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ORIGINAL RESEARCH

Pilot-scale cultivation of red microalga *Porphyridium purpureum* culture in a greenhouse for assessment of production potential under mid-latitude climate conditions

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Abstract The growth of red marine microalga *Porphyridium purpureum* and the production of B-phycoerythrin (B-PE) pigment were examined during pilot cultivation in a laboratory and in a greenhouse. The studies of *P. purpureum* cultivation in a greenhouse were conducted under mid-latitude climate conditions during different seasons. Maximum B-PE content (56 $mg \cdot g^{-1}$) and productivity (655.2 $mg \cdot m^{-2} \cdot day^{-1}$) were observed under controlled laboratory conditions. A strong linear correlation was found between the total daily irradiance and areal biomass productivity across different seasons. The total biomass and B-PE yield in May were significantly higher compared to other seasons (49.6 and 1.13 $g \cdot m^{-2}$), and in December these values were minimal. Despite the highest biomass productivity (12 $g \cdot m^{-2} \cdot day^{-1}$), culture death occurred in June. The photosynthetic efficiency exhibited seasonal variation, ranging from 2.87% in May to 3.83% in November, and was significantly higher under laboratory conditions (6.94%). The spring period is generally favorable for *P. purpureum* growth under mid-latitude climate conditions, whereas culture growth in summer has a certain potential, however, the impact of high illumination and temperature conditions should be considered and controlled. Although productivity in autumn-winter period was lower and limited by light supply, the possibility of obtaining additional biomass yield was demonstrated.

Keywords *Porphyridium* . Open ponds . Productivity . Yield . Phycobiliproteins . B-phycoerythrin

Introduction

Microalgae, as well as some higher plants, are a rich source of valuable physiologically active compounds and have prospects to be used for food, animal feed, nutraceuticals and pharmaceutical substances (Khavari et al. 2021; Khalid et al. 2024). Microalgae cultivation under natural light conditions is the main method for producing its biomass commercially. Commercial cultivation of microalgae in continental mid-latitude climate is seasonal and usually lasts from May to September, but under favorable weather conditions, both in terms of light and temperature, the duration of season in greenhouse facilities can be extended. It should be taken into account that the use of artificial lighting and thermal stabilization equipment in the commercial microalgae production leads to a significant increase in the cost of the final product. However, some species of microalgae, such as *Phaeodactylum tricornutum*, *Porphyridium purpureum* and others, do not require increased temperature and illumination for their growth and synthesis of valuable substances. These species are producers of biologically active substances and have high growth rates, hence they are suitable for use in microalgae biotechnology. Organization of microalgae cultivation in the autumn-winter period utilising greenhouse facilities contributes to an increase in the medium temperature in the photobioreactors

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and allows to alleviate the instability of natural conditions during cultivation.

During the transition to the commercial microalgae cultivation, the necessary stage is the approbation of the process in pilot systems. In this case, microalgae cultivation is carried out in facilities similar to industrial ones, but of smaller volume, which allows to evaluate their productivity and chemical composition of the resulting microalgae biomass, as well as to apply the necessary adjustments when scaling up cultivation processes and avoid significant errors when introducing cultivation technology on an industrial scale.

The produced biomass of the microalga *Porphyridium purpureum* can be used as a source of a range of valuable physiologically active substances, such as extracellular sulfated polysaccharides, unsaturated fatty acids, and also pigments belonging to the group of phycobiliproteins (PBPs) (Fabregas et al. 1998; Li et al. 2019b; Haoujar et al. 2022; Stadnichuk and Kusnetsov 2023). With regard to application, the red pigment B-phycoerythrin (B-PE) is of greatest interest. A broad spectrum of therapeutic activities, including antioxidant, antimicrobial, and anticancer properties, has been reported for B-PE (Ashaolu 2024). Phycoerythrins are also used as fluorescent probes in flow cytometry and microscopy. B-PE aqueous solution has a pleasant pink color and pronounced orange fluorescence, and its protein nature and lack of information on toxicity opens wide prospects for the use of the pigment in the food, cosmetic, and medical industries (George and John 2023).

Cultivation of *P. purpureum* microalgae for production of biomass rich in PBPs may be carried out either in the batch or in the semi-continuous mode. The B-PE content can reach 85% of the total PBPs amount, and the B-PE productivity can reach 40-50 mg L^{-1} day^{-1} (Fabregas et al. 1998; Kathiresan et al. 2006; Gudvilovich and Borovkov 2014; Fuentes-Grunewald et al. 2015). Variation of cultivation parameters is known to significantly alter the metabolism and direction of biosynthetic processes in *P. purpureum* culture (Upitis et al. 1989; Fabregas et al. 1998; Gudvilovich and Borovkov 2014; Fuentes-Grunewald et al. 2015).

Despite its great potential for use in biotechnology, there are very limited data on the cultivation of *P. purpureum* on an industrial or semi-industrial scale under natural illumination. Most of these studies refer to the cultivation in tubular photobioreactors (Rebolloso Fuentes et al. 1999; Fuentes-Grunewald et al. 2015; Schoeters et al. 2023), while studies in open raceway ponds have only been performed in a Mediterranean climate (Chile) in summer (Castro-Varela et al. 2021).

Therefore, the objective of this study was to assess the productive potential of the microalga *Porphyridium purpureum* for continental mid-latitude climate under conditions of greenhouse without additional equipment (artificial lighting and thermal stabilization) in different seasons. The growth of red marine microalga *P. purpureum* and the production of B-PE pigment were examined during pilot cultivation in open ponds in a laboratory and in a greenhouse.

