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Effect of Nitrogen Limitation on the Growth and Lipid Composition of the Green Alga *Botryococcus braunii* Kütz IPPAS H-252

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Abstract—The effect of nitrogen limitation in a medium on the composition of intracellular lipids in the alga *Botryococcus braunii* Kütz IPPAS H-252 in the course of culture development was investigated. Under the conditions of nitrogen limitation, the alga under investigation accumulated lipids as triacylglycerols, and this process was accompanied by substantial changes in the total fatty acid (FA) composition, which were manifested in a decrease in trienoic acids (from 52.8–57.2 to 19.5–24.7% of total FAs) and an increase in the content of oleic (from 1.1–1.2 to 17.1–24.4%) and saturated (from 23.7–26.0 to 32.9–46.1%) acids. In the control culture, the directionality of FA redistribution was less marked, and these changes were noticed at the later stages of culture development. Under nitrogen limitation, marked changes in the FA composition of polar lipids occurred by the 13th day, and they were characterized by an increase in the content of saturated acids (up to 76.8%) and a dramatic decrease in the content of all polyenoic acids (up to 6.8%). The changes in the FA composition of triacylglycerols were noticed as early as by the 7th day; these changes consisted in an increase in the content of oleic acid, and its high content (28.4–38.4%) was maintained up to the end of culturing. In the control culture, triacylglycerols with a high content of oleic acid were found by the 13th day, although, by this time, the content of total lipids and triacylglycerols did not change.

Key words: Botryococcus - lipids - nitrogen limitation

INTRODUCTION

The green alga *Botryococcus braunii* attracts attention because of its surprising ability to form a considerable amount of liquid hydrocarbons. Their composition is determined by the nature of a respective algal race (A, B, or L), and their content is believed to depend also on growth conditions [1]. Provision of biogenic elements, mainly nitrogen, is one of the main factors affecting algal metabolism. The change in the carbon/nitrogen ratio in a medium is known to result in a change in the directionality of metabolism. In many algae, an increase in this ratio results in an accumulation of neutral lipids, mainly triacylglycerols and/or carbohydrates [2–5] and is accompanied by considerable rearrangements in FA composition. These rearrangements consist in an increase in the content of saturated acids and a decrease in polyenoic acids [4, 6, 7]. Moreover, a decrease in the concentration of chlorophyll *a* accompanied by an increase in the content of carotenoids were noticed under the conditions of nitrogen limitation [8].

Unfortunately, studies devoted to investigating the effect of nitrogen concentration on the biochemical composition of *B. braunii* are few in number and are mainly related to the production of hydrocarbons. For instance, a study showed that an increase in the nitrate content in a growth medium resulted in an increase in the duration of exponential phase of growth [9] and, consequently, in an increase in algal yield. In this case, the relative hydrocarbon content decreased; however, their total yield increased by 25% due to a higher biomass yield. On the contrary, culturing of the alga in a medium with decreased nitrogen content resulted in a decrease in both biomass and hydrocarbon content [10].

The objective of this work was to investigate the effect of nitrogen limitation on the biochemical composition (the contents of proteins, lipids, and carbohydrates), lipid class distribution, and FA composition in the lipids of the alga *Botryococcus braunii* Kütz IPPAS H-252.

MATERIALS AND METHODS

A *Botryococcus braunii* Kütz IPPAS H-252 strain obtained from the Collection of Microalgae of the Timiryazev Institute of Plant Physiology, RAS, was used in this work.

Conical 1-l flasks were used for culturing the alga at 25°C; the volume of the culture was 600 ml. The culture was illuminated (20 W/m^2, 10-h photoperiod) and

Abbreviation: FA—fatty acid.

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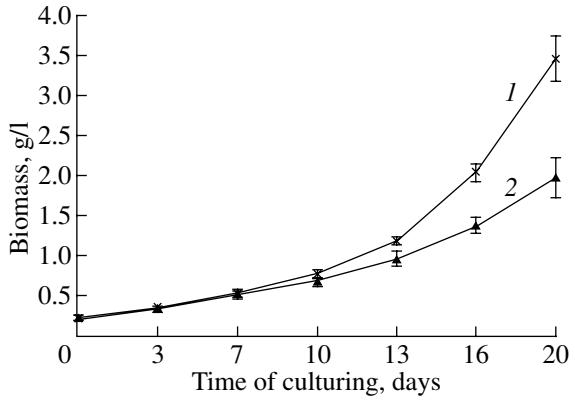

Fig. 1. Biomass accumulation in *B. braunii*. (*1*) Control; (*2*) nitrogen limitation.

