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GROWTH OF CULTURES OF MARINE MICROALGAE *PORPHYRIDIUM PURPUREUM* AND *TETRASELMIS VIRIDIS* ON MODIFIED NUTRIENT MEDIA

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Marine species of microalgae are capable of synthesizing a wide range of biologically active substances and are currently considered as the most promising sources of such compounds. Nutrient media for cultivation of microalgae are mostly prepared based on natural or artificial seawater. Modifying the nutrient medium for cultivation of marine microalgae by replacing its natural seawater base with freshwater one seems promising. Unialgal cultures of the marine microalgae *Porphyridium purpureum* and *Tetraselmis viridis* were grown under conditions of replacing sterile seawater with freshwater, with sea salt added up to a concentration of 18 and 28 g·L^{-1} for *T. viridis* and *P. purpureum*, respectively. Based on experimental data obtained, production characteristics of *P. purpureum* and *T. viridis* batch cultures were determined when grown on freshwater-based and seawater-based nutrient media. In general, a change in the density of *P. purpureum* and *T. viridis* cultures during batch cultivation both on freshwater and seawater had a unidirectional character (correlation coefficients in both cases were 0.99), and the water base of the nutrient medium had no significant effect on their growth rate. As shown experimentally, the biomass yield of *P. purpureum* and *T. viridis* using freshwater as a base of the nutrient medium was 3.2–3.4 g of dry weight *per* 1 L of the culture and generally corresponded to the similar parameter of cultures grown using seawater. Despite the fact that the mean growth rate of *T. viridis* cultured in freshwater did not differ significantly from the growth rate of the microalga cultured in seawater, higher mean rates of pigment synthesis and their total accumulation were observed in the culture grown in seawater. In the case of *P. purpureum*, the water base of the nutrient medium had no noticeable effect on B-phycoerythrin synthesis rate and content of this pigment in the culture and biomass of the microalga. The obtained results show that cultures of marine microalgae *P. purpureum* and *T. viridis* can be successfully grown without using natural seawater. It significantly reduces labor costs and biomass production costs; also, it expands geographical perspectives for their mass cultivation.

Keywords: marine microalgae, *Porphyridium purpureum*, *Tetraselmis viridis*, freshwater, productivity, biomass, pigments

Marine species of microalgae are currently considered as the most promising sources of biologically active substances due to their ability to synthesize a wide range of compounds positively affecting organisms of both humans and animals [Minyuk et al., 2008]. Those include polyunsaturated fatty acids, sulfated polysaccharides, photosynthetic pigments (chlorophylls, carotenoids, and phycobiliproteins), vitamins, and other substances with anti-inflammatory, antifungal, immunomodulatory, and antioxidant

properties [Borowitzka, 2013; Chauton et al., 2015; Gaignard et al., 2019; Geada et al., 2021; Li S. et al., 2019]. This allows using marine microalgae biomass and biosynthesis products as dietary supplements and applying them in cosmetology, pharmacology, and food production.

The need for polyunsaturated fatty acids has risen due to the development of aquaculture: for all hydrobionts, microalgae are a key source of valuable long-chain omega-3 fatty acids [Borowitzka, 2013]. Also, marine microalgae are a food supplement for fish. Those contain essential fatty acids, amino acids, polysaccharides, antioxidants, vitamins, and minerals. All these compounds stimulate growth and survival of fish larvae and improve quality of final products [Chauton et al., 2015; Gargouch et al., 2018; Ma, Hu, 2024; Tredici et al., 2009].

The cultivation of marine microalgae in coastal zones is generally less costly due to lower investment, logistics, and operating expenses. However, certain constraints due to competition from recreational, fishing, and fish farming areas and due to urban development force to relocate marine microalgae cultivation complexes to areas far from the shore.

Most nutrient media for microalgae cultivation are prepared on the base of natural or artificial seawater. Various nutrient media for cultivation of microalgae species have been described: F/2, Artificial Seawater Medium, Pm, *etc.* [Fuentes-Grunewald et al., 2015; Gargouch et al., 2018; Kathiresan et al., 2006; Lelekov et al., 2016; Raes et al., 2013; Strizh et al., 2004]. Their composition allows maintaining microalgae cells in a vegetative state. Nevertheless, the culture growth rate and biomass yield may differ significantly depending on starting concentrations of mineral nutrients and cultivation conditions.