Materials and methods

Microalga strain and culture medium

The unialgal culture of the red microalga *Porphyridium purpureum* (Bory) K.M.Drew & R.Ross 1965 was used in the present study. The studied strain was IBSS-70 from the Collection of Hydrobionts of the World Ocean from the Scientific and Educational Center for Collective Use of A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS (IBSS). The cultivation was carried out using nutrient medium for marine red algae according to Trenkenshu et al. (1981) with the following composition ($g \cdot L^{-1}$): $NaNO_3$ – 1.2; $NaH_2PO_4 \times 2H_20$ – 0.45; EDTA-Na_2 – 0,037; $FeC_6H_5O_7 \times 3H_20$ – 0.0265; $MnCl_2 \times 4H_20$ – 0,004; $Co(NO_3)_2 \times 6H_20$ – 0,0031; $(NH_4)_6Mo_7O_{24} \times 4H_20$ – 0,0009; $K_2Cr_2(SO_4)_2 \times 4H_20$ – 0,0017. The nutrient medium was prepared using sterilized Black Sea seawater with 18 ‰ salinity and addition of 15 $g \cdot L^{-1}$ NaCl. Sterilization was carried out by UV sterilizer Prime 55 W (Prime, China) for 4 hours.

Experimental setup and cultivation conditions

The laboratory cultivation of the red microalga *P. purpureum* was carried out in the photobioreactors, which were rectangular plastic ponds measuring 1.25 × 0.6 m (0.75 m^2) covered with 150 μm thick polyethylene

film (Figure 1a). Cultivation of *P. purpureum* in the greenhouse algobiotechnological unit (Sevastopol, Russia, N. 44°61.564′, E. 33°50.463′) was carried out in the autumn-winter (from 29.10 to 22.11 and from 10.12 to 29.12) and spring-summer (from 18.05 to 25.05, and from 28.05 to 07.06); the photobioreactors consisted of 0.5 × 0.5 m square ponds lined with 150 μm-thick polyethylene film laid on the leveled ground surface (Figure 1b).

The culture of *P. purpureum* in a laboratory and in a greenhouse unit was grown in batch mode. During cultivation, the culture was constantly mixed using aquarium electric pumps "BARBUS PUMP 006". The culture depth in the ponds was maintained at the level of 8-10 cm, by adding fresh water daily up to the initial level. Throughout all experiments, the pH of the medium value was within 8-9 units. Under laboratory conditions, DRL-700 lamps were used for illumination, the average surface irradiance was 40 $W \cdot m^{-2}$, which provided 3.45 $MJ \cdot m^{-2} \cdot day^{-1}$ total irradiance; and the culture temperature was 26–28 °C. Cultivation in the greenhouse unit was carried out at the natural illumination level depending on the season; illumination on the culture surface was determined with a light meter Li-250A (LI-COR, USA) with Li-190R quantum sensor. The average total irradiance of the operating surface of the ponds during the daytime was 1.37 $MJ \cdot m^{-2} \cdot day^{-1}$ in the autumn, 0.73 $MJ \cdot m^{-2} \cdot day^{-1}$ in the winter, and 7.7 $MJ \cdot m^{-2} \cdot day^{-1}$ in the spring-summer period (Figure 2 a,b).

The culture temperature in the experiment varied in the range of 15–25 °C in the autumn-winter period, and from 22 to 33 °C in the spring-summer period (Figure 2 c,d). During cultivation in December, the temperature was maintained in the range from 24 to 25 °C, in other cases, no additional thermal regulation was applied.

Analytical procedures and calculation

Microalgal culture samples to determine the culture density and its biochemical composition were collected from the photobioreactors during cultivation once a day. Sampling from the ponds was carried out from different points; 5 mL of microalgae cell suspension was taken at each point, thus obtaining an average sample of 20 mL. Biomass dry weight concentration was measured photometrically. Microalgal suspension optical density at 750 nm (D_{750}) was measured by Unico 2100 photometer (United Products & Instruments, USA) in 5 mm pathway cuvettes; absolute measurement error did not exceed 1.0 %. Optical density units (o.d.u.) (D_{750}) conversion to biomass dry weight (*DW*) values was expressed as follows: $DW = k \times D_{750}$, where DW is biomass dry weight; D_{750} is culture optical density; k is the conversion factor. The empirical conversion factor ($k = 1.4$ g L^{-1} o.d.u.$^{-1}$) was applied for calculation (Borovkov et al. 2023).

The concentration of B-PE in the algal culture was determined spectrophotometrically. The sampled suspension of *P. purpureum c*ulture was centrifuged for 10 min, the supernatant was drained, and the precipitated biomass was used to determine pigments concentration. For quantitative determination of PBPs in biomass, extraction with phosphate buffer (0.05 M; pH = 7–7.5) was carried out. The spectra

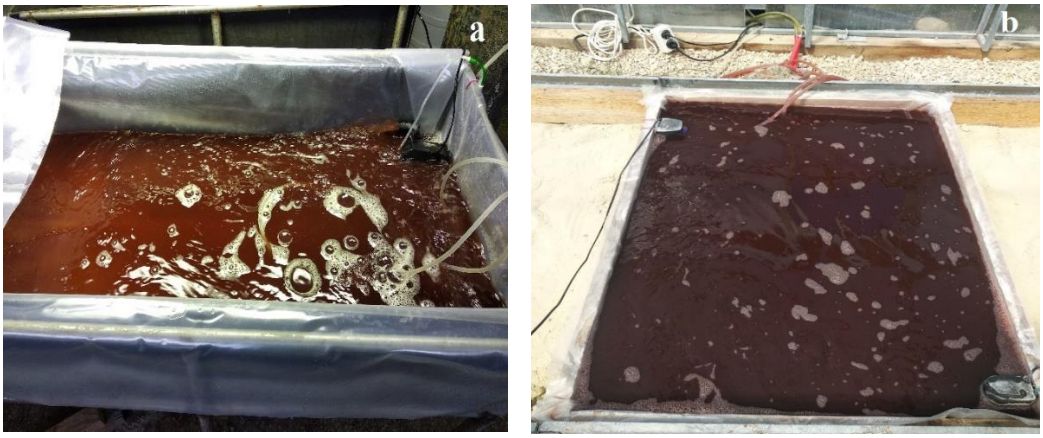


Fig. 1 General view of the photobioreactors for the pilot-scale cultivation of *P. purpureum* microalga culture in the laboratory (a) and in the greenhouse unit (b)

