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EXPERIMENTAL ARTICLES

The Effect of Temperature on the Lipid Composition of the Green Alga *Botryococcus*

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Abstract—The lipid composition of the green alga *Botryococcus* was studied at three different cultivation temperatures: suboptimal (18°C), optimal (25°C), and supraoptimal (32°C). Cultivation at the supraoptimal temperature was found to considerably inhibit the synthesis of nearly all intracellular lipids, except for triacylglycerides, and to influence their fatty acid composition. In particular, the content of trienoic fatty acids was significantly lower at the supraoptimal than at the optimal cultivation temperature. At the same time, the fatty acid composition of the extracellular lipids of the alga virtually did not depend on cultivation temperature.

Key words: green alga, lipids, fatty acids, temperature.

The ability of the green colonial alga *Botryococcus* to synthesize liquid hydrocarbons [1] has attracted researchers' attention to the study of the synthesis of fatty acids, which serve as the hydrocarbon precursors in this alga [2]. It is known that cultivation temperature considerably influences the metabolism of lipids, including membrane lipids, in algae [3]. The alga *Botryococcus* has not yet been investigated in this respect.

The aim of the present work was to study the effect of cultivation temperature on the qualitative and quantitative composition of the extracellular and intracellular lipids of *Botryococcus.*

MATERIALS AND METHODS

The alga *Botryococcus braunii* (green variety), which is deposited in the culture collection at the Cambridge University as *B. braunii* Kutz No LB 807/1 Droop 1950 H-252, was obtained from the collection of unicellular algae at the Institute of Plant Physiology of the Russian Academy of Sciences. The alga was cultivated as described earlier [4, 5]. A collection culture maintained on Prat agar was activated through three subsequent aerobic luminostat subcultures in 1-l flasks containing 0.5 l of liquid Prat medium. The medium was inoculated with mid-linear-growth-phase cells at a concentration of 90–100 mg dry wt/l. The alga was cultivated at 20-W/m^2 illumination for 24-h periods of 10-h night and 14-h day. The culture was aerated with air containing 1 vol % carbon dioxide. The cultivation temperature was maintained, to an accuracy of 0.2°C, at three levels: 18°C (suboptimal), 25°C (optimal), and 32°C (supraoptimal). The dry weight of the biomass was evaluated by the standard procedure, using Vladipor membrane filters with 0.85- to 0.95-μm pore sizes. The biomass was dried at 70°C for 24 h.

Lipids were analyzed as follows: Cells from 50–100 ml of algal culture were harvested by centrifugation, washed with 30 ml of 0.2% NaCl, suspended in 20 ml of hot isopropanol, and boiled for 3 min to inactivate lipases. After cooling, this suspension was mixed with 20 ml of chloroform and kept for 12 h at room temperature. Then the chloroform extract was mixed with 10 ml of water and allowed to separate into phases. The lower chloroform phase and the upper aqueous phase were collected separately. The aqueous phase was again extracted with chloroform. The two chloroform extracts were pooled, dehydrated with anhydrous sodium sulfate, and placed in a weighed flask. After the removal of the solvent with a rotary vacuum pump, lipids were dried further in a desiccator, and quantified by weighing them together with the flask.

Extracellular lipids were extracted thrice with chloroform from the culture liquid supernatant. The extracts were pooled and processed in the same way as described above.

Lipid extracts were subjected to thin-layer microchromatography [6] on the KSK silica gel in a solvent system for neutral lipids (hexane–diethyl ether–acetic acid mixture in a volume proportion of 85 : 15 : 1) [7]. The separated lipids were identified by comparing their R_f values with those of the respective authentic samples: di- and triacylglycerides, fatty acids, methyl esters of fatty acids, sterols, and sterol esters purchased from Serva (Germany) and Sigma (United States). Lipids

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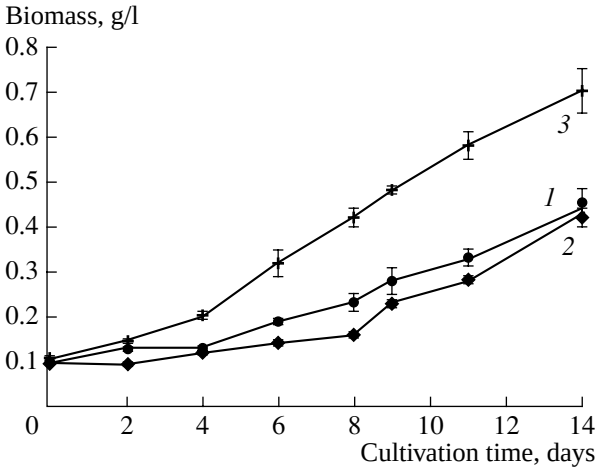

Fig. 1. Growth of the green alga *B. braunii* at (*1*) 18, (*2*) 25, and (*3*) 32°C.

from various classes were quantified by the dichromate method, measuring absorbance at 350 nm against distilled water in 1-cm pathlength cuvettes [8].

