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GROWTH OF THE CRYPTOPHYTE MICROALGA *RHODOMONAS SALINA* (WISLOUCH) D. R. A. HILL & R. WETHERBEE, 1989 UNDER DIFFERENT CULTIVATION CONDITIONS

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Temperature and irradiance effect was studied on the specific growth rate and biomass accumulation of the cryptophyte alga *Rhodomonas salina*. Optimal conditions for its cultivation were determined allowing to obtain the maximum biomass. *R. salina* was cultivated on the Conway medium (in our own modification) at a temperature of (20 ± 1), (24 ± 1), and (28 ± 1) °C and irradiance of 13, 67, 135, and 202 μmol photons·m^{-2}·s^{-1}. As shown, an increase in temperature up to above-optimal values resulted in a decrease of the microalga growth rate and biomass. For *R. salina*, there were no significant differences in growth rates at irradiance of 135 and 202 μmol photons·m^{-2}·s^{-1} (μ values were of (0.69 ± 0.04) and (0.64 ± 0.02) day^{-1}, respectively). The microalga growth slowed down at low irradiance (13 μmol photons·m^{-2}·s^{-1}) (μ value was of (0.33 ± 0.03) day^{-1}). The maximum biomass [(3.74 ± 0.28) g·L^{-1}] was obtained at the optimal temperature [(24 ± 1) °C] and irradiance of 135 μmol photons·m^{-2}·s^{-1}. Under optimal cultivation conditions, maximum accumulation of proteins was registered at the exponential growth phase (29 %), and maximum accumulation of lipids was recorded at the stationary phase (41 %).

Keywords: microalga *Rhodomonas salina*, cultivation, temperature, irradiance, growth rate, biomass

The cryptophyte alga *Rhodomonas salina* (Wislouch) D. R. A. Hill & R. Wetherbee, 1989 is widely used in aquaculture, as well as in food and cosmetic industries. It is the main food object when culturing larvae and juveniles of commercial molluscs (oysters, scallops, and mussels) and has high nutritional value (Kholodov et al., 2017 ; Zhang et al., 2013). The microalga provides zooplankton with vitamins, fatty acids, and pigments that are transmitted through food chains (Vu et al., 2016). Larvae and juveniles of bivalve molluscs reared in a nursery are most vulnerable during the metamorphosis period (this is the time when their mortality can be maximum). *R. salina* inclusion in mollusc diet contributes to a significant increase in the growth rate of larvae and spat due to their accumulation of a sufficient amount of total lipids (Tremblay et al., 2007 ; Videla et al., 1998 ; Whyte et al., 1989).

Moreover, the microalga *R. salina* is a promising object for phycoerythrin production, and phycoerythrin can be used as a natural dye for food and cosmetics (Chaloub et al., 2015).

When cultivating *R. salina*, irradiance and temperature are the main factors affecting its growth rate (Ladygina, 2010), biomass accumulation, and biochemical composition – protein, carbohydrate, lipid, and phycoerythrin content. With temperature increasing +20 to +32 °C, *R. salina* maximum

growth rate is known to decrease; with irradiance rising 15 to 150 μmol photons·m^{-2}·s^{-1}, it increases (Chaloub et al., 2015). An optimal irradiance range for photosynthesis and alga growth is 60–100 μmol photons·m^{-2}·s^{-1} (Vu et al., 2016). Irradiance of 200 μmol photons·m^{-2}·s^{-1}, as well as high concentrations of nitrates (3.529 mM) and phosphates (0.144 mM) – regardless of temperature – contributed to an increase in *R. salina* growth rate. Moreover, high concentrations of nitrates and phosphates – regardless of irradiance and temperature – resulted in maximum accumulation of protein in algal cells (Guevara et al., 2016 ; Silva et al., 2009). Analysis of the results of several studied shows that *R. salina* biomass accumulation and biochemical composition are sensitive to changes in cultivation conditions.

The aim of the work is to determine optimal conditions for *R. salina* cultivation for its use as a food object in aquaculture.

MATERIAL AND METHODS

The studied microalga was *Rhodomonas salina* – strain CCAP 978127 obtained in 2011 from the collection of IFREMER (Institut Français de Recherche pour l'Exploitation de la Mer) (France).