Cultivation of marine microalgae becomes unprofitable far from the coastline in case of using nutrient media based on natural seawater. Its mineral composition is unique, and it cannot be properly reproduced under artificial conditions: in addition to mineral salts and trace elements, seawater contains a large number of free ions. One of the ways to reduce the cost of the resulting microalgae biomass is cultivation on nutrient media based on artificial seawater, but its preparation implies additional material and labor expenses. Replacing the base of a nutrient medium with freshwater with natural sea salt added is the way to obtain a base close to natural seawater in its characteristics. Such modification excludes dependence on a natural source of seawater and reduces the cost of biomass; obviously, it is crucial when elevating the efficiency and expanding the area of intensive microalgae cultivation.

A red microalga *Porphyridium purpureum* (Bory) K. M. Drew & R. Ross, 1965 is of interest as a source of sulfated exopolysaccharides, essential fatty acids, and pigments of the phycobiliprotein group. Their biotechnological potential is actively used in producing nutraceuticals, pharmaceuticals, food, and cosmetics; moreover, it is applied in biomedical research and even in clinical diagnostics [Gaignard et al., 2019; Li S. et al., 2019; Manirafasha et al., 2016]. A green microalga *Tetraselmis viridis* (Rouchijajnen) R. E. Norris, Hori & Chihara, 1980 is capable of accumulating significant amounts of polyunsaturated fatty acids. Those play a key role in organisms of humans by participating in metabolic processes. High productivity and nutritional value make the alga promising for production of biologically active substances and food supplements for humans and animals [Borowitzka, 2013; Raes et al., 2013]. Microalgae of the genus *Tetraselmis* are widely used in aquaculture as valuable food enriched with polyunsaturated fatty acids and protein [Borowitzka, 2013; Ma, Hu, 2024; Tredici et al., 2009]. Interestingly, lipids can accumulate in these species in high concentrations (up to 22%) even with relatively high protein levels (31–36%) [Barka, Blecker, 2016].

Accordingly, it seems promising to culture these biotechnologically valuable species of marine microalgae on a nutrient medium modified by replacing its natural seawater base with freshwater one and adding sea salt. Therefore, the aim of this work was to test the cultivation of two marine microalgae, *Tetraselmis viridis* and *Porphyridium purpureum*, on a freshwater-based nutrient medium.

MATERIAL AND METHODS

Objects of the study were unialgal cultures of marine microalgae *P. purpureum* (Rhodophyta) and *T. viridis* (Chlorophyta) from IBSS core facility "Collection of hydrobionts of the World Ocean."

Cultivation was carried out on nutrient media of the following composition [Trenkenshu et al., 1981]:

- For *P. purpureum*: $NaNO_3$, 1.2 g·L^{-1}; $NaH_2PO_4 \times 2H_2O$, 0.45 g·L^{-1}; EDTA-Na_2, 0.037 g·L^{-1}; $FeC_6H_5O_7 \times 3H_2O$, 0.0265 g·L^{-1}; $MnCl_2 \times 4H_2O$, 0.004 g·L^{-1}; $Co(NO_3)_2 \times 6H_2O$, 0.0031 g·L^{-1}; $(NH_4)_6Mo_7O_{24} \times 4H_2O$, 0.0009 g·L^{-1}; and $K_2Cr_2(SO_4)_2 \times 4H_2O$, 0.0017 g·L^{-1}.
- For *T. viridis*: $NaNO_3$, 1.8 g·L^{-1}; $NaH_2PO_4 \times 2H_2O$, 0.3 g·L^{-1}; EDTA-Na_2, 0.037 g·L^{-1}; $FeC_6H_5O_7 \times 3H_2O$, 0.042 g·L^{-1}; $MnCl_2 \times 4H_2O$, 0.008 g·L^{-1}; $Co(NO_3)_2 \times 6H_2O$, 0.00625 g·L^{-1}; $(NH_4)_6Mo_7O_{24} \times 4H_2O$, 0.00183 g·L^{-1}; and $K_2Cr_2(SO_4)_2 \times 4H_2O$, 0.00238 g·L^{-1}.

A nutrient medium for each species was prepared on sterile Black Sea water (for *P. purpureum*, 10 g·L^{-1} of sea salt was added). For modification, seawater was replaced with freshwater to which sea salt (manufactured by Galit production cooperative) was added to concentrations of 18 and 28 g·L^{-1} for *T. viridis* and *P. purpureum*, respectively.