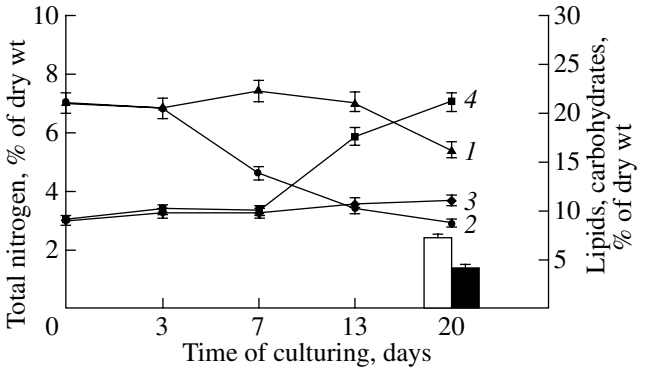

Fig. 2. Contents of (*1*, *2*) total nitrogen and (*3*, *4*) lipids (*1*, *3*) in the control and (*2, 4*) at nitrogen limitation in *B. braunii*. Columns—carbohydrate content by the 20th day (□ control; ■ nitrogen limitation).

continuously bubbled with air enriched with CO_2 (1 vol %) using a membrane compressor delivering 1 l/min. A Prat medium modified by us [11] was used in the control culture; nitrogen limitation was created by decreasing the potassium nitrate concentration in the medium fourfold.

Culture samples (25 ml) for analyses were regularly taken in the course of experiments. Biomass concentration was determined after filtering the samples through preliminary weighed Vladipor filters (pore diameter of 0.85–0.95 μm). The filters were dried to constant weight at 70°C and reweighed.

Total nitrogen was estimated by Kjeldahl micromethod, and carbohydrates, by anthrone method [12]. For extracting lipids, an algal suspension aliquot was centrifuged, the biomass sediment was washed with 0.2% NaCl, fixed with boiling propan-2-ol, and successively extracted three times with a mixture of chloroform and propan-2-ol (1 : 1, v/v) [13].

Lipid extracts were separated by microTLC using silica gel–gypsum glass plates in a system for neutral lipids (hexane : diethyl ether : acetic acid = 85 : 15 : 1, v/v/v) [13, 14]. The lipids were identified by comparing them with standards as regards their R_f values, and diacylglycerols, triacylglycerols, FAs, FA methyl esters, sterols, and sterol esters (Serva, Germany, and Sigma, United States) were used as the standards. Individual lipid classes were quantified using a dichromate method and subsequent measuring of optical density at 350 nm against distilled water with the optical-path length of 1 cm [15]. FA methanolysis was performed in a mixture of methanol and sulfuric acid (50 : 1, v/v) at 90°C for 2 h. FA methyl esters were analyzed using a GCD Plus chromatomass spectrometer (Hewlett Packard, United States) equipped with an HP-5 capillary column of 30-m length and 0.25-mm internal diameter (Hewlett Packard) under following conditions: carrier gas, helium at 1 ml/min; sample-injection temperature, 230°C; initial column temperature, 100°C; temperature increase to 230°C at the rate of 8°C/min; and detector temperature, 230°C. The samples were injected using gas-flow splitting (1 : 50). FAs were identified by comparing their mass spectra and retention times with those of available standards (from Serva and Sigma). Double-bond positions in monoenoic acids were determined using the mass spectra of dimethyl-disulfide derivatives of respective FA methyl esters [16]. FA content was referred to as mol %.

The experiments were carried out in three replications. The Fischer criterion and Excel package were used for assessing the significance of differences.

RESULTS

After 20 days of culturing *B. braunii* under the conditions of nitrogen limitation, the biomass yield was virtually two times less as compared to the control (Fig. 1); the content of nitrogen-containing compounds decreased from 6.8 to 2.9% per dry biomass, and that of total lipids increased up to 21% (Fig. 2). At the same time, by the end of the experiment, the content of carbohydrates was 1.7-fold less than in the control, being equal to 4.2%.

