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**ESTIMATION OF THE PRODUCTION CHARACTERISTICS  
AND CHEMICAL COMPOSITION OF *SCENEDESMUS RUBESCENS*  
WITH VARYING AVAILABILITY OF MINERAL SUBSTRATE**

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Biotechnology of microalgae is a promising field for obtaining renewable sources of biomass rich in proteins, lipids, and pigments; this makes the search for optimal cultivation conditions highly relevant. Representatives of the genus *Scenedesmus* Meyen, 1829 feature rapid growth, resistance to environmental changes, and high content of biologically active compounds, which determines their potential for use in the food, pharmaceutical, and energy industries. The aim of the study was to analyze the growth performance and photosynthetic pigment content in a culture of *Scenedesmus rubescens* (P. J. L. Dangeard) E. Kessler, M. Schafer, C. Hummer, A. Kloboucek & V. A. R. Huss, 1997 on three types of mineral nutrient media: complete Tamiya medium, its half-strength modification (Tamiya ½), and Bold's basal medium (BBM) (variants No. 1, 2, and 3, respectively). In the experiment, *S. rubescens* was cultured for 16 days under continuous illumination and additional carbon dioxide supply. On BBM and Tamiya medium, a high specific growth rate ( $\mu$ ) was recorded: 0.48 and 0.49 day<sup>-1</sup>, respectively. When using Tamiya ½ nutrient medium, *S. rubescens* specific growth rate was lower: 0.33 day<sup>-1</sup>. Linear growth on all three media was observed up to the 4<sup>th</sup> day. During this period, the cell density of *S. rubescens* in experimental variants No. 1 and 3 increased nearly 7-fold, and in variant No. 2, by 3.7 times. The maximum productivity of *S. rubescens* for the experimental variants No. 1, 2, and 3 was 0.35, 0.18, and 0.31 g dry weight·L<sup>-1</sup>·day<sup>-1</sup>, respectively, with no morphological changes in cells. Significant differences were revealed in the biochemical and kinetic growth characteristics of *S. rubescens* cultured on the three nutrient media. High concentrations of chlorophyll *a* and *b* were recorded in cells grown on Tamiya medium and BBM. The obtained results indicate very similar growth characteristics of *S. rubescens* for variants No. 1 and 3 during the exponential and linear growth phases. This suggests that cultivation on BBM can provide productivity and accumulation of valuable compounds comparable to those reached on Tamiya medium.

**Keywords:** *Scenedesmus rubescens*, Bold's basal medium, Tamiya nutrient medium, batch culture, productivity, photosynthetic pigments

Microalgae biotechnology contributes much to solving global problems related to the population growth, environmental pollution, depletion of fossil fuel reserves, and need for sustainable food and energy resources. As a renewable source of a range of biologically active substances with numerous potential applications, microalgae offer enormous opportunities for innovation. In other words, microalgae attract attention due to the multifunctionality of products obtained and significant potential for use in various industries (in land reclamation, as well as in the production of dietary supplements, feed, fuel, and cosmetics) [Levasseur et al., 2020; Suresh, Benor, 2020].

The growth rate of microalgae allows for the production of enormous amounts of biomass (10–20 times higher than that of corn or soybeans *per unit area*), with a high content of proteins, pigments, lipids, and microelements. The biochemical composition of biomass can be controlled by changing the physical and chemical parameters of the habitat. Representatives of the genus *Scenedesmus* Meyen, 1829 (the class Chlorophyceae) are often revealed in freshwater bodies. These immobile colonial green microalgae consist of cells about 8  $\mu\text{m}$  wide and 14  $\mu\text{m}$  long and are of high nutritional value. Many species of this genus are used worldwide in various commercial projects because of their rapid growth, ease of cultivation, and ability to adapt to different environmental conditions.

The nutritional value of *Scenedesmus* green microalgae is due to high content of protein, polyunsaturated fatty acids, vitamins, and biologically active compounds [Chu, 2012]. Interestingly, each species has its own ratio of lipids, carbohydrates, and proteins [Nur et al., 2014]. *Scenedesmus rubescens* (P. J. L. Dangeard) E. Kessler, M. Schafer, C. Hummer, A. Kloboucek & V. A. R. Huss, 1997 was used as a model organism for the production of  $\beta$ -carotene, omega-3 fatty acids, and glycerol [Jo et al., 2020]; those are important in combating various diseases, *inter alia* cardiovascular pathologies, chronic inflammation, atherosclerosis, and cancer, as well as in delaying aging processes.