of pigment extracts were measured on a recording spectrophotometer SF-2000 in the wavelength range of 400–800 nm with a step of 0.1 nm. The optical density of the obtained extracts was recorded in the area of characteristic absorption maxima of B-PE (545 nm), as well as at 750 nm (to account for nonspecific absorption of the solution). The concentration of pigments in the aqueous extract was calculated according to Borovkov et al. (2023) using the optical density values for the corresponding wavelengths.

The maximum biomass productivity (P_m) was calculated by approximating growth curve at the linear growth phase part on the basis of biomass (B) by the following equation:

$$B = B_l + P_m \times (t - t_l) \quad (1)$$

where B_l is culture density at the beginning of linear growth phase; t_l is the time at the beginning of linear growth phase (Lelekov et al. 2022).

Total biomass and phycoerythrin yields were calculated as the difference between final and initial values of corresponding concentrations expressed per m^2. Areal yields and productivity were calculated on the basis of volumetric values taking into account the culture depth.

Photosynthetic efficiency was calculated from the daytime areal productivity (g·m^{-2}·day^{-1}) multiplied by the mean caloric content and divided by the light energy input per unit ground area (kJ·m^{-2}·day^{-1}), given the caloric content of the *Porphyridium* biomass was 20.5 kJ·g^{-1} (Tibbets et al. 2015) and all light energy was absorbed by the dense algal culture.

Statistical analysis

Arithmetic means ($\overline{x}$)), standard deviation (SD), and confidence intervals for the means ($\Delta\overline{x}$) were calculated. All calculations were performed in the Libre Office and Scidavis software for a significance level of $\alpha = 0.05$. Triplicate biological and analytical samples were used in the experiments. The results were subjected to the test of normality and homogeneity of variance, followed by the analysis of variance (ANOVA); and upon detecting statistical differences, means were compared using the Duncan test at a 5% probability

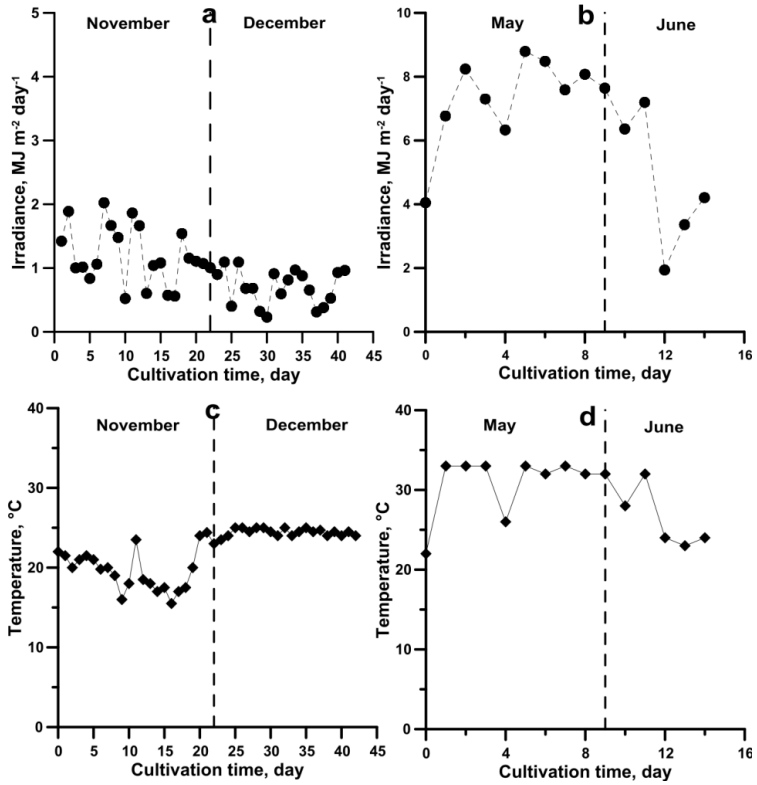


Fig. 2 The environmental conditions during cultivation of *P. purpureum* in the greenhouse unit. Daily irradiance on the photobioreactor surface in the autumn-winter (a) and spring-summer period (b), culture temperature dynamics in the autumn-winter (c) and spring-summer period (d); dashed line represents the end of one experiment and the start of another (autumn/winter and spring/summer)

level. Student's t-test was applied to compare the means between two groups. The tables and figures display the mean values and calculated confidence intervals for the three replicates (n = 3).

Results

Production characteristics of *Porphyridium purpureum* culture during its pilot cultivation in a laboratory unit

Firstly, the production characteristics of *P. purpureum* culture were investigated in the laboratory unit (Figure 1a). The microalgal culture was cultivated in a batch mode; the culture density dynamics in the course of growth is presented in Figure 3. The lag-phase corresponding to the culture adaptation period was short and lasted for one day. After that, linear culture growth was observed during 7 days until the end of the experiment. The observed maximum productivity during this period was 11.7 g of dry biomass per m² per day. The total yield over 8 days of cultivation was 81.2 $g \cdot m^{-2}$ in terms of biomass and 4.55 $g \cdot m^{-2}$ in terms of B-PE. The content of B-phycoerythrin (B-PE) in the *P. purpureum* biomass was 5.6%. Taking into consideration the biomass productivity, the photosynthetic efficiency during cultivation in the laboratory unit was 6.94 %. The obtained data indicates the absence of significant limitation both in terms of mineral nutrient elements and in the illumination of the microalgae culture cells.