The effect of nitrogen limitation on individual lipid classes was as follows. After three days of nitrogen limitation, as also in the control, polar lipids comprised more than a half of algal lipids (Fig. 3). After 13 days of nitrogen limitation, i.e., in the middle of the experiment period, triacylglycerol content increased virtually threefold, from 8 to 25% of total lipids, while in the control, this fraction increased from 5 to 16% of total lipids only by the 20th day. An increase in triacylglycerol content was accompanied by a decrease in that of the polar lipid fraction, both in the experiment (from 51 to 30%) and in the control (from 55 to 45%). In the course of nitrogen limitation, the content of sterol fraction increased from 5.8 to 11.8%. At the same time, both under nitrogen limitation and in the control, there were no significant changes in the content of other lipid classes, such as free FAs, alcohols, sterol esters, and hydrocarbons. Such tendency for a redistribution of

individual lipid classes became most pronounced after recalculating their content per dry weight (Table 1). Under nitrogen limitation, an increase in the content of total lipids occurred mainly due to triacylglycerols. By the end of the experiment, triacylglycerol content per unit dry weight increased fivefold, while there were no significant changes in the absolute content of polar lipids and other fractions. However, it must be emphasized that, by the 13th day, the content of polar lipids decreased from 4.5 to 3.8%. In the control, the increase in the content of triacylglycerols up to 1.7% was observed only by the 20th day.

In line with earlier evidence [17], the FA composition at the active-growth stage of *B. braunii* was characterized by a high content of C_{16} and C_{18} polyenoic acids (from 65 to 77% of total FAs) (Table 2), while the monoenoic acid content ranged from 4.0 to 6.6%. Monoenoic to polyenoic acid and monoenoic to dienoic acid ratios were minimal, comprising 0.10 and 0.3–0.5, respectively. By the end of the experiment (i.e., by the 20th day), there were a decrease in the content of trienoic acids (from 48.5–61.7 to 38.7%) and an increase in that of oleic acid (from 1.0–1.2 to 13.1%). All these changes were statistically significant. The relative content of linoleic acid remained unchanged, while that of another dienoic acid, $C_{16:2}$, decreased about twofold. Meanwhile, a monoenoic to dienoic acid ratio increased threefold, and a monoenoic to polyenoic acid ratio, fourfold. As a whole, under nitrogen limitation, the directionality of FA redistribution was more pronounced, and the changes were observed at earlier stages of culture development. Thus, as early as at the 13th day, the culture of *B. braunii* grown under nitrogen limitation was characterized by a virtually threefold decrease in the unsaturation index of lipids. Such change occurred due to an increase in the contents of saturated acids (from 23.7–26.0 to 46.1%) and oleic acid (from 1.1 to 17.0%) and a decrease in the content of all polyenoic acids (from 67.3–70.8 to 26.9%), including linoleic acid. The content of α-linolenic acid decreased virtually threefold. The monoenoic to dienoic acid ratio increased tenfold, and that of monoenoic to dienoic acid, six–sevenfold. The changes in algal lipid FA composition under nitrogen limitation remained up to 20th day.

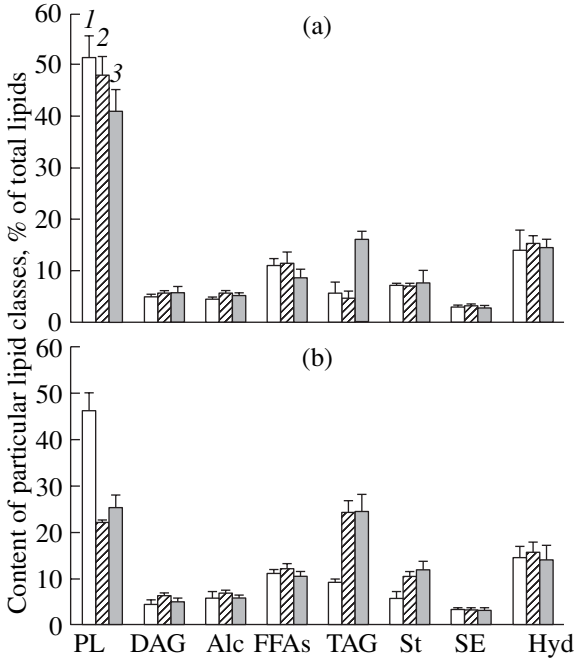

Fig. 3. Intracellular lipid composition in *B. braunii*.
(a) Control; (b) nitrogen limitation; (*1*) 1st–3rd days; (*2*) 13th day; (*3*) 20th day; PL—polar lipids; DAG—diacylglycerols; Alc—alcohols; FFAs—free FAs; TAG—triacylglycerols; St—sterols; SE—sterol esters; Hyd—hydrocarbons.