*Scenedesmus* biomass is rich in chlorophylls and carotenoids which can significantly improve overall health of humans and animals. These pigments effectively inhibit microbial growth; they are antioxidants; and they promote the health of eyes and skin, and boost the immune system. The pigments are of great importance in the food industry, as they expand the possibilities for the functional agriculture and improve nutritional profiles of various products [Fan et al., 2013; Guedes et al., 2013; Ishaq et al., 2016; Patil, Kaliwal, 2019]. Due to this fact, *Scenedesmus* algae have significant commercial potential for use in medicine and in food and cosmetics industries. In addition, *Scenedesmus* biomass is one of the main live feeds that ensure the proper development of hydrobionts: rotifers, zooplankton representatives, as well as fish and shrimp larvae [Mayeli et al., 2004; Rudenko, Tkacheva, 2021].

However, mass production of microalgae involves overcoming many obstacles. One of the problems is the high cost of macro- and microelements for culture medium preparation: according to data provided in [Fasaei et al., 2018], it accounts for about 34% of the total production cost, while harvesting cost is about 20–30%. While problems associated with biomass harvesting can be solved by using efficient and low-cost methods, such as flocculation [Rahman et al., 2022], the search for a productive, sustainable, and cost-effective culture medium remains crucial for reducing the overall cost of microalgal production. The results of many experimental studies, including our own, have shown that *Scenedesmus* representatives can noticeably boost their growth rate when mineral components of nutrient media are partially or completely replaced with wastewater [Ishaq et al., 2016; Muluye et al., 2021]. This can really cut down the cost of media preparation. However, in most cases, it is advisable to use microalgal biomass obtained in this way to extract valuable substances, to fertilize agricultural crops, or to make a feed supplement for animals (not for humans) [Gorbunova, Gudvilovich, 2020; Gorbunova, Zubko, 2010].

When organizing production of microalgae, not only their productivity is important, but also quality of the obtained yield. This fact governs the search for the optimal nutrient medium for *Scenedesmus*. To date, various types of nutrient media have been described in literature and are actively used worldwide for the cultivation of microalgae of the family Scenedesmaceae. These are Uspensky medium, Bold's basal medium (BBM), Chu's media No. 10 and 13, Tamiya medium, Tamiya 1/2 medium, Šetlík medium, and BG-11 medium. Although all of them are suitable for *Scenedesmus* culturing, the growth rate of a microalga, its yield, and pigment content can vary significantly due to different composition and concentration of chemical compounds used for media preparation.

Thus, rapid growth, high concentration of antioxidants, and physiological plasticity make *Scenedesmus* representatives a promising target for biotechnology. However, it is possible to realize the commercial potential only by improving strategies and optimizing microalgae cultivation parameters, and these are the key goals of researchers. The aim of this study was to analyze the growth characteristics and pigment content in the culture of *Scenedesmus rubescens* (Chlorophyta: Scenedesmaceae, Scenedesmus) grown on three mineral media: Tamiya, Tamiya ½, and BBM.

## MATERIAL AND METHODS

The object of the study was the green microalga *S. rubescens*, strain IBSS-91 from IBSS core facility “Collection of Hydrobionts of the World Ocean” (Sevastopol). The microalga was grown on three nutrient media: Tamiya [Tamiya, 1957], Tamiya ½ [Hase et al., 1957; Sandmann et al., 2022], and BBM [Bischoff, Bold, 1963] (hereinafter variants No. 1, 2, and 3 respectively). These media differ from each other not only in composition and levels of concentration of chemical compounds, but also in the cost (Table 1).

**Table 1.** Average market cost of mineral nutrient media for industrial cultivation of *Scenedesmus rubescens*

Chemical compound	Price per 1 kg, rubles	Cost of a reagent for preparation of 1,000 L of the medium, rubles		
		Tamiya medium	Tamiya ½ medium	BBM
NaNO <sub>3</sub>	285	–	–	71.25
KNO <sub>3</sub>	357	1,785	892.5	–
MgSO <sub>4</sub> ·7H <sub>2</sub> O	134	335	167.5	10.05
NaCl	95	–	–	2.38
K <sub>2</sub> HPO <sub>4</sub>	693	–	–	52
KH <sub>2</sub> PO <sub>4</sub>	534	667.5	333.75	93.45
CaCl <sub>2</sub> ·2H <sub>2</sub> O	172	–	–	4.3
Na <sub>2</sub> EDTA·2H <sub>2</sub> O	790	29.2	14.6	39.5
FeSO <sub>4</sub> ·7H <sub>2</sub> O	433	1.3	0.65	2.15
KOH	254	–	–	7.85
H <sub>3</sub> BO <sub>3</sub>	344	0.98	0.5	0.39
MnCl <sub>2</sub> ·4H <sub>2</sub> O	865	1.57	0.78	1.25
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	365	0.08	0.04	3.25
MoO <sub>3</sub>	2,500	0.04	0.02	1.8
NH <sub>4</sub> VO	9,400	0.22	0.11	–
CuSO <sub>4</sub> ·5H <sub>2</sub> O	555	–	–	0.87
Co(NO <sub>3</sub> ) <sub>2</sub> ·5H <sub>2</sub> O	2,376	–	–	1.16
In total		2,822	1,411	292