Fatty acids were subjected to methanolysis in a methanol–sulfuric acid (50 : 1) mixture at 90°C for 2 h. After incubation, two volumes of water were added, and the methyl esters of fatty acids were extracted with hexane. The extract was washed with distilled water to a neutral reaction and dehydrated with anhydrous sodium sulfate. The hexane was evaporated using the rotary vacuum pump, and the residue was dissolved in benzene. The benzene solutions were stored at –20°C for future analysis.

The methyl esters of fatty acids were analyzed with a GCD Plus gas chromatograph–mass spectrometer (Hewlett Packard, United States) equipped with a (30 m × 0.25 mm ID) HP-5 column. The carrier gas was helium at a flow rate of 1 ml/min. The injector and detector were kept at 230°C. The initial temperature of the column was 100°C. Then the column temperature was raised to 230°C at a rate of 8°C/min. The separated fatty acid esters were identified by analyzing their mass spectra and by comparing their retention times with those of the respective authentic samples (the methyl esters of saturated, branched, and monoenoic C10–C24 acids, as well as C18:2, α-C18:3, and γ-C18:3 acids) purchased from Serva (Germany) and Sigma (United States)). The position of double bonds in monoenoic fatty acids was determined by analyzing their sulfoderivatives with the gas chromatograph–mass spectrometer as described by Christie [9]. Fatty acids were quantified using heptadecanoic acid as the internal standard. The acid was added to samples before the extraction of lipids. The synthesis rate of particular fatty acids was determined as described in the publications [10, 11].

All the experiments were performed in triplicate. Data were statistically processed by the methods described in the textbook [12] using the Excel software package.

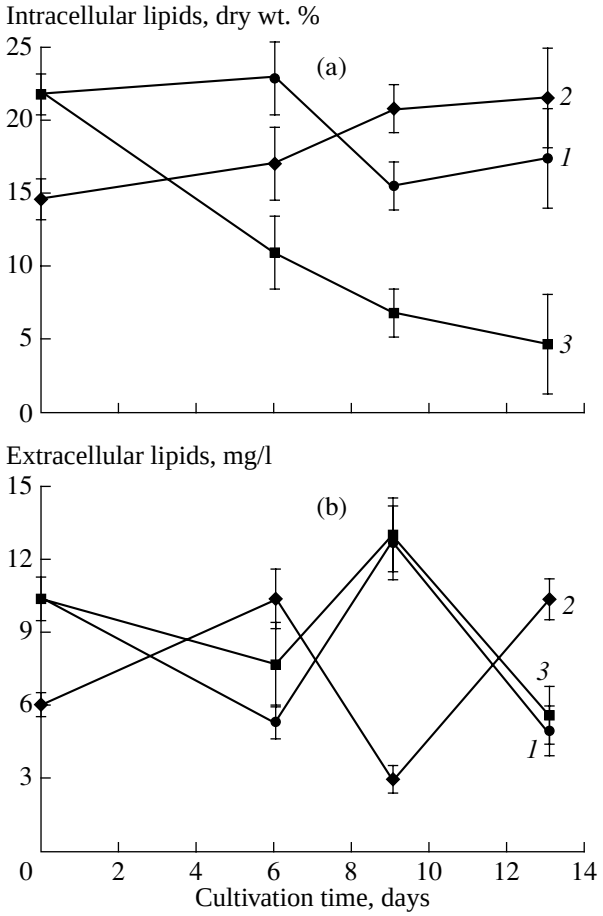

Fig. 2. The content of (a) intracellular and (b) extracellular lipids in the *B. braunii* culture grown at (*1*) 18, (*2*) 25, and (*3*) 32°C.

RESULTS AND DISCUSSION

As can be seen from Fig. 1, the *Botryococcus* biomass accumulated at the cultivation temperatures 25 and 18°C was almost the same (0.43 and 0.44 g/l, respectively). At the same time, cultivation at the supraoptimal temperature augmented the biomass yield to 0.7 g/l.

We failed to reveal any statistically significant differences in the content of intracellular lipids in *B. braunii* cells grown at 18 and 25°C (Fig. 2a). At the same time, after 13 days of cultivation at 32°C, the content of intracellular lipids in the *Botryococcus* culture decreased from 22 to 5 dry wt %. The decrease in lipids was accompanied by the accumulation of polysaccharides. This is in agreement with the data from Casadevall *et al.* [1], who found that some *Botryococcus* strains accumulate polysaccharides in the stationary growth phase, while they actively synthesize hydrocarbons in the exponential and early linear growth phases.