Thus, the growth of the red microalgae *P. purpureum* in the laboratory pilot unit occurred at a high growth rate, and the content of B-phycoerythrin in the biomass corresponded to the initial stage of active growth (linear growth phase). The obtained data demonstrate the possibility of producing *P. purpureum*

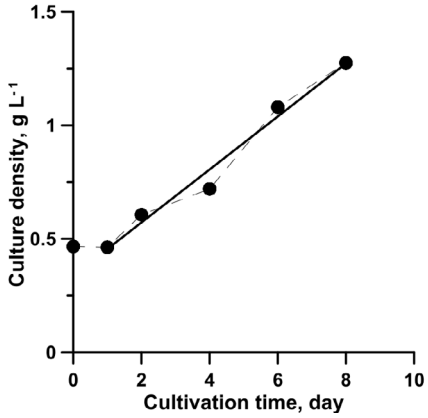


Fig. 3 Dynamics of *P. purpureum* culture density in a laboratory pilot unit. Solid line is approximation by equation (1)

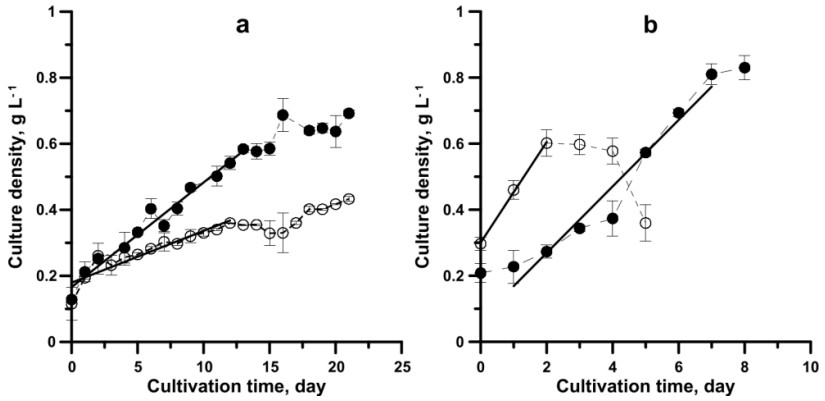


Fig. 4 Dynamics of *P. purpureum* culture density in a pilot greenhouse unit. Autumn-winter period (a): November (black circles), December (white circles); spring-summer period (b): May (black circles), June (white circles). Solid lines are approximation by equation (1)

biomass with bioactive substances determining the biotechnological value of the studied species under conditions of intensive cultivation.

Production characteristics of *Porphyridium purpureum* culture grown in a greenhouse pilot unit during the spring-summer and autumn-winter periods

The dynamics of biomass and B-PE accumulation in the *P. purpureum* batch culture was studied under greenhouse conditions during November, December, May and June. No artificial lighting was applied, and no thermal regulation was used except for December. Figure 4. demonstrates *P. purpureum* growth curves in a greenhouse unit during different seasons. The initial density of the *P. purpureum* culture when grown in November and December was 0.12 g DW·L^{-1} (9.6 g DW·m^{-2}) (Figure 4a). The initial density of *P. purpureum* culture in May and June was higher than in the autumn-winter period, amounting 0.2 and 0.3 g DW·L^{-1} (16.6 and 24 g DW·m^{-2}, respectively) (Figure 4b).

Based on the obtained experimental data, the production characteristics of *P. purpureum* in terms of biomass and phycoerythrin were calculated when grown in different seasons of the year. The total biomass and B-PE yield along with cultivation conditions during corresponding periods are given in Table 1.

The production characteristics of *P. purpureum* culture in the course of the linear growth phase, including maximum productivity, B-PE content, and photosynthetic efficiency, are given in Table 2. The values of production characteristics under laboratory conditions are also provided in these Tables for comparison. The highest production characteristics, B-PE content in biomass, and photosynthetic efficiency were registered under laboratory conditions. Regarding outdoor conditions, the highest biomass and B-PE total yield and biomass and B-PE productivity per day were observed in May, and the lowest values were in December.

The average productivity in November during the linear growth phase was 2.56 g DW·m^{-2}·day^{-1}, which is more than twice as high as the productivity in December (1.26 g DW·m^{-2}·day^{-1}) (Table 2). Thus, the maximum density of *P. purpureum* culture by the 22nd day of cultivation in November was 55.4 g DW·m^{-2}, which is 1.6 times higher than in December (34.6 g DW·m^{-2}). Examining the dynamics of biomass accumulation in the course of the daylight hours in December, it was noted that the increase in culture density occurred until 13:00, after which time biomass density remained constant until the end of the day.

For the first two days in May, the growth of the culture was insignificant. Apparently, there was an adaptation period of the laboratory culture of *P. purpureum* to the greenhouse conditions. After this stage, the average biomass productivity in May at the linear growth phase was close to 9 g DW per m² per day, and the maximum culture density by the 8th day reached 66 g DW·m². When grown in June, the culture was already pre-adapted to the greenhouse conditions, and the maximum productivity (12 g DW·m^{-2}·day^{-1}) was

Table 1 Cultivation conditions and total yield of *P. purpureum* culture when grown in the spring-summer and autumn-winter periods