Both under nitrogen limitation and in the control, the FA composition of *B. braunii* polar lipids was characterized by the presence of C_{12}–C_{26} acids (Table 3),

Table 1. The composition of major lipid classes in *B. braunii* grown on a complete medium or under nitrogen limitation, % of dry weight

Class	Control				Nitrogen limitation			
	3rd day	13th day	20th day	F_a	3rd day	13th day	20th day	F_b
Polar lipids	5.0 ± 0.1	5.2 ± 0.8	4.4 ± 0.1	0.7	4.5 ± 0.2	3.8 ± 1.0	5.3 ± 0.5	1.5
Diacylglycerols	0.5 ± *m*	0.6 ± 0.1	0.5 ± *m*	1.4	0.5 ± 0.1	1.1 ± 0.4	1.0 ± 0.1	2.0
Alcohols	0.4 ± *m*	0.6 ± 0.2	0.6 ± 0.1	0.8	0.6 ± 0.1	1.2 ± 0.4	1.2 ± 0.1	1.9
Free FAs	1.1 ± 0.2	1.2 ± 0.1	0.9 ± 0.2	1.5	1.1 ± 0.2	2.0 ± 0.3	2.1 ± 0.4	3.1
Triacylglycerols	0.5 ± 0.2	0.5 ± *m*	1.7 ± 0.1	204.7	1.0 ± 0.2	4.1 ± 1.0	5.1 ± 0.2	14.7
Sterols	0.7 ± *m*	0.7 ± 0.1	0.8 ± *m*	1.4	0.6 ± 0.2	1.7 ± 0.2	2.5 ± 0.6	6.5
Sterol esters	0.3 ± *m*	0.3 ± 0.1	0.3 ± *m*	0.8	0.3 ± *m*	0.5 ± 0.1	0.6 ± *m*	4.2
Hydrocarbons	1.4 ± 0.4	1.6 ± 0.1	1.6 ± 0.1	1.3	1.5 ± 0.2	2.7 ± 0.8	2.9 ± 0.2	2.4

Notes: M—mean; *m*—standard error ($m < 0.1$); F_a—Fischer criterion calculated for the control; F_b—Fischer's criterion calculated for the nitrogen limitation experiment; $F = 8.02$, standard value for $P \le 0.01$.

Table 2. FA composition of total lipids of *B. braunii* grown on a complete Prat medium and at nitrogen limitation, % of total FAs

Acid	Control					Nitrogen limitation				
	3rd day	7th day	13th day	20th day	F_a	3rd day	7th day	13th day	20th day	F_b
16:0	16.2 ± 2.2	12.4 ± 0.3	12.8 ± 2.4	22.4 ± 2.6	8.2	14.8 ± 0.5	15.9 ± 1.8	22.5 ± 1.8	19.1 ± 1.2	12.7
16:1ω7	1.2 ± 0.1	0.9 ± 0.4	0.8 ± 0.2	0.4 ± 0.2	3.1	1.0 ± 0.1	0.7 ± 0.1	1.5 ± 0.1	0.8 ± 0.1	18.1
16:1ω13*tr*	0.1± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	10.1	0.3 ± *m*	0.3 ± *m*	traces	0.1 ± *m*	285.8
16:2	3.1 ± 0.4	3.7 ± 0.2	1.7 ± 0.4	1.9 ± 0.2	15.5	3.4 ± 0.4	3.8 ± 0.7	1.6 ± 0.1	2.3 ± 0.3	8.1
16:3	10.8 ± 1.1	16.6 ± 1.1	7.9 ± 1.9	7.5 ± 0.8	18.9	11.6 ± 0.6	12.5 ± 1.6	5.6 ± 1.4	5.7 ± 0.7	21.6
18:0	3.4 ± 0.4	1.9 ± 0.3	5.2 ± 0.5	2.6 ± 0.5	20.4	2.7 ± 0.4	2.0 ± 0.3	7.3 ± 0.5	4.8 ± 0.1	129. 6
18:1ω9	1.0 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	13.1 ± 2.3	55.0	1.1 ± 0.1	1.2 ± 0.1	17.1 ± 3.3	24.4 ± 1.0	192.7
18:2	9.9 ± 0.7	11.2 ± 0.5	9.3 ± 0.2	10.4 ± 0.2	5.1	11.1 ± 0.3	9.8 ± 0.7	5.8 ± 0.6	8.4 ± 0.2	49.0
18:3ω3	40.2 ± 1.4	45.1 ± 1.4	40.6 ± 1.0	31.2 ± 0.6	43.0	41.2 ± 0.5	44.7 ± 0.9	13.9 ± 5.1	19.0 ± 1.2	139.9
Other FAs*	14.1 ± 2.3	6.7 ± 1.1	20.3 ± 3.5	10.0 ± 2.4	–	12.8 ± 3.1	9.1 ± 1.1	24.7 ± 2.9	15.4 ± 1.3	–
Total unsaturated/total saturated	2.5 ± 0.2	4.4 ± 0.4	1.9 ± 0.1	2.5 ± 0.2	31.3	2.7 ± *m*	3.2 ± 0.4	1.1 ± 0.2	2.0 ± *m*	39.6
Total monoenoic/total polyenoic	0.1 ± *m***	0.1 ± *m*	0.1 ± *m*	0.4 ± *m*	189.2	0.1 ± *m*	0.1 ± *m*	1.0 ± 0.4	0.9 ± *m*	32.9
Total monoenoic/total dienoic	0.5 ± 0.1	0.3 ± 0.1	0.4 ± *m*	1.6 ± 0.1	96.9	0.4 ± *m*	0.4 ± 0.1	3.6 ± 0.8	2.8 ± *m*	85.2