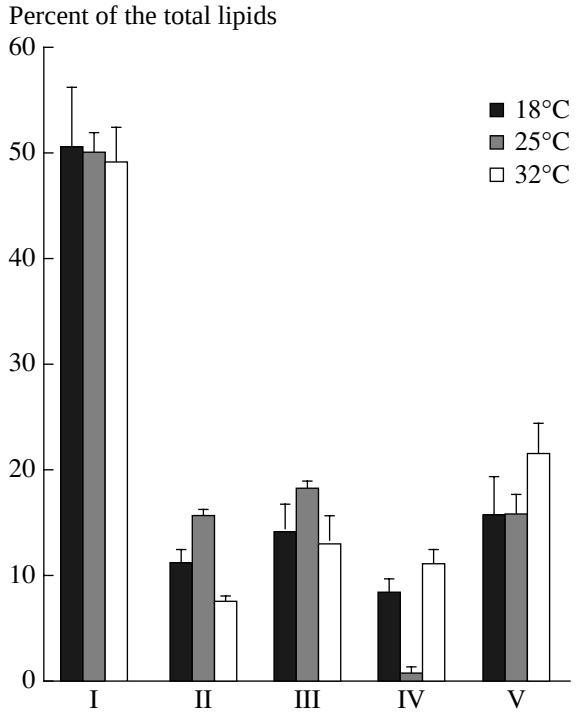

Fig. 3. The composition of the intracellular lipids of *B. braunii* grown at different cultivation temperatures: I, polar lipids + diacylglycerides; II, sterols; III, alcohols + free fatty acids; IV, triacylglycerides; and V, hydrocarbons.

Irrespective of the cultivation temperature, the lipid fraction of *Botryococcus* cells contained polar lipids and diacylglycerides, which amounted to 50% of the total intracellular lipids (Fig. 3). The content of other lipid classes (sterols, alcohols, free fatty acids, and hydrocarbons) comprised 15–20% of the total lipids of algal cells grown at 25°C and exhibited insignificant changes in the course of cultivation. Triacylglycerides, which are reserve lipids in many green algae [13], were present in the cells grown at 25°C in trace amounts in the early cultivation terms and in an amount of up to 2% after 13 days of cultivation. The content of triacylglycerides in the cells grown at the supra- and suboptimal temperatures was 9.2–13.8 and 7.6–9.3%, respectively. The increase in triacylglycerides was accompanied by a twofold decline in sterols. The content of alcohols and free fatty acids also decreased. The content of intracellular polar lipids and hydrocarbons virtually did not depend on the growth temperature.

At all cultivation temperatures, the concentration of extracellular lipids showed a considerable variation, changing from 2 to 15 mg/l in the course of cultivation (Fig. 2). The extracellular lipids were represented by polar lipids, alcohols, free fatty acids, triacylglycerides, and hydrocarbons. The effect of cultivation temperature on the composition of extracellular lipids was statistically insignificant.