Cultivation season	Day length	Average solar irradiance, MJ·m^{-2}·day^{-1}	Average culture temperature, °C	Total yield for the entire cultivation period	
				Biomass, g·m^{-2}	B-PE, g·m^{-2}
November (22 days)	9 h 40 min	1.55	19.8	41.6±3.4 ^{c}	0.815±0.017 ^{c}
December (22 days)	8 h 53 min	1.07	24.5	22.4±1.8 ^{d}	0.124±0.002 ^{d}
May (9 days)	15 h 03 min	7.78	30.6	49.6±0.6 ^{b}	1.132±0.04 ^{b}
June (6 days)	15 h 20 min	7.06	27.2	23.8±1.9 ^{d}	—
Laboratory unit (8 days) (control)	24 h 00 min	3.45	27.0	81.2±3.12 ^{a}	4.547±0.19 ^{a}

Different superscript letters within the same column indicate a statistically significant difference by Duncan test ($P < 0.05$)

Table 2 Production characteristics of *P. purpureum* culture at the linear growth phase in different seasons

Cultivation season	Maximum productivity at the linear growth phase		B-PE content in biomass (linear growth phase), mg·g^{-1}	Photosynthetic efficiency, %
	biomass, g·m^{-2}·day^{-1}	B-PE, mg·m^{-2}·day^{-1}		
November	2.56±0.15 ^{c}	52.7±0.65 ^{c}	22.8±1.0 ^{b}	3.83±0.07 ^{b}
December	1.26±0.25 ^{d}	31.7±7.5 ^{c}	23.2±2.3 ^{b}	3.54±0.05 ^{c}
May	9.0±0.05 ^{b}	161.7±4.8 ^{b}	23.1±0.33 ^{b}	2.37±0.11 ^{d}
June*	12±0.8 ^{a}	187.2±1.2 ^{b}	15.65±0.15 ^{c}	3.48±0.28 ^{c}
Laboratory unit (control)	11.7±0.4 ^{a}	655.2±24.6 ^{a}	56.0±3.31 ^{a}	6.94±0.14 ^{a}

* In June, the linear growth phase lasted two days, culture death was observed after that.
Different superscript letters within the same column indicate a statistically significant difference by Duncan test ($P < 0.05$)

observed in the first two days. However, after 3 days, the growth of *P. purpureum* ceased, and after 5 days, a phase of culture death was observed (Figure 4b). Apparently, the negative impact on the culture was caused by increased temperature and illumination at the beginning of the experiment when the cell concentration was minimal.

When *P. purpureum* was grown in November, the concentration of B-PE in the culture began to increase significantly only after 4 days. By the end of the cultivation, the B-PE concentration increased 2.6 times (up to 1.17 $g \cdot m^{-2}$) compared to the initial values (Table 3). In December, the concentration of B-PE in the culture increased from 495 to 688 $mg \cdot m^2$ during the first 7 days and then remained relatively constant throughout the experiment. When growing *P. purpureum* in May, an increase in B-PE concentration in the culture by 3.8 times was observed by the end of cultivation (7^{th} day) (up to 1.53 $g \cdot m^{-2}$). In June, its content decreased by 2.5 times by the end of the experiment compared to the initial values (Table 3). The highest increase in PE concentration over the entire cultivation period was observed in May, which also corresponded to higher biomass productivity during this period.

Discussion

Approximately 70–100 mg of nitrogen is required for the synthesis of 1 g of microalgal biomass (Upitis et al. 1989). Therefore, the estimated yield of *P. purpureum*, considering the initial dilution of the culture, could reach 2 g of biomass per 1 liter of culture. Nevertheless, the maximum biomass yield of the culture obtained in November and May was 3–4 times lower than the calculated values. Therefore, the most probable factors that limited the growth of *P. purpureum* in the experiment were light and temperature conditions of the environment, rather than the concentration of mineral nitrogen.

The calculations showed that when *P. purpureum* is cultivated during the autumn-winter period, all production characteristics of the culture in November were significantly higher than in December. It should be noted that environmental conditions in December are characterized by a decrease in both the duration of daylight (by 8 %) and the level of solar irradiance (1.5 times) compared to November. In consideration of the aforementioned factors, the production characteristics of the *P. purpureum* culture in December significantly declined: the culture productivity in the linear stage decreased twice, the total biomass yield decreased by 1.85 times, and the total B-PE yield decreased by 6.5 times compared to the corresponding values in November (Table 1, Table 2).

It is worth noting that a decrease in the production characteristics of *P. purpureum* in December occurred in spite of maintaining the temperature at a level optimal for the growth of this culture (24–25 °C). When cultivating *P. purpureum* in November, the average daily temperature in the ponds (around 20 °C) was slightly lower than in December; however, it remained mostly within the optimal range for this species (Figure 2). Thus, stabilization of temperature at an optimal level during the winter period was not a factor determining the productivity of *P. purpureum* in the autumn-winter period.

Most laboratory studies have shown that *P. purpureum* performs best at temperatures ranging from 20 to 25 °C (Gaignard et al. 2019; Li et al. 2019a), but it can also grow at lower temperatures (10-15 °C) (Durmaz et al. 2007; Guihéneuf and Stengel 2015). For example, a slight culture growth (0.051 $g \cdot L^{-1} \cdot day^{-1}$) was observed during the winter period under a temperature of 10 °C (Schoeters et al. 2023), which potentially provides opportunities for growing *P. purpureum* in colder periods.

When cultivating *P. purpureum* during the spring-summer period, the production characteristics of the culture in May significantly exceeded the similar values of June: the total biomass yield was twice as high, and the total B-PE yield was an order of magnitude greater (Table 1).