Microalgae were cultured in glass plane-parallel photobioreactors, 25 × 50 cm each. Working thickness was of 2 and 5 cm, and volume was of 1 and 3 L for *T. viridis* and *P. purpureum*, respectively. These volumes were maintained throughout the experiment with daily adding distilled water before sampling to compensate for evaporation. A grid of 18-W fluorescent lamps served as a light source; the mean irradiance intensity at the surface of the photobioreactors was 20 and 40 W·m^{-2} for *P. purpureum* and *T. viridis*, respectively. Irradiance intensity on the surface of the photobioreactors was recorded with a LI-250A light meter with a pyranometer (LI-COR, the USA). Temperature was maintained at +26…+28 °C, and pH of a medium varied from 8 to 10 during cultivation. Cultures were bubbled with air with a Hailea ACO-308 compressor; the rate of air supply was about 0.5 L·L^{-1} culture *per* minute. To increase solubility of atmospheric carbon dioxide, air bubbling was carried out *via* an atomizer: a plastic tube 5 cm long and 5 mm in diameter, with a pore diameter of < 0.1 mm. The experiments lasted for 18 and 10 days for *P. purpureum* and *T. viridis*, respectively.

The optical density of cultures (D_{750}) was measured with a Unico 2100 spectrophotometer in cuvettes with a working length of 5 mm at a wavelength of 750 nm. Dry weight content (DW) was determined by a photometric method [Metody, 1975] by equation $DW = k \times D_{750}$. *Prior* to it, empirical conversion factors from the optical density of the cultures to DW were established (1.4 and 0.8 for *P. purpureum* and *T. viridis* respectively) [Borovkov, Gevorgiz, 2005; Borovkov et al., 2023].

The maximum productivity (P_m) was calculated by approximating empirical data on the linear phase of microalgae cumulative curve using equation (1):

$$B = B_l + P_m \cdot t\,, \tag{1}$$

where B_l is culture density at the beginning of linear growth phase, g·L^{-1};

t is time, days.

Pigment content was recorded spectrophotometrically. Sampling was carried out at different growth phases of a batch culture after thorough mixing. A suspension was centrifuged for 10 min, the supernatant was drained, and the precipitated biomass was used for pigment determination. The spectra of pigment extracts were measured with a SF-2000 spectrophotometer (Russia). For quantitative determination of B-phycoerythrin (hereinafter B-PE), *P. purpureum* biomass was extracted with a phosphate buffer (0.05 M; pH 7–7.5). The optical density of the obtained extracts was registered in the area of characteristic absorption maxima of B-PE (545 nm), R-phycocyanin (615 nm), and allophycocyanin (650 nm), as well as at 750 nm (to account for non-specific absorption of the solution). B-PE concentration in the aqueous extract was calculated according to [Stadnichuk, 1990] using optical density values for corresponding wavelengths:

$$B-PE = 0.1 \times D_{545} - 0.063 \times D_{615} + 0.023 \times D_{650}\,. \quad (2)$$

Chlorophylls and carotenoids were extracted from cells with 100% acetone. Concentrations of chlorophylls and total carotenoids were determined by formulas from [Wellburn, 1994] using optical density values at wavelengths corresponding to absorption maxima of similar pigments:

$$\text{Chl}\,a = 11.75 \times D_{662} - 2.35 \times D_{645}\,;$$

$$\text{Chl}\,b = 18.61 \times D_{645} - 3.96 \times D_{662}\,;$$

$$\text{Carotenoids} = (1,000 \times D_{470} - 2.27 \times \text{Chl}\,a - 81.4 \times \text{Chl}\,b)/227\,.$$

Arithmetic mean ($\bar{x}$), standard deviations (S), standard errors of the mean, and confidence intervals for means ($\Delta\bar{x}$) were established. Calculations were carried out in LibreOffice and SciDAVis (at the significance level, α, of 0.05). In order not to clutter the graphs, statistical indicators characterizing variability of the studied features were omitted without compromising the perception of the results obtained. The tables provide mean values and determined confidence intervals ($\bar{x} \pm \Delta\bar{x}$) for three replications.

RESULTS

Initial culture density for each of two variants (seawater-based and freshwater-based nutrient media) was about 0.2 g·L^{-1} for *P. purpureum* and 0.13 g·L^{-1} for *T. viridis*. Throughout the cultivation, *P. purpureum* biomass increased by more than 15 times compared to the initial one, and that of *T. viridis*, by 25 times (Fig. 1).

During batch cultivation, microalgae culture cells are maintained in a vegetative state due to initial stock of mineral nutrition elements, with the culture density gradually rising and reaching the maximum value which determines the total biomass yield. Throughout the entire period of *P. purpureum* and *T. viridis* cultivation, the character of changes of cumulative curves for seawater- and freshwater-based media was almost the same and had a high correlation (correlation coefficients were of 0.99 for both *P. purpureum* and *T. viridis*). Total biomass growth for analyzed variants also had no significant differences (Fig. 1). Based on data obtained, production characteristics of *P. purpureum* and *T. viridis* batch cultures by biomass on freshwater- and seawater-based nutrient media were determined (Table 1).