Experiments on the effect of cultivation conditions on the microalga production parameters were carried out in two stages:

1. Determining optimal temperature. *R. salina* was batch-cultivated on the Conway medium in our own modification (Kholodov et al., 2017), at a temperature of (20 ± 1), (24 ± 1), and (28 ± 1) °C, 24-hour irradiance with Philips TL-D 36W/965 lamps, and continuous air bubbling with a microcompressor. The microalga was cultivated in 2-L flasks, and irradiance was the same – 67 μmol photons·m^{-2}·s^{-1}.
2. Determining optimal irradiance. The microalga was cultivated at optimal temperature of (24 ± 1) °C (this value was obtained during the first stage of the experiment), 24-hour irradiance of the flask surface of 13, 67, 135, and 202 μmol photons·m^{-2}·s^{-1}, and continuous air bubbling.

The experiments were carried out in triplicate. The concentration of algal cells was counted daily in a Goryaev chamber in four fields of view under an MBI-6 microscope. The microalga growth rate was determined according to the formula (Vonshak, 1986):

$$\mu = \frac{\ln N_1 - \ln N_0}{T_1 - T_0},$$

where N_0 is the concentration of algal cells at the beginning of cultivation;

N_1 is the concentration of algal cells at the end of the selected cultivation interval;

$T_1 - T_0$ is the cultivation interval (days).

Biochemical analysis of the alga (protein, carbohydrate, and lipid content) was carried out at each growth phase at a temperature of (24 ± 1) °C and irradiance of 135 μmol photons·m^{-2}·s^{-1}. To obtain *R. salina* dry biomass, a certain volume of the culture with a known cell concentration was centrifuged for 3 minutes on an OPN-3 centrifuge at 3000 rpm; then, it was washed twice with isotonic NaCl solution (9 g·L^{-1}). Later, raw biomass was dried to constant weight at +105 °C for 24 hours. The mass fraction of total protein, lipids, and carbohydrates in dry matter (%) was determined by photocolorimetric methods. Total protein content was analyzed according to Lowry *et al.* (1951); lipid content, using a phospho-vanillin reagent; and carbohydrate content, by a color reaction with L-tryptophan reagent (Metody gidrokhimicheskikh issledovanii, 1988). The data were statistically processed using

standard Microsoft Excel software packages. All the calculations were performed for a significance level $\alpha = 0.05$. In the text and on the graphs, mean values are given, and the boundaries of a confidence interval are indicated (Lakin, 1990).

RESULTS AND DISCUSSION

R. salina cells are motile, with two flagella and one chloroplast. Mean cell size is as follows: width, (7 ± 0.35) μm; length, (12 ± 0.58) μm; and volume, (527 ± 0.43) μm^3 (Fig. 1).