Table 3 The concentration of B-PE in *P. purpureum* culture at the beginning and in the end of cultivation in different seasons

Cultivation period	The concentration of B-PE in culture, $mg \cdot L^{-1}$		
	Initial	Final	P-value
May	5.01±0.10	19.16±0.03	$P < 0.001$
June	7.24±0.21	2.95±0.06	$P = 0.0011$
November	5.6±0.30	14.61±0.73	$P < 0.001$
December	6.19±0.12	8.6±0.31	$P = 0.002$

P-value was determined by Student's t-test. Differences within the row are significant for $P < 0.05$

The most likely that the increase in both the photoperiod length and the level of solar irradiance (by 7.5%) in June negatively affected culture growth and the biosynthesis processes of pigments compared to the values obtained in May. The start of the experiment in June coincided with sunny weather; the temperature data (see Figure 2d) correspond to the morning hours, while at midday the temperature in the pools was significantly higher than optimal for the active growth of this species, which apparently had a negative impact on the culture growth.

Despite Chang et al. (2017) reported growth of *P. purpureum* up to 35 °C without significant issues, most researchers noted that temperatures of 30 °C and above had a negative impact on the *P. purpureum* culture (Golueke and Oswald 1962; Schoeters et al. 2023). Thus, during the two-year cultivation of *P. purpureum*, exposure to elevated temperatures of 34–35 °C for several days was critical, leading to growth inhibition and even rapid death of the culture during the summer period. Moreover, the growth of *P. purpureum* also stopped in April after the temperature in the greenhouse increased to 30-35 °C (Schoeters et al. 2023).

The calculations showed that the highest productivity of the *P. purpureum* culture was observed in May, demonstrating the possibility to obtain approximately 50 g of *P. purpureum* biomass per 1 m² and, accordingly, up to 1 g of phycoerythrin contained in it, over a 7-day technological cycle. Despite the fact that the productivity of *P. purpureum* in the autumn-winter period was significantly lower than in May (2 and 3 times lower in November and December, respectively), due to the increased cultivation time in autumn, comparable total yield values were obtained for the entire growing period (Table 1). Thus, when cultivating the red microalgae *P. purpureum* in a pilot unit during the late autumn period, it is possible to obtain more than 40 $g \cdot m^{-2}$ of biomass per square meter and about 0.7 g of phycoerythrin contained in it over an 18-day technological cycle.

Under comparable conditions of growing *P. purpureum* (spring-summer period, similar type of ponds, operating depth, and cultivation regime), the average culture productivity was 3.19 $g \cdot m^{-2} \cdot day^{-1}$, and the content of B-PE in the biomass was 10.2 $mg \cdot g^{-1}$ (Castro-Varela et al. 2021). These production characteristics of the culture, as well as the B-PE content in the obtained biomass, are more than two times lower than those in the current research (Table 1, Table 2). It should be noted that the concentration of nitrogen in the nutrient medium used by (Castro-Varela et al. 2021) was twice as high as in the medium used in the experiment, and the maximum culture density achieved was 0.30 $g \cdot L^{-1}$, which was more than 2.5 times less than in this study, and also indicates a deficiency in other mineral nutrients or suboptimal cultivation conditions.

The possibility to achieve maximum productivity of *P. purpureum* specifically during the spring period is also confirmed by the data (Schoeters et al. 2023) from the year-round cultivation of the culture over two years in a greenhouse in a tubular photobioreactor. At the same time, the maximum productivity and growth rate (0.195 $g \cdot L^{-1} \cdot day^{-1}$ and 0.233 day^{-1}, respectively) were achieved in mid-May. The productivity data of *P. purpureum* obtained in the current study during the spring period also correlate well with the data obtained for *Dunaliella salina* under similar conditions, where the possibility of achieving a productivity of 7 $g \cdot m^{-2} \cdot day^{-1}$ by this culture was demonstrated (Borovkov et al. 2021).

Upon comparing the relationship between productivity and irradiance in the laboratory unit and under natural light conditions, it was noted that in laboratory conditions the photosynthetic efficiency was more than 2-fold higher than under natural light (Table 2). This may be partly due to the fact that under natural illumination night loss of microalgae biomass occurs due to dark respiration (Rebolloso Fuentes et al. 1999). Also, temperature and light were stabilized and optimal under laboratory cultivation. Higher photosynthetic efficiency also corresponded to the significantly higher B-PE content in biomass. Higher pigment content under laboratory conditions may be due to decreased irradiance in spite of continuous illumination.

The photosynthetic efficiency in November and December, when the total daily irradiance was at a minimum level, was 50-65 % higher than in May, when the total daily irradiance was highest (Table 1). However, at the same time, the photosynthetic efficiency was lower in December than in November despite maintaining the temperature at 24–25 °C. Temperature maintenance could have provided a higher growth rate, but increased temperature may have contributed to faster biomass breakdown during the dark period (Edmundson and Huesemann 2015).

The light intensity is one of the most critical factors that affect the growth and quantitative composition of microalgal pigments. In the literature, the following trend is observed: microalgae that possess phycobilisomes and phycobiliproteins in their plastids generally grow better under low light conditions

(~10-50 µmol photons $m^{-2} \cdot s^{-1}$), whereas other types of algae, such as dinoflagellates or green algae, typically require higher light intensity (~60-100 µmol photons $m^{-2} \cdot s^{-1}$) (John et al. 1984; Algarra and Ruediger 1993; Sosa-Hernández et al. 2019; Stadnichuk and Kusnetsov 2023). It is known that *P. purpureum*, in general, prefers low light levels. Thus, it was shown that with an increase in illumination intensity (from 70–80 to 300–400 $W \cdot m^{-2}$) in the experiments, inhibition of *P. purpureum* growth, bleaching of B-PE pigment, and even culture death at low density (about 0.2 $g \cdot L^{-1}$ dry biomass) (Upitis et al. 1989) were observed.

In the present study, a strong linear correlation was observed between total daily irradiance (I_{day}, MJ m^{-2} day^{-1}) and maximum areal biomass productivity (P_m) of *P. purpureum* within the studied irradiance range during different seasons of the year. The relationship between these parameters can be expressed as follows (R^2=0.89):

$$P_m = 1.41 \cdot I_{day}. \quad (2)$$

The coefficient of productivity-irradiance relationship under natural conditions allows prediction of *P. purpureum* production depending on climatic conditions within the range of temperature and irradiance studied.