Notes: $F = 8.02$, standard value for $P \leq 0.01$.

* 12:0, 14:0, 14:1, 15:0, 16:1ω6, 18:1ω7, 20:0, 20:4, 20:5, 22:0, 24:0, 26:0.

** $m < 0.1$.

and both C_{16} and C_{18} polyenoic acids, mainly hexadecatrienoic acid (from 1.2 to 17.8% of total FAs) and α-linolenic acid (from 2.4 to 44.7%), as well as a saturated palmitic acid C–16:0 (from 11.6 to 39.9%) predominated among these acids. It must be pointed out that polyenoic acids exceeding 18 carbon atoms in their chain length, such as $C_{20:4}$ and $C_{20:5}$, were located only in polar lipids. At the stage of an active culture growth, the oleic acid content, which ranged from 0.5 to 8.9%, was minimum, while the polyenoic acid content during this period was maximum (from 56.9 to 76.8%). The 13-day-old culture grown under nitrogen limitation substantially differed from all other samples taken both before and after this time in the FA acid composition of polar lipids. This culture was characterized by a dramatic decrease in the content of all polyenoic acids (to 6.8%) and an increase in the saturated acid content (up to 73.8%). However, by the end of the experiment, the polar lipid FA composition in the nitrogen-limited culture was more consistent with the control one. Nevertheless, the content of trienoic acids decreased 1.4-fold, and that of monoenoic acids increased from 2.6 to 3.3-fold as compared to the culture at the stage of active growth.

Both under nitrogen limitation and in the control, the FA composition of triacylglycerols in *B. braunii* was represented by C_{12}–C_{26} FAs, and saturated acids (45–70%) as well as oleic acid (15–40%) predominated among them (Table 4). The content of polyenoic acids, mainly linoleic acid, ranged from 3.6 to 11.6%. By the 3rd day, both under nitrogen limitation and in the control, saturated acids predominated in triacylglycerols. However, by the 7th day under nitrogen limitation, the content of oleic acid increased, and its content remained high up to end of the experiment. The same changes in the FA composition of triacylglycerols were observed in the 13-day-old culture, and this trend persisted up to the end of the experiment.

DISCUSSION

The work presented here was devoted to the effect of nitrogen on both *B. braunii* Kütz IPPAS H-252 growth and chemical composition. It should be pointed out that *B. braunii* Kütz IPPAS H-252 strain studied here is more alike to another *Botryococcus* species, viz., the *Botryococcus sudeticus*, a representative of green algae, in its key indices, such as hydrocarbon and FA acid composition. The results of this investigation were published in [18].