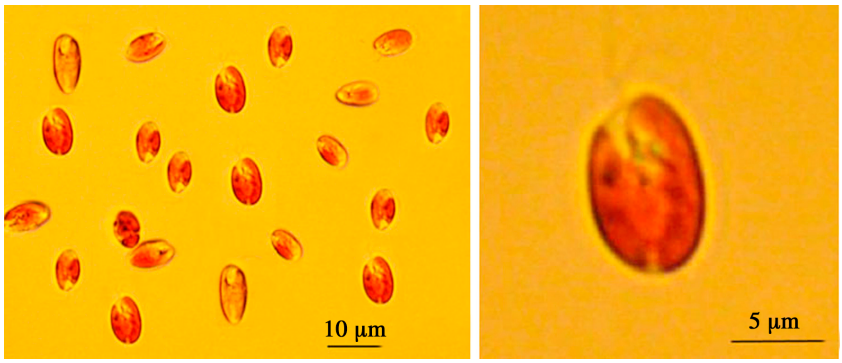

Fig. 1. Microalga *Rhodomonas salina*

Optimal temperature value affecting the growth rate, nutrient uptake, and cell chemistry is species-specific. When cultivating the microalga *R. salina* under different temperatures, the highest rate of cell division was recorded at (24 ± 1) °C; at a temperature of (28 ± 1) °C, it sharply decreased. The maximum culture density (5.43×10^6 cells·mL^{-1}) was obtained at (24 ± 1) °C on the 9^{th} day of cultivation (Fig. 2). At (20 ± 1) and (28 ± 1) °C, maximum cell concentrations were significantly lower – 3.28×10^6 and 3.72×10^6 cells·mL^{-1}, respectively. At (20 ± 1) °C, *R. salina* exponential growth lasted for 8 days; at (24 ± 1) °C, for 6 days; and at (28 ± 1) °C, for 4 days. The period of alga cultivation was the longest at low temperatures – when the culture entered the stationary phase on the 11^{th}–12^{th} day. The cultures kept at (28 ± 1) °C entered the stationary phase on the 7^{th} day; at (24 ± 1) °C, on the 9^{th} day. Under such cultivation conditions, *R. salina* linear growth was observed for 4–7 days; then, a decrease in the cell concentration was recorded, and the culture entered the stationary phase (after 7–11 days of cultivation). A linear dependence of cell concentration on temperature was obtained, with the coefficient R^2 at (20 ± 1), (24 ± 1), and (28 ± 1) °C being 0.85, 0.94, and 0.77, respectively.

The maximum mean daily growth – 0.79×10^6 cells·mL^{-1}·day^{-1} – was recorded at a temperature of (24 ± 1) °C. The values were significantly lower at (20 ± 1) and (28 ± 1) °C – 0.27×10^6 and 0.39×10^6 cells·mL^{-1}·day^{-1}, respectively. The growth rate at a temperature of (24 ± 1) °C is more than 2 times higher than the value at (28 ± 1) °C (Table 1). The obtained results are comparable with the data of other researchers (Brown et al., 1997); according to them, an increase in temperature +26 to +32 °C during *R. salina* cultivation on the F/2 medium results in a decrease in its maximum growth rate.

The dynamics of *R. salina* biomass accumulation under different temperatures was similar to the change in cell density in the culture. Maximum algal biomass – 2.87 g·L^{-1} – was obtained at (24 ± 1) °C. At (20 ± 1) and (28 ± 1) °C, the values differed slightly and amounted to 1.73 and 1.99 g·L^{-1}, respectively (see Table 1). Therefore, a temperature of (24 ± 1) °C was optimal for *R. salina* cultivation under conditions of our experiment.

Table 1. Indicators of the microalga *Rhodomonas salina* growth at different temperatures

Growth indicator	Temperature, °C		
	20 ± 1	24 ± 1	28 ± 1
Mean daily growth, $\times 10^6$ cells·mL^{-1}·day^{-1}	0.27 ± 0.04	0.79 ± 0.06	0.39 ± 0.05
Growth rate, day^{-1}	0.19 ± 0.05	0.53 ± 0.07	0.24 ± 0.03
Maximum biomass (raw), g·L^{-1}	1.73 ± 0.15	2.87 ± 0.24	1.99 ± 0.11

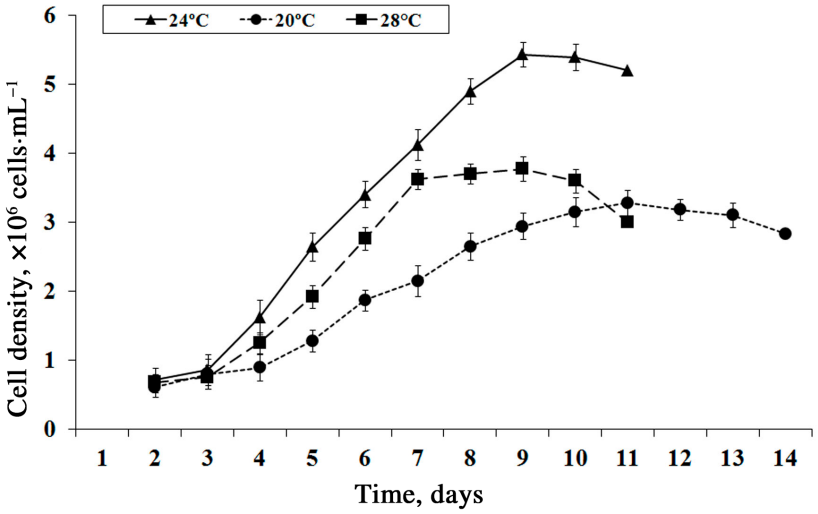

Fig. 2. Dynamics of the microalga *Rhodomonas salina* growth at different temperatures

Irradiance significantly affected *R. salina* growth. This dependence is shown in Fig. 3. At optimal cultivation temperature [(24 ± 1) °C] and different irradiance [13, 67, 135, and 202 μmol photons·m^{-2}·s^{-1}], the growth rate was maximum [(0.69 ± 0.04) day^{-1}] at 135 μmol photons·m^{-2}·s^{-1}.