Despite the revealed dependence of *P. purpureum* productivity on daily irradiance, when cultivating *P. purpureum* during late spring and summer periods, it is necessary to take into account the negative impact of excessive solar irradiance and increased temperature on the culture, especially at the time of cultivation start. To reduce the degree of stress impact of light and temperature factors on the culture of *P. purpureum*, it is necessary to apply shading of ponds, cooling, and to increase their depth. In the autumn-winter period, productivity directly depended on both the duration of daylight and the level of solar radiation; the culture yield significantly decreased in December as compared to November, even with temperature stabilization at the level optimal for culture growth (Table 1). And thus, in the late autumn period, the level of solar irradiation was a factor significantly limiting the productivity of *P. purpureum*. During this period, it may be suggested to reduce the working depth of the ponds and to use less concentrated nutrient media considering the actual productivity of *P. purpureum* in order to reduce labor costs.

Conclusion

The production characteristics of the red microalga *P. purpureum*, the producer of a wide range of valuable substances, have been determined during its cultivation in the autumn-winter and spring-summer periods in the continental mid-latitude climate. The main factors limiting the growth and impacting the productivity of microalgae cultures were primarily light and temperature conditions, which imposes additional requirements to the organization of the production process. In general, lower temperatures were well-tolerated by *P. purpureum*, while higher temperatures, especially above 35 °C, were detrimental. A strong linear correlation was found between total daily irradiance and maximum biomass productivity. The spring period is favorable for the intensive growth of the *P. purpureum* culture. Whereas cultivation in the summer period has certain potential; however, due to weather conditions, there is a risk of the culture death. Although the productivity in the autumn-winter period is lower than in the spring, it is possible to obtain an additional biomass yield. The obtained results demonstrated that the cultivation of *P. purpureum*, which does not require high illumination and temperature, can become an alternative during the off-season when production facilities are no longer used for the cultivation of more light-tolerant and thermophilic species in the continental mid-latitude climate.

List of abbreviations

B-PE	B-phycoerythrin
DW	dry weight
PBPs	phycobiliproteins

Competing interests The authors declare that they have no competing interests.

Authors' contributions ABB, conceptualization, methodology, formal analysis and investigation; ING, methodology, formal analysis and investigation, writing - original draft; ALA, formal analysis and investigation, writing - review and editing; SYuG, formal analysis and investigation, writing - review and editing. All authors contributed to the discussion and interpretation of the results and approved the final manuscript.

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References

Algarra P, Ruediger W (1993) Acclimation processes in the light harvesting complex of the red alga *Porphyridium purpureum* (Bory) Drew et Ross, according to irradiance and nutrient availability. Plant Cell Environ 16(2):149–159. https://doi.org/10.1111/j.1365-3040.1993.tb00856.x

Ashaolu TJ (2024) The powerful phycobiliproteins-phycocyanin and phycoerythrin: Pleiotropic applications and biofunctional uses. Algal Res 82:103636. https://doi.org/10.1016/j.algal.2024.103636

Borovkov AB, Gudvilovich IN, Avsiyan AL, Lantushenko AO, Rylkova OA, Memetshaeva OA, Degtyar IV, Chekushkin AA (2021) Productivity and morphometric parameters of the microalga *Dunaliella salina* IBSS-2 under pilot cultivation in continental mid-latitude climate in spring. 3 Biotech 11(10):13. https://doi.org/10.1007/s13205-021-02982-7

Borovkov AB, Gudvilovich IN, Lelekov AS, Avsiyan AL (2023) Effect of specific irradiance on productivity and pigment and protein production of *Porphyridium purpureum* (Rhodophyta) semi-continuous culture. Bioresource Technol 374:128771. https://doi.org/10.1016/j.biortech.2023.128771

Castro-Varela P, Sáez K, Gómez PI (2021) Effect of urea on growth and biochemical composition of *Porphyridium purpureum* (Rhodophyta) and scaling-up under non-optimal outdoor conditions. Phycologia 60(6):572–581. https://doi.org/10.1080/00318884.2021.1953305

Chang J, Le K, Song X, Jiao K, Zeng X, Ling X, Shi T, Tang X, Sun Y, Lin L (2017) Scale-up cultivation enhanced arachidonic acid accumulation by red microalgae *Porphyridium purpureum*. Bioproc Biosyst Eng 40:1763–1773. https://doi.org/10.1007/s00449-017-1831-x

Durmaz Y, Monteiro M, Bandarra N, Gökpinar Ş, Işik O (2007) The effect of low temperature on fatty acid composition and tocopherols of the red microalga, *Porphyridium cruentum*. J Appl Phycol 19:223-227. https://doi.org/10.1007/s10811-006-9127-6

Edmundson SJ, Huesemann MH (2015) The dark side of algae cultivation: characterizing night biomass loss in three photosynthetic algae, *Chlorella sorokiniana*, *Nannochloropsis salina* and *Picochlorum* sp. Algal Res 12:470–476. https://doi:10.1016/j.algal.2015.10.012

Fabregas J, Garcia D, Morales E, Dominguez A, Otero A (1998) Renewal rate of semicontinuous cultures of the microalga *Porphyridium cruentum* modifies phycoerythrin, exopolysaccharide and fatty acid productivity. J Ferment Bioeng 86(5):477–481. https://doi.org/10.1016/S0922-338X(98)80155-4