At earlier stages of culturing (until the 10th day), a decrease in the concentration of potassium nitrate in the medium to 25% of the control one did not affect the rate of algal growth as compared to the control. However, further culturing on a nitrogen-limited medium resulted in a decrease in both algal growth and biomass yield (Fig. 1). Such decrease was accompanied by changes in the biochemical composition, such as a decrease in the content of nitrogen compounds and carbohydrates, as well as in an enhanced lipid synthesis (Fig. 2). The results presented here demonstrate that total lipid content increased due to triacylglycerols (Table 1). This evidence is consistent with earlier results for some algae, in which an increase in the triacylglycerol content as a response to nitrogen limitation was usually observed. When culturing *Scenedesmus obliquus* [3] and *Chlamydomonas reinhardtii* [19], a significant increase in the triacylglycerol content as a response to nitrogen limitation was also observed.

Other lipid components, such as wax esters, are also known to act as reserve substances in algae [20]. Hydrocarbons formed by *B. braunii* can hardly be regarded as reserve substances because a number of researchers claim that hydrocarbon synthesis in a given alga is maximal during exponential growth stage [21]. Published data on the effect of nitrogen limitation on the hydrocarbon synthesis available at present are very contradictory. Thus, it was demonstrated [9, 10] that a higher initial concentration of nitrate in the medium caused a higher hydrocarbon yield. However, an opposite result was obtained later [22]. It was demonstrated that nitrogen limitation stimulated hydrocarbon synthesis. Our data show that nitrogen limitation does not enhance hydrocarbon synthesis, and they are consistent with a view that these substances do not function as reserve compounds in the *B. braunii* strain under investigation.

The FA composition of microalgal lipids varies depending on the physiological state of cells, which substantially changes in the course of culture development. The content of polyenoic acids in the actively-photosynthesizing algal cells is known to increase, and a transition of an alga to a stationary phase is accompanied by an increase in the content of saturated and monoenoic acids (mainly oleic acid) and a decrease in the content of polyenoic acids [23, 24]. By the 20th day, the FA composition of the control culture was characterized by an increase in the oleic acid content accompanied by a decrease in the content of polyenoic acids, first of all, α-linolenic acid, and these changes could be regarded as an indicator of the transition of the culture to a stationary phase of growth. Similar rearrangement of the FA composition of *B. braunii* lipids in the course of the development of the alga was pointed out by us earlier [17].

Changes in the FA composition of lipids in the alga under investigation caused by nitrogen limitation proceeded mainly at the expense of a decrease in the content of α-linolenic acid accompanied by an increase in the content of oleic acid as a more saturated acid, and these changes are consistent with the evidence obtained on other algae [4, 25–27].

Reserve acyl-containing lipids are known to differ in their FA composition from the membrane lipids. The membrane lipids include sulfolipids, phospholipids, and galactolipids, and the latter are mainly represented by monogalactosyldiacylglycerols and digalactosyldia-

Table 3. FA composition of polar lipids of *B. braunii* grown on a complete Prat medium and at nitrogen limitation, % of total FAs