For each variant, the experiment was conducted in three replicates. The nutrient media were prepared on distilled water. *S. rubescens* was pre-adapted to each medium for 5 days. The microalga was grown in 5 cm thick plane-parallel glass cultivators [Trenkenshu et al., 2017]. The working volume of the cultivator was 3 L. The culture was grown under continuous illumination; a light grid consisting of 18-W fluorescent lamps was used. The light intensity on the surface of the photobioreactors was recorded using

a LI-250A light meter with a photometric sensor (Li-COR, the USA). The mean illumination was 10 klx. The cultures were bubbled with air; a Hailea ACO-308 air compressor pump was used. The mean air flow rate was about 1 L·L<sup>-1</sup> of culture *per* minute (with additional carbon dioxide supply to stabilize pH and introduce a carbon source into the medium). An Aqua Medic pH controller (Germany) was used to maintain the optimal pH level, 6.5–7, in the cultivators. The temperature was stabilized in a range of +28...+30 °C. In order to compensate for evaporation, water volume was maintained in the cultivators throughout the experiment by adding distilled water to a 3-L mark once a day before measurements. The water volume we added averaged 60 mL.

To inoculate experimental cultivators, a culture pre-adapted to a specific medium was used, with an initial density of 0.16 g dry weight·L<sup>-1</sup>.

The culture density was assessed by an optical method. The optical density of the microalgal culture (D) was calculated using the formula:

$$D = -\lg(T) , \quad (1)$$

where T is the transmittance value determined with a Unico photometer at a wavelength of 750 nm in cuvettes with a working length of 0.5 cm (an absolute error did not exceed 1.0%).

The cuvettes were placed as close as possible to the photodetector to reduce the effect of light scattering on the instrument readings.

The optical density units were converted to microalgal dry weight (DW) according to the equation:

$$DW = k \times D_{750} , \quad (2)$$

where k is an empirically determined conversion factor equal to 0.77 g·L<sup>-1</sup>·D<sup>-1</sup>.

To determine k, a series of samples was taken from all the experimental cultivators. The series included at least three replicates, 15 mL each. For each sample, optical density (D) and dry weight were measured in parallel. *Prior* to determining D, the microalgal suspension in a sample was thoroughly mixed. To measure dry weight, an aliquot of the suspension (10 mL) was placed in test tubes pre-heated to constant weight in a drying oven at (+105 ± 3) °C for 24 h. Using a laboratory portable centrifuge OPn-3, with a maximum separation factor of  $g = 1.870$  at 3,000 rpm, cells were precipitated for 15 min, and then the supernatant was carefully decanted. Microalgal cells were washed from salts with the same volume of distilled water and centrifuged again. At the final stage, the test tubes with biomass were dried in an oven at (+105 ± 3) °C for 24 h. The difference between the initial and final weight of the test tubes was used to determine dry weight of *S. rubescens* in the filtered volume; then, it was calculated to 1 L.

The specific growth rate ( $\mu$ ) was assessed by approximating the biomass growth curve in the exponential phase using the formula:

$$\mu = \frac{\ln B_2 - \ln B_1}{t_2 - t_1} , \quad (3)$$

where  $\ln B_1$  and  $\ln B_2$  are biomass values at the beginning and end of the exponential growth phase;

$t_1$  and  $t_2$  are time values at the beginning and end of the exponential growth phase [Trenkenshu, 2019].

The maximum productivity of the culture ( $P$ ) for each experimental variant was calculated by approximating the biomass growth curve in the linear growth phase by the formula:

$$P = \frac{B_2 - B_1}{t_2 - t_1}, \quad (4)$$

where  $B_1$  and  $B_2$  are biomass values at the beginning and end of the linear growth phase [Trenkenshu, 2005].

The culture yield for each experimental variant ( $H$ ) was determined using the formula:

$$H = B_{max} - B_0, \quad (5)$$

where  $B_0$  is a biomass value at the beginning of the experiment;

$B_{max}$  is the maximum biomass during the experiment [Lelekov, Trenkenshu, 2007].