The nutrient media used for *P. purpureum* and *T. viridis* cultivation were expected to produce about 4 g of biomass *per* 1 L of culture [Trenkenshu et al., 1981; Upitis et al., 1989]. The calculated biomass growth for the entire cultivation period, taking into account both nitrogen concentration in a medium and dilution, could be about 3–3.5 g·L^{-1} which was consistent with data obtained in the experiment (Table 1).

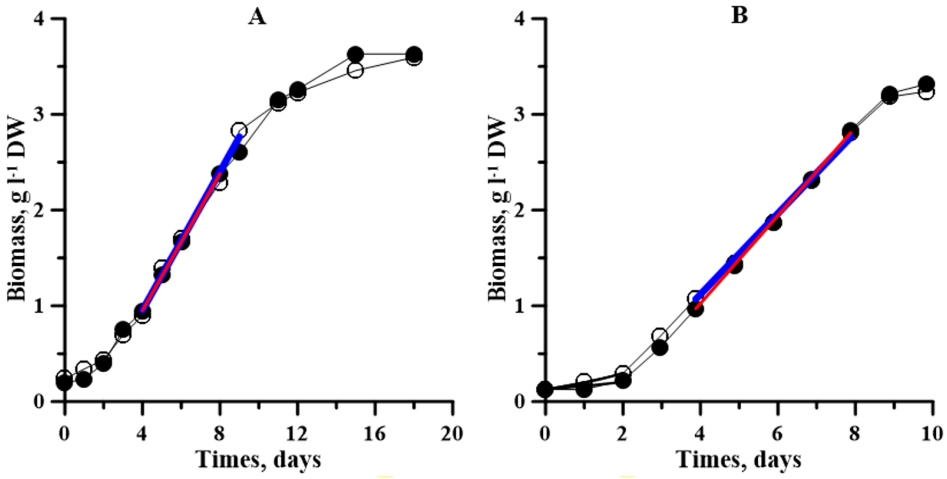

Fig. 1. Dynamics of *Porphyridium purpureum* (A) and *Tetraselmis viridis* (B) batch culture density when grown on a nutrient medium: ●, based on freshwater; ○, based on seawater. Solid lines are an approximation of the linear phase by equation (1) (red, based on freshwater; blue, based on seawater). The values of the coefficients (maximum productivity) are given in Table 1

Biomass productivity of *P. purpureum* and *T. viridis* at the linear growth phase when cultured on freshwater did not differ noticeably from that of corresponding cultures grown on seawater. Both maximum and mean growth rates were slightly higher in *T. viridis* compared to *P. purpureum* which seems to be due to individual characteristics of a culture. However, the specific growth rate of *T. viridis* when cultured on freshwater was lower by almost 2 times compared to the same parameter of the culture grown on seawater (Table 1). *T. viridis* culture was previously grown on an unmodified medium; accordingly, it can be assumed as follows: during the first two days, algal cells adapted to new conditions which is confirmed by some differences in the shape of the accumulation curve (Fig. 1B). In general, a change in *P. purpureum* and *T. viridis* density during batch cultivation on both freshwater and seawater had a unidirectional character, and the productivity of cultures was not much affected by a water base of a nutrient medium.

Table 1. Production characteristics of *Porphyridium purpureum* and *Tetraselmis viridis* batch cultures when grown on freshwater-based and seawater-based nutrient media

Culture	Water base of the nutrient medium	Specific growth rate, day^{-1}	Maximum productivity, $g \cdot L^{-1} \cdot day^{-1}$	Productivity at the linear growth stage, $g \cdot L^{-1} \cdot day^{-1}$	Total biomass growth, $g \cdot L^{-1}$
Porphyridium purpureum	Freshwater	0.45	0.36	0.27 ± 0.02	3.44 ± 0.23
	Seawater	0.35	0.36	0.29 ± 0.01	3.36 ± 0.02
Tetraselmis viridis	Freshwater	0.23	0.46	0.43 ± 0.01	3.19 ± 0.02
	Seawater	0.42	0.46	0.42 ± 0.006	3.11 ± 0.01

During batch cultivation, biochemical composition of resulting microalgae biomass is determined by many parameters, and the key ones are concentration of mineral nutrition elements and cultivation conditions. Therefore, the effect of replacing a water base of a nutrient medium with freshwater on synthesis rate and total pigment accumulation in *P. purpureum* and *T. viridis* was investigated as well. Under these conditions, *P. purpureum* growth was accompanied by a change in B-PE content both in the culture and its cells (Fig. 2).