Acid	Control					Nitrogen limitation				
	3rd day	7th day	13th day	20th day	F_a	3rd day	7th day	13th day	20th day	F_b
16:0	11.6 ± 1.4	20.7 ± 3.1	33.6 ± 9.9	27.0 ± 6.1	4.0	28.2 ± 9.7	23.0 ± 7.0	39.9 ± 3.4	22.6 ± 1.9	2.8
16:1ω7	1.0 ± 0.5	0.9 ± 0.1	0.7 ± 0.3	0.3 ± *m*	1.7	1.0 ± *m*	0.7 ± 0.2	0.4 ± 0.1	1.4 ± 0.4	5.9
16:1ω13*tr*	0.2 ± 0.1	1.4 ± 0.4	1.2 ± 0.4	0.6 ± 0.2	6.8	0.9 ± 0.4	0.7 ± 0.3	0.1 ± 0.1	1.1 ± 0.2	3.3
16:2	5.1 ± 0.2	4.4 ± 0.3	2.3 ± 0.9	2.6 ± 0.5	10.2	4.1 ± 0.5	4.6 ± 1.1	0.4 ± 0.1	2.1 ± 0.2	16.7
16:3	17.8 ± 1.4	15.8 ± 2.0	8.8 ± 3.8	10.1 ± 1.9	5.4	11.4 ± 3.9	14.4 ± 2.3	1.2 ± 0.4	7.9 ± 0.7	10.5
18:0	1.6 ± *m***	2.7 ± 0.7	5.6 ± 2.3	1.0 ± 0.3	4.8	2.6 ± 0.9	2.2 ± 0.2	17.1 ± 1.5	3.8 ± 0.8	88.1
18:1ω9	0.5 ± *m*	0.5 ± *m*	1.2 ± 0.5	0.6 ± 0.1	3.0	0.6 ± *m*	0.7 ± *m*	8.9 ± 1.9	5.5 ± 2.9	9.5
18:2	9.2 ± 0.7	9.5 ± 0.5	7.3 ± 2.4	11.3 ± 0.5	2.8	9.5 ± 1.8	8.7 ± 1.2	2.8 ± 0.5	9.1 ± 0.8	11.8
18:3ω3	44.7 ± 3.3	36.3 ± 2.7	25.0 ± 9.0	39.4 ± 3.5	4.2	31.9 ± 7.9	36.4 ± 4.4	2.4 ± 0.5	28.8 ± 6.4	12.8
Other FAs*	8.3 ± 1.7	7.8 ± 1.6	14.3 ± 2.2	7.1 ± 0.9	–	9.8 ± 1.7	8.6 ± 1.9	26.8 ± 4.1	17.7 ± 5.1	–
Total unsaturated/total saturated	5.1 ± 0.6	2.8 ± 0.7	1.8 ± 1.2	2.4 ± 0.5	5.8	2.4 ± 1.0	2.9 ± 0.9	0.2 ± 0.1	1.9 ± 0.4	4.5
Total monoenoic/total polyenoic	0.1 ± *m*	0.1 ± *m*	0.2 ± *m*	0.1 ± *m*	3.2	0.1 ± *m*	0.1 ± *m*	1.9 ± 0.1	0.4 ± 0.1	198.4
Total monoenoic/total dienoic	0.3 ± 0.1	0.4 ± 0.1	0.9 ± 0.4	0.4 ± 0.1	3.3	0.5 ± 0.2	0.5 ± 0.1	3.9 ± 0.1	1.5 ± 0.4	70.4

Note: $F = 8.02$, standard value for $P \le 0.01$.

* 2:0, 14:0, 14:1, 15:0, 16:1ω6, 18:1ω7, 20:0, 20:4, 20:5, 22:0, 24:0, 26:0.

** $m < 0.1$.

Table 4. FA composition of triacylglycerols of *B. braunii* grown on a complete Prat medium and at nitrogen limitation, % of total FAs

Acid	Control					Nitrogen limitation				
	3rd day	7th day	13th day	20th day	F_a	3rd day	7th day	13th day	20th day	F_b
16:0	41.8 ± 2.2	36.5 ± 3.5	29.9 ± 7.9	34.1 ± 2.5	1.9	38.7 ± 2.5	32.6 ± 2.2	35.7 ± 3.3	32.2 ± 2.3	2.3
16:1ω7	3.0 ± 0.3	4.2 ± 0.8	2.8 ± 1.0	1.5 ± 0.1	5.4	3.7 ± 0.2	2.4 ± 0.4	3.0 ± 0.2	2.0 ± 0.3	10.6
16:2	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	1.0 ± 0.3	11.2	0.2 ± 0.1	1.1 ± 0.3	1.1 ± 0.2	1.7 ± 0.3	12.8
16:3	0.3 ± 0.1	0.2 ± *m***	0.3 ± 0.2	3.4 ± 0.7	32.2	0.2 ± 0.1	2.1 ± 0.7	1.3 ± 0.5	1.8 ± 0.5	5.6
18:0	11.2 ± 3.0	11.7 ± 1.8	12.3 ± 2.7	6.6 ± 0.5	2.4	16.2 ± 3.5	5.5 ± 0.7	8.1 ± 0.7	7.7 ± 0.7	10.9
18:1ω9	14.4 ± 4.9	21.0 ± 2.8	31.0 ± 7.4	40.0 ± 1.6	9.7	14.7 ± 2.5	34.2 ± 0.6	28.4 ± 5.1	38.4 ± 3.2	16.7
18:2	3.2 ± 0.9	5.7 ± 1.1	3.9 ± 0.7	6.4 ± 1.1	4.4	3.2 ± 0.7	8.4 ± 1.4	3.7 ± 0.7	5.2 ± 0.8	10.5
18:3	1.6 ± 0.8	0.7 ± 0.5	traces	traces	4.1	traces	traces	traces	0.7 ± 0.7	1.7
Other FAs*	24.3 ± 3.2	19.9 ± 2.6	19.5 ± 4.2	7.0 ± 1.5	–	23.1 ± 4.2	13.7 ± 1.9	18.7 ± 1.3	10.3 ± 1.8	–
Total unsaturated/total saturated	0.5 ± 0.2	0.8 ± 0.1	1.0 ± 0.2	1.3 ± 0.1	6.3	0.4 ± *m*	1.3 ± 0.1	1.0 ± 0.1	1.3 ± 0.1	34.2
Total monoenoic/total polyenoic	6.6 ± 2.2	5.7 ± 0.9	10.2 ± 1.5	4.2 ± 0.2	5.9	8.4 ± 2.2	4.2 ± 1.0	7.5 ± 1.2	5.3 ± 1.3	2.8
Total monoenoic/total dienoic	8.7 ± 1.4	6.4 ± 0.8	11.0 ± 1.4	6.3 ± 0.7	6.6	9.0 ± 2.5	5.0 ± 1.0	9.4 ± 1.3	7.1 ± 1.3	2.6