The condition of *S. rubescens* cells and the purity of the microalgal culture were monitored under a Carl Zeiss Axiostar Plus light microscope (Germany) at magnification of 400×.

The content of pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) was determined by spectrophotometry. Samples were taken in triplicate on the 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 10<sup>th</sup>, and 16<sup>th</sup> days of the experiment after thorough culture mixing. The suspension was centrifuged for 10 min at 3,000 rpm; the supernatant was decanted; and the precipitated biomass was frozen for 24 h and used for determining pigments. The latter ones were extracted from microalgal cells with 100% pure acetone (“EKOS-1” JSC, Russia). Absorption spectra of acetone extracts were recorded on a SF-2000 spectrophotometer (“OKB SPECTR” LLC, Russia) in a range of 400–800 nm in quartz cuvettes with an optical path length of 1 cm.

Concentrations of chlorophyll *a* and *b* and the total content of carotenoids were calculated using the formulas proposed by [Wellburn, 1994], based on the optical density ( $D$ ) values at wavelengths corresponding to the absorption maxima of these pigments:

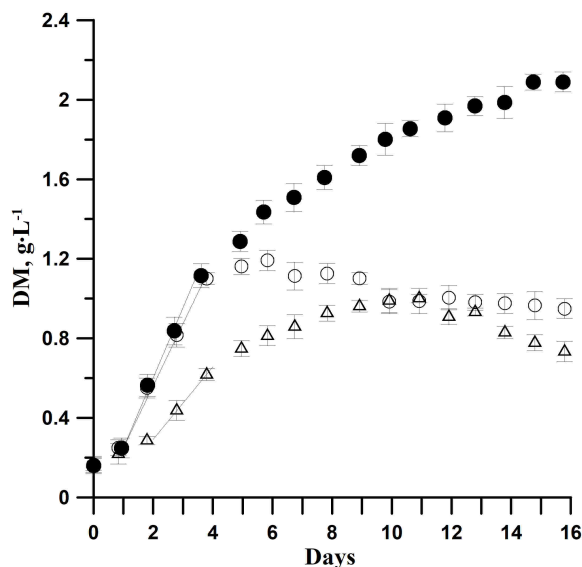
$$\begin{aligned} Chl\ a &= 11.75 \times D_{662} - 2.35 \times D_{645}; \\ Chl\ b &= 18.61 \times D_{645} - 3.96 \times D_{662}; \\ Carotenoids &= (1,000 \times D_{470} - 2.7 \times Chl\ a - 81.4 \times Chl\ b)/227. \end{aligned} \quad (6)$$

We determined arithmetic means ( $\bar{x}$ ), standard deviations ( $S$ ), standard errors of a mean, and confidence intervals for a mean ( $\Delta\bar{x}$ ). Calculations involved LibreOffice and SciDAVis software; a significance level  $\alpha$  was 0.05. The tables show mean values and established confidence intervals ( $\bar{x} \pm \Delta\bar{x}$ ) for three replicates.

## RESULTS

For 16 days, the microalga was grown on three types of nutrient media in three laboratory cultivators under continuous illumination. The dynamics of microalgal culture density is described by cumulative curves (Fig. 1).

The specific growth rate ( $\mu$ ) on Tamiya nutrient medium and BBM was calculated for the microalga based on biomass concentrations during the exponential phase [see Equation (3)]. The data on determined kinetic characteristics for *S. rubescens* during the cultivation on three media is presented in Table 2.



**Fig. 1.** Dynamics of the density of *Scenedesmus rubescens* batch culture when grown on Tamiya nutrient medium (●), BBM (○), and Tamiya ½ nutrient medium (△)