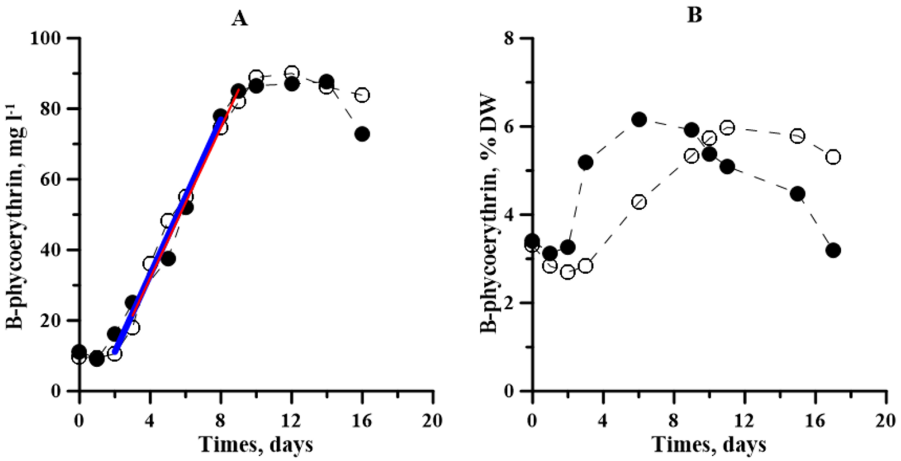

Fig. 2. B-phycoerythrin content in a batch culture (A) and in cells (B) of *Porphyridium purpureum* when grown on a nutrient medium: ●, based on freshwater; ○, based on seawater. Solid lines are an approximation of the linear phase (red, based on freshwater; blue, based on seawater). The values of the coefficients (maximum synthesis rate) are given in Table 2

B-PE content during *P. purpureum* cultivation on both freshwater and seawater gradually increased (9.2–9.5 times) and reached maximum values (88–90 mg·L^{-1}) on the 10th–14th day from the beginning of the experiment (Fig. 2A). A change in B-PE concentration in the culture was unidirectional for both variants of the medium, and the correlation coefficient was of 0.98. An increase in B-PE content in *P. purpureum* cells was less pronounced: it was about 2 times over 7 days and reached 6% of DW on average for each variant (Fig. 2B). We recorded a decrease in B-PE concentration in *P. purpureum* cells in the second half of batch cultivation; such a change has been reported by many researchers and explained by a direct dependence of pigment concentration on nitrogen content in a medium which can drop to minimum values by the final cultivation stage [Fuentes-Grunewald et al., 2015; Li T. et al., 2019]. Data characterizing the rate of B-PE accumulation in the algal culture when grown on freshwater- and seawater-based nutrient media are provided in Table 2.

Table 2. B-phycoerythrin (B-PE) production characteristics of *Porphyridium purpureum* batch culture when grown on freshwater-based and seawater-based nutrient media

Water base of the nutrient medium	Specific synthesis rate (0–3rd days), day^{-1}	Maximum B-PE synthesis rate, mg·L^{-1}·day^{-1}	Mean B-PE synthesis rate (2nd–9th days), mg·L^{-1}·day^{-1}	Total B-PE accumulation, mg·L^{-1}
Freshwater	0.3	12.1	9.8 ± 1.75	77.9 ± 4.7
Seawater	0.2	12.3	10.2 ± 2.3	80.5 ± 5.4

The calculation results showed that the maximum rate of B-PE synthesis in *P. purpureum* culture both on freshwater and seawater was almost the same. The mean rate of B-PE synthesis at the linear stage and the level of pigment accumulation are slightly higher in the culture grown on seawater, but these differences are not significant (Table 2). Despite close values of the culture growth rate (Table 1) and observed rate of B-PE synthesis in two variants, a certain temporal discrepancy was recorded in dynamics of B-PE content in *P. purpureum* biomass (Fig. 2B). This can be explained by a noticeable difference between specific rates of pigment synthesis in two variants in the first three days of the experiment. During this period, the rate of pigment synthesis in a culture grown on freshwater with sea salt added was 1.5 times higher compared to that of a culture grown on seawater. In this case, B-PE could accumulate in *P. purpureum* cells much faster not only in the first day, but also in the next few days.