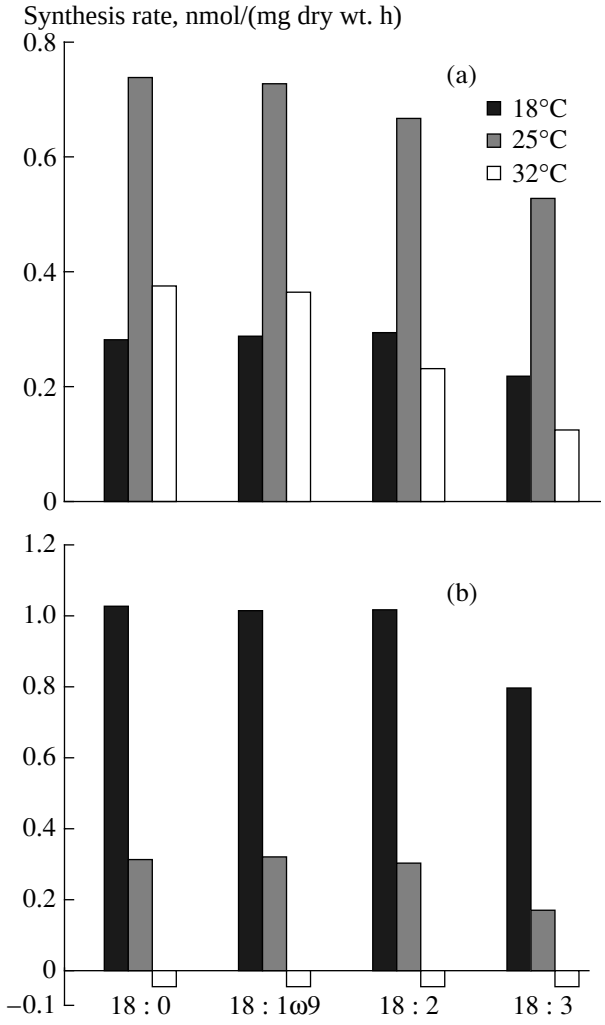

Fig. 4. The synthesis rates of fatty acids in *B. braunii* cells grown at different cultivation temperatures. The rates were calculated for cultivation periods between (a) the 6th through 9th day and (b) the 9th through 13th day of cultivation.

At the same time, analysis showed that cultivation temperature exerted a statistically significant effect on the fatty acid composition of the intracellular lipids of the alga, which is an indication of the state of cellular membranes. As can be seen from the data presented in Table 1, the intracellular lipids of *Botryococcus* have a fatty acid composition typical of green algae [13], being dominated by palmitic (C16:0) and stearic (C18:0) acids. The monoenoic fatty acids of the alga were represented by three isomers of hexadecenoic (C16:1) acid and two isomers of octadecenoic (C18:1) acid. The major isomer of hexadecenoic acid gave rise to two ionic fragments with *m/z* ratios equal to 217 and 145, which correspond to the position of the double bond at C9 atom from the carboxyl terminus of the molecule. The minor isomer of hexadecenoic acid gave rise to ionic fragments with *m/z* ratios equal to 231 and 131,

Table 1. The fatty acid composition (% of the total fatty acids) of the intracellular lipids of *B. braunii* grown at different cultivation temperatures