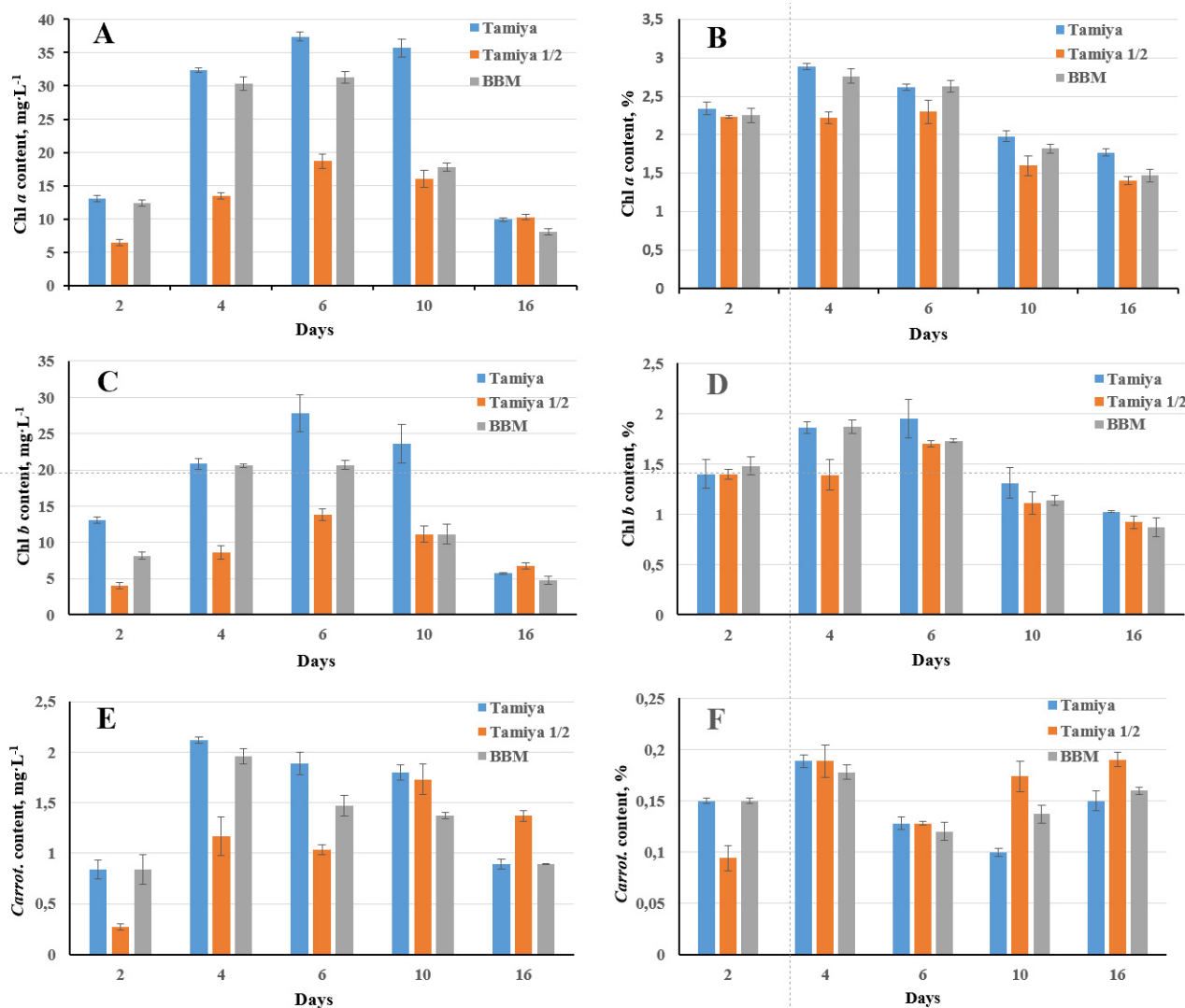
**Table 2.** Kinetic characteristics of *Scenedesmus rubescens* growth

Parameter	Tamiya medium	Tamiya ½ medium	BBM
Biomass yield, g dry weight·L <sup>-1</sup>	1.94 ± 0.06	0.84 ± 0.02	1.03 ± 0.04
Maximum productivity, g dry weight·L <sup>-1</sup> ·day <sup>-1</sup>	0.35 ( $R^2 = 0.97$ )	0.18 ( $R^2 = 0.98$ )	0.31 ( $R^2 = 0.99$ )
Specific growth rate, day <sup>-1</sup>	0.48 ( $R^2 = 0.99$ )	0.31 ( $R^2 = 0.99$ )	0.49 ( $R^2 = 0.99$ )

For all the experimental variants, *S. rubescens* linear growth was recorded from the 1<sup>st</sup> to the 4<sup>th</sup> day. Biomass values for this period were used to calculate the growth rate of the alga. After 6 days, the growth of the culture on BBM stopped, and the phase of the microalgal death began. By that point, the biomass increase was 1.03 g·L<sup>-1</sup>. Notably, during the linear growth phase on Tamiya ½ medium, *S. rubescens* growth rate was almost twice as low as that for the other variants. On the 10<sup>th</sup> day of the experiment, the density of the cultures in variants No. 2 and 3 became equal. Then, the phase of the microalgal death was recorded on Tamiya ½ medium.

Biochemical analysis of the microalgal biomass when grown on complete Tamiya nutrient medium showed as follows: chlorophyll *a* content in its cells rose during cultivation from 2.34% at the beginning of the linear growth phase to 2.62% (Fig. 2). Then, the concentration of this pigment gradually decreased; a minimum value of 1.77% was noted on the 16<sup>th</sup> day of the experiment.

