch, this coupled with the increased dry cell weight led to a final volumetric concentration of 360 ± 37 mg L^{-1}, with this value being one of the highest reported (values in the literature range from 60–250 mg L^{-1} (Rebolloso Fuentes et al. 2000; Li et al. 2019; Latsos et al. 2021; Borovkov et al. 2023)) The phycoerythrin productivity calculated on the final day of the batch was 13 ± 1.4 mg L^{-1} day^{-1}, this being slightly lower than the value for the 5*f* medium (15 ± 1.7 mg L^{-1} day^{-1}). Unsurprisingly the productivity values for the batch system are less than those reported for continuous cultures (20–60 mg L^{-1} day^{-1}) (Fábregas et al. 1998; Latsos et al. 2021; Borovkov et al. 2023).

In considering the production of phycoerythrin there is likely to be both a minimum and maximum specific content; these values being constrained by the biology of the organism (i.e. there is likely to be a minimum amount needed for photosynthesis, while there is a maximum amount which can be accumulated within the cell). Values from this work can provide an idea of these bounds. Results from the flask screening provide an indication of the minimum value as the growth conditions at the end of the cultivation (i.e. nutrient depletion, a short path length and relatively low cell density and hence a relatively high light intensity) are unlikely to favour high specific phycoerythrin contents. Contrastingly, conditions in the PBRs (i.e. excess nutrients, relatively long path lengths and high cell densities) favour a high specific phycoerythrin content. Hence, values of 19 ± 2 to 170 ± 34 mg g^{-1} for *P. purpureum* represent reasonable estimates of the minimum and maximum specific phycoerythrin contents; values in the literature generally fall within this range (Table 1).

As previously noted, from a production perspective goal is to increase the cell density while maintaining a high specific phycoerythrin content. Latsos et al. (2021) found an inverse relationship between the biomass productivity and specific phycoerythrin content for *R. salina*; such a relationship was not observed in this work. It was found that it was possible to maintain a relatively high specific phycoerythrin content provided the culture did not undergo nitrogen depletion. This is consistent with the fact that nitrogen is needed to synthesise phycoerythrin, and that cells may use phycoerythrin as a store of nitrogen.

An obvious avenue for future work is to apply the learnings from this work to a larger scale continuous culture; a phycoerythrin productivity of 180 mg L day^{-1} is plausible (assuming a cell density of 3 g L^{-1}, a specific growth rate of 0.6 day^{-1} and a specific phycoerythrin concentration of 100 mg g^{-1}). While this work has focussed on phycoerythrin production there is considerable interest in the use of red algae for the production of other products (e.g. pigments, polysaccharides) (Guihéneuf and Stengel 2015; Gaignard et al. 2019); the results from this work can be readily applied to such processes.

Conclusions

The aim of this work was to screen a range of species which could be used for the production of phycoerythrin and then use this knowledge to develop and optimize a scalable production process. Five different species of red algae and three species of cyanobacteria were screened at the flask scale; significant variation (3.7 ± 0.6 − 175 ± 40 mg g^{-1}) in the total phycobilin content was observed. Phycoerythrin made up 2–20% of the total phycobilins for

Table 3 Summary of results from 3 L PBR experiments. Reported values are averages, with the variation being the standard deviation about the mean for the dry cell weight, the specific phycoerythrin concentration and the volumetric phycoerythrin concentration. Error values for the specific growth rate indicate the 95% confidence intervals in the regression used to determine the value. Biomass productivity values were calculated for the duration of the batch (i.e. using the dry cell weight on the final day of cultivation)

Species	Growth conditions	Final dry cell weight ($g\ L^{-1}$)	Specific growth rate (days 0–6, day^{-1})	Biomass productivity ($mg\ L^{-1}\ day^{-1}$)	Maximum specific phycoerythrin concentration ($mg\ g^{-1}$)	Final volumetric phycoerythrin concentration ($mg\ L^{-1}$)
P. purpureum	*f* medium 140 µmol photons $m^{-2}\ s^{-1}$	0.65 ± 0.38	0.61 ± 0.13	46 ± 27	52 ± 28 (day 6)	7.0 ± 3.3
R. violacea	*f* medium 140 µmol photons $m^{-2}\ s^{-1}$	0.47 ± 0.29	0.57 ± 0.16	33 ± 21	94 ± 56 (day 8)	4.7 ± 1.1
T. oligopyrenoides	*f* medium, 140 µmol photons $m^{-2}\ s^{-1}$	0.27 ± 0.08	0.35 ± 0.13	19 ± 6	49 ± 23 (day 14)	12 ± 5.2
P. purpureum	5*f* medium 450 µE light	1.72 ± 0.16	0.62 ± 0.06	120 ± 11	170 ± 34 (day 10)	210 ± 25
R. violacea	5*f* medium 450 µmol photons $m^{-2}\ s^{-1}$	1.77 ± 0.33	0.65 ± 0.10	130 ± 23	120 ± 60 (day 14)	220 ± 124
P. purpureum	20*f* medium 390 µmol photons $m^{-2}\ s^{-1}$ days 0–12 650 µmol photons $m^{-2}\ s^{-1}$ for days 12–14 980 µmol photons $m^{-2}\ s^{-1}$ for days 14–16 1200 µmol photons $m^{-2}\ s^{-1}$ from day 16 onwards	3.46 ± 0.36	0.37 ± 0.16	130 ± 13	120 ± 55 (day 18)	360 ± 37

the species of cyanobacteria examined, while for the red algae this value was 40–70%.

Scale-up experiments were performed using 3 L bubble column photobioreactors. It was found that the growth rates of *P. purpureum* and *R. violacea* were similar (of the order 0.6 day^{-1}), with *T. oligopyrenoides* growing more slowly (0.35 ± 0.13 day^{-1}). Due to its tendency to form clumps growth of *F. sanguinara* was more challenging in the PBRs used. Increasing the light intensity from 140 to 450 µmol photons $m^{-2}\ s^{-1}$ did not increase the specific growth rate for *P. purpureum* and *R. violacea*, suggesting the reported values may be close to the maximum. It was found that supplying the cultures with sufficient nitrogen was a key factor in maintaining a high specific phycoerythrin content through the batch. Further increases in the light intensity (to a maximum of 1200 µmol photons $m^{-2}\ s^{-1}$) and nutrient concentrations (by a factor of 20) increased the biomass concentration (3.46 ± 0.36 g L^{-1}) and volumetric phycoerythrin concentration (360 ± 37 mg L^{-1}), however phycoerythrin productivity values did not improve (15 ± 1.7 mg $L^{-1}\ day^{-1}$ for 5*f* medium compared with 13 ± 1.4 mg $L^{-1}\ day^{-1}$ for 20*f* medium).

The results from this work provide valuable information about the phycoerythrin content in red algae, as well as knowledge about their behavior when cultivated in a scalable PBR design. This includes species for which few data exist in the open literature. It was found that of the species examined *P. purpureum* and *R. violacea* were the most suitable candidates for large-scale phycoerythrin production due to their relatively high growth rates and phycoerythrin contents. The maximum cell densities (3.46 ± 0.36 g L^{-1}) and volumetric phycoerythrin concentrations (360 ± 37 mg L^{-1}) are among the highest reported in the open literature. Results from this work can be used to form the basis of further scale-up, with the use of a larger-scale continuous reactor being an obvious avenue for future work.

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Data availability The experimental data underlying this work have been uploaded here: https://doi.org/https://doi.org/10.17633/rd.brunel.25880428.v1.

Declarations

Competing interests The authors declare no competing interests.

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