Fatty acid	6th day			9th day			13th day		
	18°C	25°C	32°C	18°C	25°C	32°C	18°C	25°C	32°C
12 : 0	0.10 ± 0.01	0.27 ± 0.07	0.55 ± 0.15	0.39 ± 0.06	0.15 ± 0.05	0.36 ± 0.18	0.37 ± 0.17	0.21 ± 0.12	0.36 ± 0.10
14 : 1	0.09 ± 0.02	0.16 ± 0.03	0.41 ± 0.05	0.24 ± 0.06	0.8 ± 0.02	0.20 ± 0.08	0.17 ± 0.05	0.16 ± 0.14	0.29 ± 0.06
14 : 0	1.00 ± 0.32	1.49 ± 0.26	3.40 ± 0.38	1.82 ± 0.42	0.99 ± 0.09	2.06 ± 0.68	1.19 ± 0.23	1.24 ± 0.55	1.25 ± 0.59
ai-15 : 0	0.20 ± 0.08	0.15 ± 0.03	0.33 ± 0.04	0.21 ± 0.07	0.10 ± 0.03	0.23 ± 0.05	0.12 ± 0.01	0.12 ± 0.05	0.31 ± 0.04
i-15 : 0	0.12 ± 0.03	0.15 ± 0.02	0.35 ± 0.05	0.16 ± 0.04	0.09 ± 0.004	0.20 ± 0.05	0.10 ± 0.01	0.12 ± 0.06	0.27 ± 0.08
15 : 0	0.71 ± 0.21	0.68 ± 0.13	1.62 ± 0.25	0.87 ± 0.32	0.44 ± 0.06	0.94 ± 0.15	0.52 ± 0.05	0.42 ± 0.21	1.44 ± 0.19
16 : 2	1.47 ± 0.30	4.30 ± 0.58	8.60 ± 0.90	1.44 ± 0.29	4.35 ± 0.38	8.35 ± 1.98	2.59 ± 0.47	4.73 ± 0.31	10.72 ± 1.49
16 : 3	13.99 ± 1.89	12.59 ± 2.96	10.18 ± 0.91	16.64 ± 0.58	16.66 ± 2.22	9.08 ± 2.04	18.90 ± 2.30	16.93 ± 0.34	6.84 ± 0.83
16 : 1ω7	0.47 ± 0.18	2.76 ± 0.40	4.01 ± 0.55	0.88 ± 0.43	3.58 ± 0.83	2.87 ± 0.18	1.22 ± 0.37	2.54 ± 1.67	3.24 ± 0.80
16 : 1ω6	0.37 ± 0.22	0.62 ± 0.08	0.43 ± 0.22	0.52 ± 0.16	0.47 ± 0.26	0.69 ± 0.24	0.38 ± 0.08	0.30 ± 0.16	–
16 : 1ω13*tr*	0.07 ± 0.07	0.33 ± 0.06	0.51 ± 0.17	0.21 ± 0.14	–	–	–	–	–
16 : 0	26.24 ± 2.13	24.68 ± 2.00	28.34 ± 2.25	25.81 ± 3.90	20.43 ± 3.28	28.55 ± 4.96	24.91 ± 3.58	17.80 ± 1.50	23.87 ± 2.84
18 : 2	8.28 ± 1.20	6.03 ± 0.65	8.87 ± 1.07	8.78 ± 0.95	8.05 ± 0.51	10.87 ± 1.45	9.75 ± 1.24	12.03 ± 2.91	15.57 ± 3.01
α-18 : 3	40.31 ± 2.33	25.65 ± 1.74	23.67 ± 4.00	37.33 ± 5.13	30.76 ± 3.10	18.27 ± 3.26	37.16 ± 3.75	31.69 ± 3.13	15.04 ± 0.85
18 : 1ω9	0.14 ± 0.14	14.66 ± 3.44	3.58 ± 2.16	–	9.57 ± 3.61	11.38 ± 1.92	–	8.08 ± 1.86	14.23 ± 0.97
18 : 1ω7	2.47 ± 1.13	1.52 ± 0.30	1.25 ± 0.33	1.74 ± 0.18	1.51 ± 0.17	3.01 ± 1.23	1.23 ± 0.05	1.70 ± 0.20	1.65 ± 0.17
18 : 0	2.52 ± 0.15	3.05 ± 0.51	3.22 ± 0.30	1.97 ± 0.30	2.07 ± 0.23	2.27 ± 0.43	1.02 ± 0.07	1.60 ± 0.20	3.26 ± 0.99
X	0.07 ± 0.07	–	–	–	–	–	–	–	–
20 : 0	0.28 ± 0.06	0.43 ± 0.13	0.37 ± 0.05	0.18 ± 0.04	0.29 ± 0.11	0.26 ± 0.06	0.07 ± 0.03	0.15 ± 0.02	0.26 ± 0.09
20 : 4	0.18 ± 0.12	–	–	0.18 ± 0.06	–	–	0.04 ± 0.04	–	–
20 : 5	0.25 ± 0.15	–	–	0.33 ± 0.10	–	–	0.07 ± 0.07	–	–
20 : 3	0.16 ± 0.16	–	–	–	–	–	–	–	–
22 : 0	0.22 ± 0.11	0.21 ± 0.09	0.12 ± 0.007	0.14 ± 0.04	0.19 ± 0.10	0.14 ± 0.05	0.08 ± 0.02	0.08 ± 0.05	0.19 ± 0.04
24 : 0	0.32 ± 0.29	0.29 ± 0.11	0.19 ± 0.04	0.16 ± 0.08	0.22 ± 0.17	0.28 ± 0.13	0.11 ± 0.03	0.09 ± 0.05	0.38 ± 0.16
$\Sigma_{unsat}/\Sigma_{sat}$	2.16 ± 0.21	2.21 ± 0.21	1.64 ± 0.24	2.27 ± 0.44	3.11 ± 0.48	1.96 ± 0.44	2.61 ± 0.49	3.67 ± 0.44	2.24 ± 0.50
$\Sigma_{monoen}/\Sigma_{polyen}$	0.06 ± 0.02	0.44 ± 0.13	0.21 ± 0.07	0.05 ± 0.01	0.32 ± 0.13	0.43 ± 0.15	0.04 ± 0.01	0.20 ± 0.07	0.41 ± 0.03
$\Sigma_{dien}/\Sigma_{trien}$	0.18 ± 0.04	0.27 ± 0.02	0.53 ± 0.08	0.19 ± 0.03	0.26 ± 0.02	0.72 ± 0.09	0.23 ± 0.05	0.34 ± 0.06	1.20 ± 0.18
$\Sigma_{mnoen}/\Sigma_{dien}$	0.37 ± 0.08	2.08 ± 0.67	0.60 ± 0.15	0.34 ± 0.06	1.22 ± 0.30	1.03 ± 0.29	0.24 ± 0.01	0.88 ± 0.35	0.77 ± 0.12

Note: Arithmetic means and standard deviations were calculated from the results of triplicate measurements. "–" stands for "not detected." *X* denotes an unidentified fatty acid.