When using Tamiya ½ nutrient medium, the dynamics of chlorophyll *a* in microalgal cells was similar, but the pigment concentration was 2 times lower. When culturing *S. rubescens* on BBM, chlorophyll *a* content increased from 2.25 to 2.63%. At the final stage of the experiment, when the microalga reached the stationary growth phase and the phase of death, chlorophyll *a* concentration values for the three variants, calculated *per* 1 L of culture, were almost equal.



**Fig. 2.** Dynamics of pigment accumulation: A, C, E, in *Scenedesmus rubescens* culture when grown on Tamiya nutrient medium, Tamiya  $\frac{1}{2}$  nutrient medium, and BBM,  $\text{mg}\cdot\text{L}^{-1}$ ; B, D, F, in the dry biomass of the microalga, %

## DISCUSSION

The choice of three media – Tamiya, Tamiya  $\frac{1}{2}$ , and BBM – is governed by the difference in composition and the wide range of concentrations of nutrients needed for their preparation. Thus, Tamiya nutrient medium is one of the most concentrated mineral media used to obtain intensive, dense cultures of green microalgae. In contrast, BBM requires a low mineral content. As an intermediate option in terms of concentration, Tamiya  $\frac{1}{2}$  medium was used: the media with half the nutrient composition.

Exponential growth of *S. rubescens* on Tamiya nutrient medium and BBM was observed on the 1<sup>st</sup> day, and on Tamiya  $\frac{1}{2}$  medium, within the first 2 days. As known, the specific growth rate remains constant during this period and reaches its maximum [Lelekov, Trenkenshu, 2007]. When cultured on BBM and Tamiya nutrient medium, *S. rubescens* was characterized by high specific growth rate, 0.48 and 0.49  $\text{day}^{-1}$ , while on Tamiya  $\frac{1}{2}$  medium, the value was 0.33  $\text{day}^{-1}$ . In [Nur et al., 2014], *Scenedesmus* specific growth rate on BBM was  $(0.22 \pm 0.04) \text{ day}^{-1}$ , which is 2.2 times lower than the values we obtained.

As already mentioned, *S. rubescens* linear growth was registered until the 4<sup>th</sup> day for all the variants. The growth rate of the microalga on Tamiya nutrient medium and BBM significantly exceeded that on Tamiya ½ medium. Over 4 days of our experiment, the microalgal biomass in variants No. 1 and 3 increased almost 7-fold, while in variant No. 2, it rose 3.7-fold. The maximum productivity of *S. rubescens* for variants No. 1, 2, and 3 was 0.35, 0.18, and 0.31 g dry weight·L<sup>-1</sup>·day<sup>-1</sup>, respectively. Importantly, at this stage, no changes in cell morphology were recorded in any of the experimental variants. From the 5<sup>th</sup> to the 9<sup>th</sup> day, linear growth of the culture was observed with a smaller slope of the accumulation curve for Tamiya and Tamiya ½ media. This seems to result from the shift in the growth-limiting factor.

Within a linear section, the growth rate of microalgae is known to be determined by the amount of light flux or the content of carbon dioxide: those are completely absorbed by a culture, and this results in a decrease in productivity. However, given that in our experiment, the culture was continuously bubbled with a CO<sub>2</sub>-containing gas-air mixture, it can be assumed that its content in a medium was sufficient to maintain the optimal level of photosynthesis and microalgal growth. Thus, carbon limitation of *S. rubescens* growth during this period can be ruled out. Apparently, in variants No. 2 and 3, the intensive growth of the microalga was limited by a lack of biogenic elements.

During the entire period of *S. rubescens* cultivation on BBM, the highest density was 1.2 g·L<sup>-1</sup>, and this value exceeds the maximum for *Scenedesmus* sp. reported in [Muluye et al., 2021]: 0.75 g·L<sup>-1</sup> after 7 days of cultivation on BBM without additional CO<sub>2</sub> supply. The authors also determined the maximum productivity of *Scenedesmus* sp.: 107 mg·L<sup>-1</sup>·day<sup>-1</sup>, which is 3 times lower than the value we obtained. Such a difference in kinetic characteristics of microalgal growth can be explained by the additional carbon dioxide supply to the culture in our experiments, while pH of media in all the variants did not increase and remained within a range of 6.5–7.