Fuentes-Grunewald C, Bayliss C, Zanain M, Pooley C, Scolamacchia M, Silkina A (2015) Evaluation of batch and semi-continuous culture of *Porphyridium purpureum* in a photobioreactor in high latitudes using fourier transform infrared spectroscopy for monitoring biomass composition and metabolites production. Bioresource Technol 189:357–363. https://doi.org/10.1016/j.biortech.2015.04.042

Gaignard C, Gargouch N, Dubessay P, Delattre C, Pierre G, Laroche C, Fendri I, Abdelkafi S, Michaud P (2019) New horizons in culture and valorization of red microalgae. Biotechnol Adv 37(1):193–222. https://doi.org/10.1016/j.biotechadv.2018.11.014

George R, John JA (2023) Phycoerythrin as a potential natural colourant: A mini review. Int J Food Sci Technol 58(2):513–519. https://doi.org/10.1111/ijfs.16229

Golueke CG, Oswald WJ (1962) The mass culture of *Porphyridium cruentum*. Appl Microbiol 10(2):102–107. https://doi.org/10.1128/am.10.2.102-107.1962

Gudvilovich IN, Borovkov AB (2014) Production characteristics of the microalga *Porphyridium purpureum (*Bory) Drew Ross (Rhodophyta) in batch and quasi-continuous culture. Int J Algae 16(3):271–283. https://doi.org/10.1615/InterJAlgae.v16.i3.70

Guihéneuf F, Stengel DB (2015) Towards the biorefinery concept: Interaction of light, temperature and nitrogen for optimizing the co-production of high-value compounds in *Porphyridium purpureum*. Algal Res 10:152–163. https://doi.org/10.1016/j.algal.2015.04.025

Haoujar I, Haoujar M, Altemimi AB, Essafi A, Cacciola F (2022) Nutritional, sustainable source of aqua feed and food from microalgae: a mini review. Int Aquat Res 14(3):157–167. https://doi.org/10.22034/iar.2022.1958713.1278

John W, Steinbiss J, Zetsche K (1984) Light intensity adaptation of the phycobiliprotein content of the red alga *Porphyridium*. Planta 16(6):536–539. https://doi.org/10.1007/BF00407086

Kathiresan S, Sarada R, Bhattacharya S, Ravishankar A (2006) Culture media optimization for growth and phycoerythrin production from *Porphyridium purpureum*. Biotechnol Bioeng 96:456–463. https://doi.org/10.1002/bit.21138

Khalid F, Azmat H, Khan N (2024) Ameliorative effects of *Moringa oleifera* leaf extract against arsenic induced histo-biochemical alterations in *Labeo rohita*. Ecotoxicol Environ Saf 287:117258. https://doi.org/10.1016/j.ecoenv.2024.117258

Khavari F, Saidijam M, Taheri M, Nouri F (2021) Microalgae: therapeutic potentials and applications. Mol Biol Rep 48(5):4757–4765. https://doi.org/10.1007/s11033-021-06422-w

Lelekov AS, Chernyshev DN, Klochkova VS (2022) Quantitative regularities of growth of *Arthrospira platensis* batch culture. Matematicheskaya Biologiya Bioinformatika 17(1):156–170. https://doi.org/10.17537/2022.17.156

Li S, Ji L, Shi Q, Wu H, Fan J (2019a) Advances in the production of bioactive substances from marine unicellular microalgae *Porphyridium* spp. Bioresource Technol 292:1–12. https://doi.org/10.1016/j.biortech.2019.122048

Li T, Xu J, Wu H, Jiang P, Chen Z, Xiang W (2019b) Growth and biochemical composition of *Porphyridium purpureum* SCS-02 under different nitrogen concentrations. Mar Drugs 17(2):124. https://doi.org/10.3390/md17020124

Rebolloso Fuentes MM, García *Sánchez JL, Fernández Sevilla JM,* Acién Fernández FG, Sánchez Pérez JA, Grima EM (1999) Out-

door continuous culture of *Porphyridium cruentum* in a tubular photobioreactor: quantitative analysis of the daily cyclic variation of culture parameters. J Biotechnol 70(1–3):271–288. https://doi.org/10.1016/S0168-1656(99)00080-2

Schoeters F, Spit J, Swinnen E, De Cuyper A, Vleugels R, Noyens I, Van Mier S (2023) Pilot-scale cultivation of the red alga *Porphyridium purpureum* over a two-year period in a greenhouse. J Appl Phycol 35:2095–2109. https://doi.org/10.1007/s10811-023-03045-5

Sosa-Hernández JE, Rodas-Zuluaga LI, Castillo-Zacarías C, Rostro-Alanís M, Cruz R, Carrillo-Nieves D, Salinas-Salazar C, Grunewald CF, Llewellyn CA, Olguín EJ, Lovitt RW, Iqbal HMN, Parra-Saldívar R (2019) Light intensity and nitrogen concentration impact on the biomass and phycoerythrin production by *Porphyridium purpureum*. Mar Drugs 17(8):460. https://doi.org/10.3390/md17080460

Stadnichuk IN, Kusnetsov VV (2023) Phycobilisomes and phycobiliproteins in the pigment apparatus of oxygenic photosynthetics: from cyanobacteria to tertiary endosymbiosis. Int J Mol Sci 24(3):2290. https://doi.org/10.3390/ijms24032290

Trenkenshu RP, Terskov IA, Sidko FYa (1981) Dense cultures of marine microalgae. Izv SO AN SSSR (ser Biol) 15(3):75–82. (in Russ.)

Tibbetts SM, Milley JE, Lall SP (2015) Chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors. J Appl Phycol 27:1109–1119. https://doi.org/10.1007/s10811-014-0428-x

Upitis VV, Pakalne DS, Shultse IF (1989) Optimization of the mineral nutrition of the red seaweed *Porphyridium cruentum*. Izv AN Latviiskoi SSR 505(8):95–104. (in Russ.)

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