Table 2. The bifactorial variance analysis of the fatty acid composition of the intracellular lipids of *B. braunii* grown at different cultivation temperatures

Fatty acid	F_a	F_b	F_{ast}	F_{bst}	η_a^2	η_b^2
12 : 0	2.70	164.13	3.55	99.45	18.17	0.04
14 : 1	5.77	2.41	3.55	3.55	30.06	2.16
14 : 0	4.89	2.21	3.55	3.55	22.67	10.22
ai-15 : 0	9.19	1.22	6.01	19.44	44.80	4.01
i-15 : 0	12.00	1.30	6.01	3.55	48.53	5.26
15 : 0	14.91	1.52	6.01	3.55	51.86	5.29
16 : 2	47.82	1.80	6.01	3.55	80.66	3.04
16 : 3	16.79	1.16	6.01	3.55	55.14	3.80
16 : 1ω7	9.95	57.72	6.01	19.44	48.82	0.09
16 : 1ω6	4.82	2.77	19.44	3.55	1.49	19.80
16 : 1ω13*tr*	1.24	12.16	19.44	6.01	2.55	38.24
16 : 0	3.04	1.42	3.55	3.55	21.66	10.10
18 : 2	3.11	6.22	3.55	6.01	15.69	31.34
α-18 : 3	26.32	0.26	6.01	3.55	68.54	0.68
18 : 1ω9	25.12	3.14	6.01	19.44	56.78	0.72
18 : 1ω7	2.95	1.45	19.44	19.44	2.62	5.32
18 : 0	4.74	4.32	3.55	3.55	22.26	20.26
20 : 0	2.30	5.23	3.55	3.55	13.34	30.34
22 : 0	32.82	1.45	19.44	19.44	0.27	6.03
24 : 0	2.88	5.21	19.44	19.44	3.18	1.76

Note: F_a, *F*-statistic calculated for the temperature factor; F_b, *F*-statistic calculated for the age factor; F_{ast}, the critical value of F_a; F_{bst}, the critical value of F_b; η_a^2, the degree of influence of the temperature factor (%); η_b^2, the degree of influence of the age factor (%); and $P \le 0.05$, the level of significance.

suggesting that the position of the double bond is at C10 atom. The third isomer of hexadecenoic acid was not conclusively identified, although there are grounds to believe that it is *trans*-3-hexadecenoic acid. As follows from *m/z* ratios equal to 217 and 173, the major octadecenoic acid was oleic acid, whereas the fraction of *cis*-vaccenic acid, characterized by the *m/z* ratios of its ionic fragments equal to 245 and 145, did not exceed 3% of the total fatty acids. Polyenoic fatty acids were represented by dienoic and trienoic C16 and C18 acids.

In the course of cultivation at the optimal growth temperature (25°C), the degree of lipid saturation tended to decrease due to a decline in the content of major saturated fatty acids (the content of C16:0 acid decreased from 36.4 to 17.8% and that of C18:0 acid decreased from 6.5 to 1.6% of the total fatty acids) (Table 1). By the midpoint of the linear growth phase, the unsaturation index of intracellular fatty acids increased from 2.21 to 3.67. In this case, the relative content of dienoic and trienoic acids increased from 48.6 to 65.4%, whereas that of monoenoic acids declined from 19.9 to 12.6% of the total fatty acids. As a result, the ratio of monoenoic acids to polyenoic acids fell by almost two times. The proportion between dienoic and trienoic acids remained at a level of 0.26–0.28 over a cultivation period of 9 days, but then increased to make up 0.34 by the 13th day of cultivation.

Within the 6 days of cultivation at the suboptimal cultivation temperature (18°C), the unsaturation index was at a level typical for cells grown at the optimal temperature (about 2.2), rising then to 2.6 because of the increased content of the C16:0 acid (Table 1). In general, the unsaturation index of the intracellular fatty acids was smaller than in the case of cells grown at the optimal temperature. The content of dienoic acids was the same as in the case of the optimal growth temperature and changed insignificantly in the course of cultivation. As for trienoic acids, their content considerably increased to make up 64.5–68.5% of the total fatty acids by the 6th day of cultivation and then remained at this level until the end of the cultivation period. The proportion between dienoic and trienoic acids was lower than in the case of cultivation at the optimal tem-

Table 3. The fatty acid composition (% of the total fatty acids) of the extracellular lipids of *B. braunii* grown at different cultivation temperatures