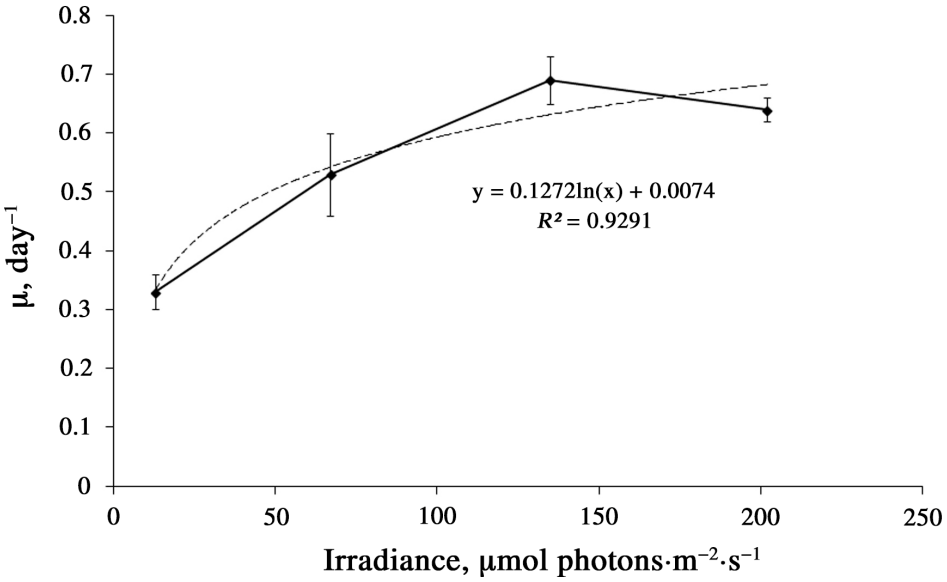

Fig. 3. Dynamics of the microalga *Rhodomonas salina* specific growth rate depending on irradiance

The maximum values of algal biomass were obtained on the 8th and 9th days of cultivation at irradiance of 135 and 202 μmol photons·m^{-2}·s^{-1} – 3.74 and 3.52 g·L^{-1}, respectively (Table 2).

Table 2. Indicators of the microalga *Rhodomonas salina* growth at different irradiance

Growth indicator	Irradiance, μmol photons·m^{-2}·s^{-1}			
	13	67	135	202
Maximum cell concentration, ×10^6 cells·mL^{-1}	2.53 ± 0.20	5.45 ± 0.38	7.10 ± 0.45	6.55 ± 0.40
Maximum biomass (raw), g·L^{-1}	1.41 ± 0.25	2.87 ± 0.24	3.74 ± 0.28	3.52 ± 0.21

There were no significant differences in *R. salina* growth rates under irradiance of 135 and 202 μmol photons·m^{-2}·s^{-1} (μ values were of (0.69 ± 0.04) and (0.64 ± 0.02) day^{-1}, respectively). The lowest growth rates were registered at 13 μmol photons·m^{-2}·s^{-1} (μ value was of (0.33 ± 0.03) day^{-1}); maximum biomass at such irradiance accounted for 1.41 g·L^{-1}.

With a rise in irradiance of the flask surface 13 to 67 μmol photons·m^{-2}·s^{-1}, the concentration of algal cells and biomass increased by 2 times (see Table 2), but the values were significantly lower than those at 135 and 202 μmol photons·m^{-2}·s^{-1} (7.10×10^6 and 6.55×10^6 cells·mL^{-1}, respectively). The maximum values of the specific growth rate and biomass were obtained at 135 μmol photons·m^{-2}·s^{-1}. Therefore, an irradiance of 135 μmol photons·m^{-2}·s^{-1} is optimal for *R. salina* batch cultivation.