The acidity of a culture medium is very important, as pH level determines availability of CO<sub>2</sub> and nutrients for a microalga, and also significantly affects its metabolism. At high pH values, carbonates are formed, and this limits the availability of carbon dioxide; as a result, we are dealing with a drop in the efficiency of photosynthesis and suppression of microalgal cell growth. As known, for each species, the optimal pH level lies within a narrow range and usually depends on both the alga itself and its specific strain [Drira et al., 2017]. This is due to the physiological characteristics of microalgae, which determine their ability to adapt to different environmental conditions. For most freshwater algae, including *Chlorella vulgaris* Beijerinck, 1890 and *S. rubescens*, pH optimum is between 7 and 8. High CO<sub>2</sub> content (> 5%) can negatively affect the rate of substrate and light absorption by a microalga and, accordingly, its growth rate and cell morphology. For example, the studies [Jena et al., 2012; Zhang et al., 2022] showed that elevated levels of carbon dioxide contributed to the formation of larger colonies of *Scenedesmus obliquus* (Turpin) Kützing, 1833. Thus, it is critical to maintain a required pH value during microalgae cultivation and have the ability to control it, as pH deviation from the optimal level can inhibit the growth or even lead to cell death. Since *S. rubescens* cultures were additionally supplied with CO<sub>2</sub>, and pH of the media was maintained at an optimal level, it can be stated that these factors did not affect the results of our study. The differences between the experimental variants were due solely to the composition of the nutrient media.

At the initial stage, in all the variants, most microalgal cells were bright green and had intact chloroplasts. Subsequently, in *S. rubescens* cells, morphological changes occurred; those appear to be related to the composition of the media used. Starting from the second week of cultivation on BBM, we observed

single cells with damaged chloroplasts. From the 6<sup>th</sup>–7<sup>th</sup> day of growth on Tamiya ½ medium, up to 10% of cells had partially fragmented chloroplasts. Starting from the 10<sup>th</sup> day of culturing on Tamiya ½ nutrient medium and BBM, the microalga became yellowish-green, which was likely to be caused by partial degradation of its pigment complex.

From the 5<sup>th</sup> day of the experiment, the content of carotenoids in *S. rubescens* cells when grown on Tamiya medium and BBM increased proportionally to a boost in biomass and then gradually decreased. The observed pattern of carotenoid accumulation in the microalga within the cultivation cycle is consistent with the results presented in [Bozhkov, Menzyanova, 1997]. When culturing *S. rubescens* on Tamiya ½ medium, with the transition of the culture to the stationary phase of growth, we noted a sharp increase in the concentration of carotenoids (see Fig. 2). As known, their elevated content in microalgae is usually related to various stress conditions. For example, when microalgae encounter a deficiency of essential nutrients (nitrogen, phosphorus, potassium, or magnesium), they can synthesize more carotenoids to protect their cells [Hu et al., 2013].

For variants No. 1 and 3, it is worth noting as follows: at the end of the linear growth phase, the cultures had equal values of biomass concentration, and the relative content of chlorophyll *a* and *b* was also characterized by almost equal values. Considering the fact that the cost of the complete Tamiya nutrient medium is an order of magnitude higher than that of BBM, it can be concluded that the use of BBM is economically feasible, but only if harvesting is organized on the 4<sup>th</sup>–5<sup>th</sup> day of batch cultivation, or if a quasi-continuous cultivation is ensured.

When culturing *S. rubescens* on Tamiya ½ medium, higher concentrations of carotenoids were recorded in its biomass during the final stages of growth than those for the other two experimental variants; however, this medium cannot be recommended for intensive *S. rubescens* cultivation for the purpose of obtaining biochemically valuable substances. It was found that the cost of preparation of Tamiya ½ nutrient medium was 5 times higher than that for BBM, while the growth rate of the microalga was 2 times lower.

After analyzing the dynamics of the relative content of carotenoids in *S. rubescens* cells grown on the three media, we revealed that their final concentration for Tamiya ½ medium was 35% higher than for variants No. 1 and 3. Considering that Tamiya ½ nutrient medium is less balanced and suitable for intensive cultivation of this species, the accumulation of carotenoids is assumed to be protective reaction of *S. rubescens* for unfavorable growth conditions. This fact once again highlights the optimal choice of Tamiya nutrient medium or BBM for intensive cultivation of this species.

The results obtained in the experiment indicate very similar growth characteristics of *S. rubescens* in variants No. 1 and 3 within the exponential and linear growth phases. This allows providing conditions that can ensure high productivity and accumulation of photosynthetic pigments on BBM, similar to those for the variant on Tamiya medium.