T. viridis growth on media both with seawater and freshwater was also accompanied by changes in pigment content (Fig. 3). Chlorophyll *a* and chlorophyll *b* concentration during *T. viridis* cultivation (on the 4th–10th days) on both freshwater and seawater gradually rose and reached maximum values by the end of the experiment: an increase by 2.4–3 and 1.7–2.2 times was registered for chlorophyll *a* and chlorophyll *b*, respectively (Fig. 3A, B). A change in content of these pigments in the culture also was unidirectional for two media, and the correlation coefficient was of 0.99. For total carotenoids, a gain in pigment concentration in the culture was recorded as well, but these changes were expressed to a lesser extent than changes in chlorophyll content (Fig. 3C). At comparable density of *T. viridis* culture for two variants, pigment concentration throughout the entire experiment was higher in the culture grown on seawater.

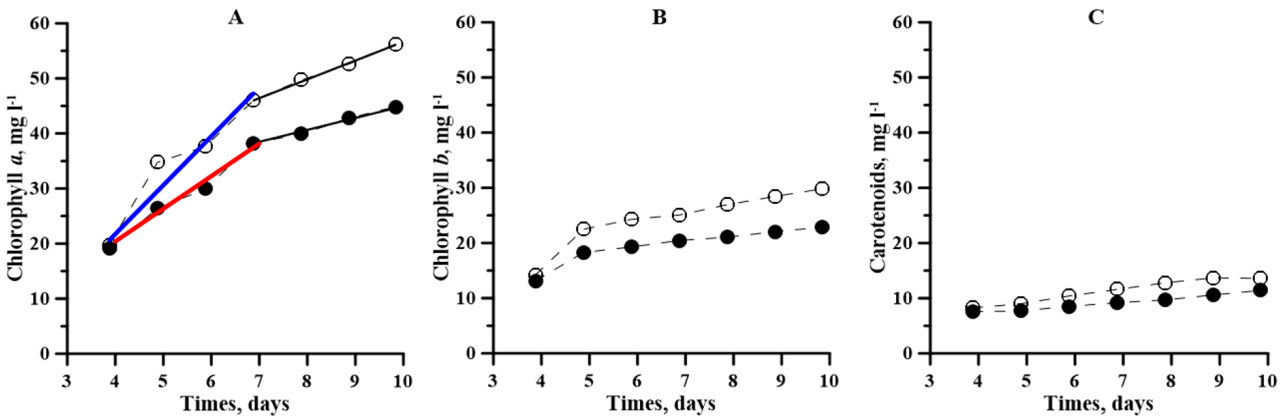

Fig. 3. Content of chlorophyll *a* (A), chlorophyll *b* (B), and carotenoids (C) in *Tetraselmis viridis* batch culture when grown on a nutrient medium: ●, based on freshwater; ○, based on seawater. Solid lines are an approximation of the linear phase (red, based on freshwater; blue, based on seawater). The values of the coefficients (maximum synthesis rate) are given in Table 3

Data characterizing features of pigment accumulation in *T. viridis* batch culture when grown on freshwater and seawater are provided in Table 3.

Table 3. Production characteristics of *Tetraselmis viridis* batch culture for chlorophyll *a*, chlorophyll *b* (chl *a* and chl *b*), and total carotenoids (car) when grown on freshwater-based and seawater-based nutrient media

Water base of the nutrient medium	Maximum pigment synthesis rate, mg·L^{-1}·day^{-1}			Pigment synthesis rate at the linear stage, mg·L^{-1}·day^{-1}			Total pigment accumulation, mg·L^{-1}		
	chl *a*	chl *b*	car	chl *a*	chl *b*	car	chl *a*	chl *b*	car
Freshwater	6.38	–	–	2.16 ± 0.53	0.96 ± 0.11	0.71 ± 0.12	25.77 ± 0.08	9.74 ± 0.62	3.44 ± 0.22
Seawater	8.76	–	–	3.41 ± 0.34	1.47 ± 0.29	1.23 ± 0.21	36.46 ± 1.27	15.60 ± 1.47	5.50 ± 0.04

Maximum and mean synthesis rates of chlorophyll *a* and synthesis rates of chlorophyll *b* and total carotenoids in *T. viridis* batch culture when grown on the seawater-based nutrient medium were more than 1.5 times higher than in case of the freshwater-based one. Such noticeable differences resulted in an increase in pigment accumulation in *T. viridis* culture grown on seawater compared

to that on freshwater by 1.4–1.6 times. Thus, the mean growth rate of *T. viridis* cultured on freshwater was not significantly different from that of the alga cultured on seawater, but higher rates of pigment synthesis and total accumulation were recorded in the culture grown on seawater.