During the alga growth, the color of the culture medium changed. At a temperature of (24 ± 1) °C and the lowest irradiance (13 μmol photons·m^{-2}·s^{-1}) on the 7th–8th day (exponential growth phase), the suspension in the flasks became red, and this color differed significantly from the color at 135 and 202 μmol photons·m^{-2}·s^{-1} (Fig. 4).

Fig. 4. Phycoerythrin accumulation in the microalga *Rhodomonas salina* cells at the exponential growth phase at irradiance of 13 μmol photons·m^{-2}·s^{-1}

As the biomass was accumulated and the culture entered the stationary phase, the suspension turned greenish which probably resulted from a change in pigment content in algal cells.

R. salina pigments are phycoerythrin, chlorophyll *a* and *b*, and carotenoids (Chaloub et al., 2015 ; Rowan, 1989). Maximum phycoerythrin accumulation in algal cells is possible at low irradiance levels on the 4th–8th days of cultivation (Bartua et al., 2002). As established (Chaloub et al., 2015),

phycoerythrin content in the alga cultivated at +20 and +26 °C was approximately 2–4 and 6–13 times higher when irradiance decreased 150 to 15 μmol photons·m^{-2}·s^{-1}, respectively. The maximum phycoerythrin concentration was obtained on the 8th day of *R. salina* cultivation at +26 °C and 15 μmol photons·m^{-2}·s^{-1}. According to the data of other researchers (Bartua et al., 2002 ; Chaloub et al., 2015), when the culture enters the stationary growth phase, phycoerythrin concentration in microalgal cells decreases, while chlorophyll concentration increases. Apparently, this contributed to the change in the color of the culture medium. Therefore, phycoerythrin biosynthesis in *R. salina* cells occurs at the exponential growth phase and under low irradiance.

A study of the biochemical composition of *R. salina* cultivated at the temperature of +24 °C and irradiance of 135 μmol photons·m^{-2}·s^{-1} showed as follows: accumulation of protein, carbohydrates, and lipids depends on the microalga growth phase. The maximum protein content [(42.8 ± 3.34) %] was recorded at the exponential phase; lipid content [(39.9 ± 4.12) %], at the stationary one. Carbohydrate concentration in algal cells is significantly lower than protein and lipid content; it peaks [(30.0 ± 1.75) %] at the end of the stationary growth phase (Fig. 5).

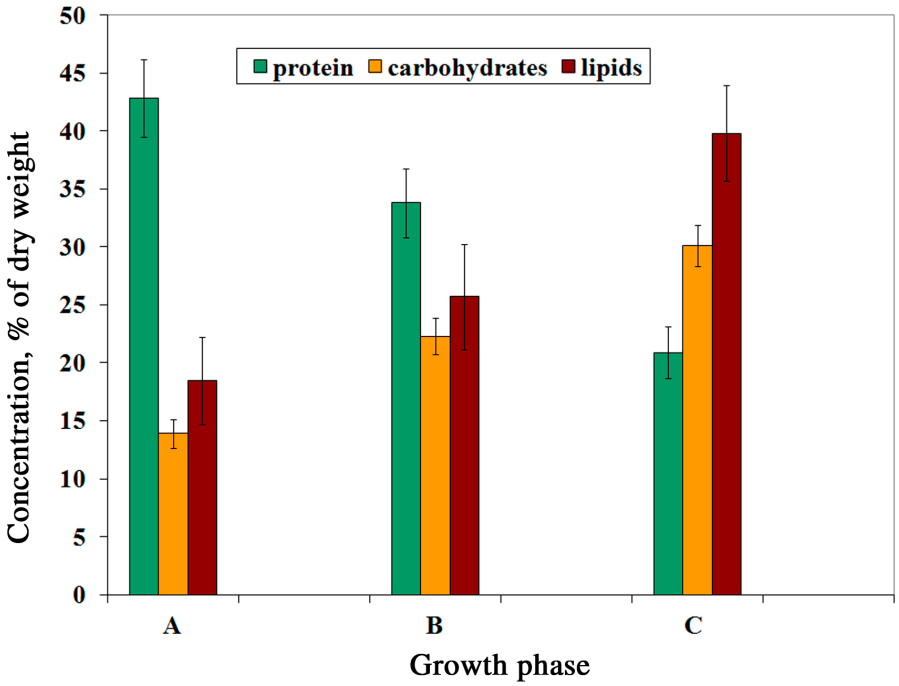

Fig. 5. Biochemical composition of the microalga *Rhodomonas salina* at different growth phases: A, exponential; B, growth retardation; and C, stationary