Fatty acid	6th day			9th day			13th day		
	18°C	25°C	32°C	18°C	25°C	32°C	18°C	25°C	32°C
9 : 0	–	0.02 ± 0.02	–	–	–	–	–	–	–
10 : 0	–	0.05 ± 0.05	–	–	–	–	–	–	–
12 : 0	0.20 ± 0.12	0.23 ± 0.06	0.82 ± 0.53	1.16 ± 0.34	0.10 ± 0.06	1.02 ± 0.12	1.46 ± 0.15	1.06 ± 0.53	0.98 ± 0.06
13 : 0	0.10 ± 0.05	0.09 ± 0.04	0.23 ± 0.13	0.47 ± 0.12	0.07 ± 0.04	0.35 ± 0.08	0.17 ± 0.17	0.33 ± 0.17	0.47 ± 0.08
14 : 1	0.00 ± 0.00	–	–	–	–	–	0.21 ± 0.21	–	0.34 ± 0.01
14 : 0	2.14 ± 0.92	4.13 ± 0.39	4.39 ± 0.37	7.53 ± 1.25	2.26 ± 0.66	5.85 ± 0.97	7.48 ± 0.75	5.40 ± 1.97	7.26 ± 0.05
ai-15 : 0	0.78 ± 0.27	0.36 ± 0.03	0.98 ± 0.15	0.92 ± 0.13	0.28 ± 0.06	1.03 ± 0.18	1.18 ± 0.17	0.67 ± 0.16	1.40 ± 0.01
i-15 : 0	0.26 ± 0.07	0.53 ± 0.03	0.63 ± 0.09	0.83 ± 0.20	0.39 ± 0.06	1.60 ± 0.56	0.96 ± 0.11	0.60 ± 0.15	1.13 ± 0.08
15 : 0	1.40 ± 0.34	2.05 ± 0.17	2.17 ± 0.37	3.59 ± 0.44	1.61 ± 0.12	4.17 ± 0.41	4.21 ± 0.35	2.89 ± 0.72	3.87 ± 0.19
16 : 2	0.33 ± 0.21	0.85 ± 0.85	–	–	0.18 ± 0.18	–	–	–	–
16 : 3	1.75 ± 0.54	0.46 ± 0.46	–	0.14 ± 0.14	0.84 ± 0.08	–	–	–	–
16 : 1ω7	2.83 ± 1.62	1.82 ± 0.25	0.74 ± 0.42	3.38 ± 1.16	1.49 ± 0.17	2.71 ± 0.55	5.76 ± 0.62	1.99 ± 1.15	2.72 ± 0.57
16 : 1ω6	0.65 ± 0.33	1.547 ± 0.39	1.74 ± 0.19	2.13 ± 0.85	1.78 ± 0.09	1.81 ± 0.53	2.57 ± 0.76	1.92 ± 0.36	3.31 ± 0.68
16 : 0	55.38 ± 1.84	52.99 ± 1.41	64.39 ± 3.81	55.67 ± 2.70	53.70 ± 3.01	55.04 ± 1.81	47.33 ± 0.96	54.94 ± 1.93	49.59 ± 1.21
18 : 2	2.28 ± 0.33	1.32 ± 0.48	0.20 ± 0.20	1.27 ± 0.23	1.62 ± 0.27	1.73 ± 0.82	1.79 ± 0.20	0.58 ± 0.44	1.24 ± 0.25
18 : 3	–	0.46 ± 0.46	–	–	–	–	–	–	–
18 : 1ω9	9.29 ± 1.18	5.77 ± 1.41	2.70 ± 1.81	6.32 ± 1.35	8.14 ± 0.60	7.17 ± 2.56	8.21 ± 1.86	4.96 ± 2.64	6.44 ± 1.71
18 : 1ω7	4.87 ± 0.88	4.77 ± 1.28	7.77 ± 0.77	4.14 ± 0.19	9.05 ± 1.57	4.68 ± 0.76	4.73 ± 0.14	4.10 ± 0.99	6.46 ± 0.11
18 : 0	16.48 ± 1.87	19.79 ± 3.29	12.35 ± 1.19	11.31 ± 0.69	16.23 ± 0.95	11.44 ± 1.32	12.51 ± 0.43	18.24 ± 3.68	13.70 ± 0.59
20 : 0	0.73 ± 0.10	1.66 ± 0.66	0.67 ± 0.15	0.45 ± 0.07	0.95 ± 0.12	0.72 ± 0.25	0.90 ± 0.21	1.34 ± 0.55	0.58 ± 0.11
22 : 0	0.39 ± 0.08	0.67 ± 0.27	0.22 ± 0.12	0.45 ± 0.14	0.58 ± 0.08	0.44 ± 0.24	0.43 ± 0.24	0.67 ± 0.26	0.40 ± 0.03
24 : 0	0.14 ± 0.08	0.42 ± 0.16	–	0.24 ± 0.12	0.74 ± 0.44	0.26 ± 0.13	0.09 ± 0.09	0.31 ± 0.07	0.11 ± 0.11
Σ_{unsat}	22.00 ± 2.89	17.02 ± 5.06	13.16 ± 2.33	17.39 ± 1.65	23.10 ± 1.60	18.09 ± 2.58	23.27 ± 2.14	13.55 ± 3.95	20.52 ± 1.75
Σ_{dat}	78.00 ± 2.89	82.98 ± 5.06	86.84 ± 2.33	82.61 ± 1.65	76.90 ± 1.60	81.91 ± 2.58	76.73 ± 2.14	86.45 ± 3.95	79.48 ± 1.75
$\Sigma_{unsat}/\Sigma_{dat}$	0.29 ± 0.05	0.21 ± 0.08	0.15 ± 0.03	0.21 ± 0.02	0.30 ± 0.03	0.22 ± 0.04	0.31 ± 0.04	0.16 ± 0.05	0.26 ± 0.03