DISCUSSION

Marine microalgae are traditionally cultured on seawater-based nutrient media [Fuentes-Grunewald et al., 2015; Kathiresan et al., 2006; Raes et al., 2013; Strizh et al., 2004]. However, there are data on cultivation of some species of marine microalgae on freshwater-based nutrient media [Gargouch et al., 2018; Lelekov et al., 2016]. Specifically, a marine microalga *Porphyridium marinum* was grown on freshwater-based nutrient medium [Gargouch et al., 2018]. The maximum density of the culture was $(4.6 \pm 0.5) \times 10^6$ cells·mL^{-1}, and B-PE content in biomass was 15.9 mg·g^{-1} in terms of DW. The maximum amount of B-PE in biomass (41 mg·g^{-1}) was registered at the second stage: with an increase in $NaNO_3$ concentration by 2 times, a drop in K_2HPO_4 content to 0 g·L^{-1}, a decrease in light intensity by 2 times, and a rise in volume of micronutrient solution by 1.5 times. However, the density of *P. marinum* batch culture, its productivity, and B-PE content in biomass (at its maximum concentration) are significantly lower than those in the experiment carried out. Moreover, the use of NaCl required adding Mg and Ca naturally contained in seawater, and the application of a two-stage cultivation mode noticeably enlarged the time of cultivation [Gargouch et al., 2018].

In our experiment, when growing *P. purpureum* and *T. viridis* on freshwater-based media, the biomass yield exceeded 3 g of DW *per* 1 L of culture, and the mean growth rate was within 0.3–0.4 g·L^{-1}·day^{-1}. These values are comparable with data obtained earlier, when *P. purpureum* and *Dunaliella salina* (Dunal) Teodoresco, 1905 were cultured on seawater: the mean productivity at the linear growth phase was of 0.37 and 0.2 g·L^{-1}·day^{-1}, respectively [Gudvilovych, Borovkov, 2017; Gudvilovich et al., 2021]. Also, these values are similar to results reported in [Li T. et al., 2019]. Moreover, our data are comparable to production parameters of *T. viridis* when grown on artificial seawater with NaCl concentration of 29 g·L^{-1}: the maximum density of the culture by the 8th day was about 12×10^6 cells·mL^{-1} [Strizh et al., 2004]. Besides, a diatom *Phaeodactylum tricornutum* Bohlin, 1897 was shown to be culturable on a freshwater-based nutrient medium with sea salt added [Lelekov et al., 2016]. In this case, the mean growth rate was of 0.3 g·L^{-1}·day^{-1} which is also comparable to results obtained in our experiment. Notably, *P. purpureum* and *T. viridis* batch cultures were grown without additional carbon: an approved technique of atmospheric air bubbling was applied [Gudvilovych, Borovkov, 2017; Gudvilovich et al., 2021].

Despite the fact that the growth rate of *T. viridis* cultured on freshwater did not differ significantly from the growth rate of this alga on seawater, this variant showed lower rates of pigment synthesis and their total accumulation which may indicate certain non-optimality of cultivation conditions. Apparently, the factor negatively affecting synthesis of *T. viridis* pigments in this case could be an elevated pH level. Throughout the entire experiment, pH of the culture grown on freshwater was higher, by 5% on average, than for the culture grown on seawater. Starting from the 5th day of *T. viridis* culturing on freshwater, when pH values were close to 10, a decrease in the rate of pigment synthesis and production was up to 30–40% in comparison with values for the alga grown on seawater. As known, pH of a medium rises during batch cultivation of microalgae, and its level is critical: it determines solubility and availability of CO_2 and nutrients and noticeably affects microalgae metabolism [Chen, Durbin, 1994; Kumar, Saramma, 2018; López-Elías et al., 2008; Qiu et al., 2017].

Specifically, the effect of pH on growth and biochemical composition of *Dunaliella bardawil* and *Chlorella ellipsoidea* was studied. As shown, a change in pH shifts the direction of biosynthesis in microalgae cells thus affecting biochemical composition of biomass obtained [Khalil et al., 2010]. Both microalgae were capable of growing over a wide pH range (4–9 for *D. bardawil* and 4–10 for *C. ellipsoidea*); however, biomass production in *D. bardawil* reached its maximum at pH of 7.5, and in *C. ellipsoidea*, at pH of 9 [Khalil et al., 2010]. For both species analyzed, the highest values of accumulation of chlorophyll *a*, chlorophyll *b*, and carotenoids were recorded at pH of 7.5. As pH increased (shifted towards alkaline side), content of these three pigments dropped significantly. Importantly, a noticeable decrease in content of all pigments for both *D. bardawil* and *C. ellipsoidea* (more than 2-fold and by 30%, respectively) was observed against the backdrop of pH rise from 9 to 11 which correlates with data obtained in our experiment with *T. viridis*.