The content of total lipids, as well as arachidonic, eicosapentaenoic, and docosahexaenoic acids, depends on the alga cultivation conditions – temperature, irradiance, and nutrient availability (Guevara et al., 2016 ; Vu et al., 2016). It was previously established that total content of fatty acids in *R. salina* is maximum at irradiance of 60–100 μmol photons·m^{-2}·s^{-1} and nutrient deficiency. Maximum concentrations of polyunsaturated fatty acids were recorded at 10–40 μmol photons·m^{-2}·s^{-1} and an excess of nutrients in the medium (Vu et al., 2016). The content of polyunsaturated fatty acids – eicosapentaenoic (C20:5ω-3) and eicosahexaenoic (C20:6ω-3) ones – is 12 and 17 %, respectively (Fernández-Reiriz et al., 1989).

Therefore, high concentrations of protein and total lipids in *R. salina* cells allow using this alga as a food object in aquaculture. Specifically, when culturing larvae of a Pacific oyster *Crassostrea gigas* (Thunberg, 1793) in the nursery, the microalga inclusion in the diet contributed to an increase in their survival and growth rate (Kholodov et al., 2017).

Conclusion. Cell density and biomass of the microalga *Rhodomonas salina* varied depending on cultivation conditions. The maximum biomass of *R. salina* (3.74 $g \cdot L^{-1}$) was obtained with a batch cultivation on the Conway medium at the temperature of (24 ± 1) °C, 24-hour irradiance of 135 μmol photons$\cdot m^{-2} \cdot s^{-1}$, and continuous air bubbling. The maximum amount of protein [(42.8 ± 3.34) %] was accumulated at the exponential growth phase; the maximum amount of lipids [(39.9 ± 4.12) %], at the stationary one.

This work was carried out within the framework of IBSS state research assignment "Investigation of mechanisms of controlling production processes in biotechnological complexes with the aim of developing scientific foundations for production of biologically active substances and technical products of marine genesis" (No. 121030300149-0).

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РОСТ КРИПТОФИТОВОЙ МИКРОВОДОРОСЛИ *RHODOMONAS SALINA* (WISLOUCH) D. R. A. HILL & R. WETHERBEE, 1989 ПРИ РАЗНЫХ УСЛОВИЯХ КУЛЬТИВИРОВАНИЯ

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Исследовано влияние температуры и освещённости на удельную скорость роста и на накопление биомассы криптофитовой микроводоросли *Rhodomonas salina*; определены оптимальные условия её культивирования для получения максимальной биомассы. *R. salina* культивировали

на питательной среде Конвея (в собственной модификации) при температуре (20 ± 1), (24 ± 1) и (28 ± 1) °С и освещённости 13, 67, 135 и 202 μмоль квантов·м$^{-2}$·с$^{-1}$. Показано, что увеличение температуры до значений выше оптимальных приводит к снижению скорости роста и биомассы микроводоросли. Существенных различий в показателях роста *R. salina* при освещённости 135 и 202 μмоль квантов·м$^{-2}$·с$^{-1}$ (значения μ — (0,69 ± 0,04) и (0,64 ± 0,02) сут$^{-1}$ соответственно) не зарегистрировано. Рост микроводоросли замедлялся при низкой освещённости (13 μмоль квантов·м$^{-2}$·с$^{-1}$) (значение μ — (0,33 ± 0,03) сут$^{-1}$). Максимальная биомасса [(3,74 ± 0,28) г·л$^{-1}$] получена при оптимальной температуре [(24 ± 1) °С] и освещённости 135 μмоль квантов·м$^{-2}$·с$^{-1}$. При оптимальных условиях культивирования максимальное накопление белка отмечено в экспоненциальной фазе роста (29 %), а липидов — в стационарной фазе (41 %).

Ключевые слова: микроводоросль *Rhodomonas salina*, культивирование, температура, освещённость, скорость роста, биомасса