Based on the studies and calculations carried out, it can be concluded that the use of BBM is economically justified, if the full yield is harvested on the 4<sup>th</sup>–5<sup>th</sup> day of batch cultivation, or a quasi-continuous cultivation mode is used. To confirm, it is necessary to test *S. rubescens* cultivation on BBM in a quasi-continuous mode with a microalgal culture density of 0.4–1.2 g·L<sup>-1</sup>.

**Conclusions.** The study analyzed the growth characteristics and photosynthetic pigment content in *Scenedesmus rubescens* cultured on three types of mineral nutrient media: complete Tamiya medium, its half-strength modification (Tamiya ½), and Bold's basal medium (BBM). Significant differences in the biochemical and kinetic characteristics of *S. rubescens* growth were established. The maximum

productivity for experimental variants No. 1, 2, and 3 was 0.35, 0.18, and 0.31 g dry weight·L<sup>-1</sup>·day<sup>-1</sup>, respectively. The highest concentrations of chlorophyll *a* and *b* were recorded when the microalga was grown on Tamiya nutrient medium and BBM. The results obtained in the experiment indicate very similar growth characteristics of *S. rubescens* culture in variants No. 1 and 3 in the range of exponential and linear growth phases. This allows us to consider BBM as an alternative to more expensive Tamiya medium for batch cultivation of *S. rubescens*. Of the types of nutrient media studied, Tamiya ½ medium was recognized as the third most effective in terms of the microalgal growth.

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## ОЦЕНКА ПРОДУКЦИОННЫХ ХАРАКТЕРИСТИК И ФОТОСИНТЕТИЧЕСКИХ ПИГМЕНТОВ *SCENEDESMUS RUBESCENS* ПРИ РАЗЛИЧНОЙ ОБЕСПЕЧЕННОСТИ БИОГЕННЫМИ ЭЛЕМЕНТАМИ

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Биотехнология микроводорослей рассматривается как перспективное направление для получения возобновляемых источников биомассы, богатой белками, липидами и пигментами, что делает актуальным поиск оптимальных условий их культивирования. Представители рода *Scenedesmus* Meуen, 1829 отличаются быстрым ростом, устойчивостью к изменениям внешней среды и значительным содержанием биологически активных соединений, что определяет их высокий потенциал для применения в пищевой, фармацевтической и энергетической отраслях. Целью настоящей работы было проанализировать параметры роста и содержания фотосинтетических пигментов в культуре *Scenedesmus rubescens* (P. J. L. Dangeard) E. Kessler, M. Schafer, C. Hummer, A. Kloboucek & V. A. R. Huss, 1997, выращиваемой на трёх типах минеральных питательных сред — на полной среде Тамия, её половинной модификации

(Тамия  $\frac{1}{2}$ ) и среде Болда (Bold's basal medium, ВВМ) (варианты № 1, 2 и 3 соответственно). В рамках исследования микроводоросли *S. rubescens* культивировали в условиях дополнительной подачи углекислого газа и непрерывного освещения в течение 16 сут. Отмечена высокая удельная скорость роста культуры ( $\mu$ ) на питательных средах ВВМ и Тамия — 0,48 и 0,49 сут<sup>-1</sup> соответственно. При использовании среды Тамия  $\frac{1}{2}$  удельная скорость роста *S. rubescens* была ниже — 0,33 сут<sup>-1</sup>. Линейный рост микроводорослей на трёх питательных средах наблюдали по 4-е сутки включительно. За этот период плотность *S. rubescens* в вариантах опыта № 1 и 3 увеличилась практически в 7 раз, в варианте № 2 — в 3,7 раза. Максимальная продуктивность *S. rubescens* для трёх вариантов эксперимента составляла 0,35, 0,18 и 0,31 г сухой массы·л<sup>-1</sup>·сут<sup>-1</sup>, при этом каких-либо изменений в морфологической структуре клеток обнаружено не было. Выявлены значимые различия в биохимических и кинетических характеристиках роста *S. rubescens*, культивируемых на трёх разных питательных средах. Высокие концентрации хлорофилла *a* и *b* зафиксированы в клетках микроводорослей, выращенных на средах Тамия и ВВМ. Полученные в эксперименте результаты свидетельствуют об очень близких ростовых характеристиках культуры *S. rubescens* в вариантах № 1 и 3 в диапазоне экспоненциальной и линейной фаз роста, что позволяет подобрать условия, способные обеспечить на среде ВВМ продуктивность и накопление ценных веществ, аналогичные таковым для варианта на среде Тамия.

**Ключевые слова:** *Scenedesmus rubescens*, питательная среда Болда, питательная среда Тамия, накопительная культура, продуктивность, фотосинтетические пигменты