Most species used in aquaculture require pH within 6–9. However, various microalgae species have their own optimal pH ranges for biomass production, often strain-specific ones [Khalil et al., 2010; Qiu et al., 2017]. A comparative assessment of production characteristics for *P. purpureum* and *T. viridis* revealed no noticeable differences in the growth rate of the batch cultures when grown on freshwater- and seawater-based nutrient media. Moreover, when using freshwater as a base for *P. purpureum* culturing, we recorded no significant differences in maximum and mean rates of B-PE synthesis and in the level of pigment accumulation in the culture. In general, all these characteristics correspond to similar parameters of B-PE biosynthesis in a culture grown on seawater, both in investigations carried out earlier and in the described experiment [Gudvilovich et al., 2021; Li T. et al., 2019].

Above-mentioned data on the experience of cultivation of *P. purpureum*, *T. viridis*, and *Ph. tricornutum* [Lelekov et al., 2016] representing different systematic groups show the need for further studies on growing other marine microalgae on freshwater-based nutrient media with sea salt added.

Conclusion. As shown, microalgae *Porphyridium purpureum* and *Tetraselmis viridis* can be successfully cultured without the use of natural seawater. Importantly, the water base of a nutrient medium does not significantly affect production parameters of these two species; in the case of *P. purpureum*, it has no effect on the rate of B-phycoerythrin synthesis and its content in both culture and biomass. When culturing *T. viridis*, a possibility of monitoring pH and adjusting it to an optimal level should be considered. The use of freshwater instead of natural seawater, no need for adding mineral salts to prepare artificial seawater, and no need for adding carbon dioxide allow maintaining high growth rate of marine microalgae cultures. It noticeably reduces labor costs and biomass production costs and also expands geographical perspectives for their mass cultivation.

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РОСТ КУЛЬТУР МОРСКИХ МИКРОВОДОРОСЛЕЙ *PORPHYRIDIUM PURPUREUM* И *TETRASELMIS VIRIDIS* НА МОДИФИЦИРОВАННЫХ ПИТАТЕЛЬНЫХ СРЕДАХ

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Морские виды микроводорослей, которые способны синтезировать широкий спектр биологически активных веществ, в настоящее время считают наиболее перспективными источниками таких соединений. Большинство питательных сред для культивирования микроводорослей приготавливают на основе природной или искусственной морской воды. Представляется перспективной модификация питательной среды для выращивания морских микроводорослей путём замены её водной основы с природной морской воды на пресную. Альгологически чистые культуры морских микроводорослей *Porphyridium purpureum* и *Tetraselmis viridis* выращивали, заменяя стерильную морскую воду на пресную, в которую добавляли морскую соль до концентрации 18 и 28 г·л$^{-1}$ для *T. viridis* и *P. purpureum* соответственно. На основании полученных экспериментальных данных определены продукционные характеристики накопительных культур *P. purpureum* и *T. viridis* при их выращивании на пресной и морской водной основе питательной среды. В целом изменение плотности культур *P. purpureum* и *T. viridis* при накопительном культивировании как на пресной, так и на морской воде имело однонаправленный характер (коэффициенты корреляции в обоих случаях 0,99), а водная основа питательной среды не оказывала существенного влияния на скорость их роста. Показано, что выход биомассы *P. purpureum* и *T. viridis* при использовании пресной воды в качестве основы питательной среды составляет 3,2–3,4 г с 1 л культуры (в пересчёте на сухое вещество) и в основном соответствует аналогичному параметру культур, выращенных с применением морской воды. Несмотря на то, что средняя скорость роста *T. viridis* при выращивании на пресной воде

существенно не отличалась от скорости роста культуры на морской воде, отмечены повышенные средние скорости синтеза пигментов и их суммарное накопление у культуры, выращиваемой на морской воде. Для *P. purpureum* водная основа питательной среды не оказывала заметного влияния на такие характеристики, как скорость синтеза B-фикоэритрина и содержание этого пигмента в культуре и в биомассе микроводоросли. Результаты работы показывают, что культуры морских микроводорослей *P. purpureum* и *T. viridis* можно успешно выращивать без использования природной морской воды, что существенно снижает трудозатраты и себестоимость получаемой биомассы, а также расширяет географические перспективы их массового культивирования.

Ключевые слова: морские микроводоросли, *Porphyridium purpureum*, *Tetraselmis viridis*, пресная вода, продуктивность, биомасса, пигменты