Note: Arithmetic means and standard deviations were calculated from the results of triplicate measurements. “–” stands for “not detected.”

perature, showing, however, a slight increase (from 0.18 to 0.23) by the end of the cultivation period. The content of palmitoleic acid in the cells grown at 18°C (0.5–1.2% of the total fatty acids) was lower than in the cells grown at 25°C (2.5–3.6%). The most drastic changes were observed in the content of oleic acid (the precursor of C18:2 and C18:3 acids), whose concentration in the cells grown at the suboptimal temperature was close to zero. The ratio of monoenoic to polyenoic acids in the cells grown at 18°C was as low as 0.04–0.06 throughout the cultivation period.

Thus, cultivation at the suboptimal temperature stimulated the synthesis of trienoic acids, as well as C20:4 and C20:5 acids. Although the ability of the alga *Botryococcus* to synthesize long-chain polyenoic acids has long been known [14], we detected C20:4 and C20:5 acids in *B. braunii* for the first time.

Within 6 days of cultivation at the supraoptimal temperature (32°C), the relative content of intracellular saturated fatty acids slightly increased, showing a decline with a concurrent increase in the unsaturation index from 1.6 to 2.2, which was mostly due to the rise in the content of monoenoic acids (from 10.2 to 19.3%) and dienoic acids (from 17.5 to 26.3%). At the same time, the content of the trienoic C16:3 acid declined from 10.2 to 6.8% and that of C18:3 acid declined from 23.7 to 15.0%, as a result of which the monoenoic/polyenoic and dienoic/trienoic proportions increased from 0.21 to 0.43 and from 0.53 to 1.20, respectively. In general, cultivation at the supraoptimal temperature led to a more than twofold decrease in the relative content of intracellular trienoic acids.

To evaluate the degree of influence of the cultivation temperature and the physiological state (i.e., the growth phase or culture age) of *Botryococcus* cells on their fatty acid composition, we performed bifactorial variance analysis of relevant data and found that the cultivation temperature exerted on the fatty acid composition of *Botryococcus* cells a more statistically significant effect than the culture age (Table 2).

To assess the promptness of the cell response to cultivation temperature with respect to changes in the fatty acid composition, we calculated the synthesis rate of C18 fatty acids from relevant data on their content in the dry biomass (this calculation method was suggested by Klyachko–Gurvich *et al.*). Calculations were performed for two cultivation periods, between the 6th through 9th day and between the 10th through 13th day of cultivation. As can be seen from the data presented in Fig. 4a, the first cultivation period was characterized by high synthesis rates of all C18 fatty acids in cells grown at the optimal temperature, whereas these rates were 2–2.5 times lower in the case of cells grown at the suboptimal temperature. The synthesis rate of C18:3 fatty acid was the same as those of more saturated fatty acids (C18:2, C18:1, and C18:0). During cultivation at 32°C, C18:0 and C18:1 acids were synthesized more quickly and C18:2 and C18:3 acids more slowly than during cultivation at the optimal growth temperature.

In the cells grown at the optimal temperature, all C18 fatty acids were synthesized two to three times more slowly over the second cultivation period (i.e., in older cells) (Fig. 4b) than over the first cultivation period (Fig. 4a). Conversely, the synthesis rates of C18 fatty acids at the suboptimal temperature were 4 to 5 times higher in the older than in the younger cells. At the supraoptimal growth temperature (32°C), the synthesis rates of all C18 fatty acids in the older cells were close to zero, which is in agreement with the low total content of lipids in these cells.

Thus, cultivation at the supraoptimal temperature considerably inhibited the synthesis of almost all intracellular lipids, except for triacylglycerides, and changed their fatty acid composition. In particular, the content and the synthesis rate of intracellular trienoic fatty acids were significantly lower at the supraoptimal than at the optimal growth temperature. The extracellular lipids of *Botryococcus* exhibited a high content of saturated C16:0 and C18:0 fatty acids (Table 3). The fatty acid composition of the extracellular lipids of the alga depended insignificantly on the cultivation temperature.

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