Note: $F = 8.02$, standard value for $P \le 0.01$.

* 12:0, 14:0, 14:1, 15:0, 16:1ω6, 20:0, 22:0, 24:0, 26:0.

** $m < 0.1$.

cylglycerols. The FA composition of polar lipids is, for the most part, represented by polyenoic acids [28]. Therefore, an enhanced synthesis of just oleic acid-rich triacylglycerols can affect the composition of total FAs.

Are these changes indeed related to increased triacylglycerol content in the biomass, or to changes in the FA composition of all acyl-containing lipids, which are characterized by a functional importance for a cell? The distribution of FAs in the polar lipid and triacylglycerol fractions was considered just to solve this problem.

Triacylglycerols considerably differed from polar lipids in their FA composition. Triacylglycerols virtually lacked α-$C_{18:3}$, $C_{20:4}$, $C_{20:5}$, and $C_{16:1\omega13tr}$ acids, and their content in $C_{16:3}$ and dienoic acids was manifold less than that in the polar lipids. At the active growth stage, saturated acids predominated, and an increase in the lipid content in the biomass was accompanied by the synthesis of triacylglycerols characterized by high oleic acid content. It is notable that the three-day-old control and treated cultures were quite similar in the FA composition of triacylglycerols, and this composition was characteristic of actively growing cultures. The nitrogen-limited seven-day-old culture did not differ from the control one in its biomass, and, at this time, was characterized by an onset of synthesis of the triacylglycerols with high oleic acid content. Triacylglycerols of this type persisted during further culturing under nitrogen limitation, while, in the control, they appeared only in the 13-day-old culture, although the content of total lipids and triacylglycerols in the culture did not change by this time.

Other algae, such as *Neochloris oleoabundans, Chlorella vulgaris*, and *Scenedesmus obliquus*, are also capable of storing oleic acid-rich triacylglycerols under nitrogen limitation [3, 29]. However, these studies did not consider changes in the FA composition of triacylglycerols.

At the stage of an active culture growth, both control and nitrogen-deficient *B. braunii* cells did not differ in the FA composition of their polar lipids, which was similar to that of total lipids. This is quite understandable, because, during this growth period, polar lipids predominated among the total ones. Under nitrogen limitation, the 13-day-old culture was characterized by a considerable increase in the content of saturated acids, which was accompanied by a decrease in the content of all polyenoic acids. Moreover, by the 13th day, the color of the culture changed from green to yellowish-brown. It can be assumed that this change was caused by an increase in the carotenoid/chlorophyll ratio, and this increase could be related to a partial degradation of chloroplast membranes, which caused a decrease in the content of polar lipids during this period. Termination of protein synthesis in the absence of micronutrients in nutrient medium is known to affect the integrity of cellular structures. A breakdown of chloroplast membrane structure causes termination of synthesis of galactolipids, the major lipids of photosynthetic membranes [30, 31], and such breakdown seems to have occurred in our experiment under nitrogen limitation. It could be assumed that the FA pool of polar lipids was, as a result of catabolism of polar lipids, redirected to the triacylglycerol synthesis, and the content of the latter during this period increased several-fold. The redistribution of carbon from polar lipids toward the neutral ones under nitrogen limitation in a medium was observed in other algae as well [3]. However, in our experiments, by the 20th day of nitrogen limitation, there were an increase in the content of polar lipids and polyenoic acids acylating them and restoration of the initial green color of the culture. Therefore, it could be assumed that these processes were caused by some profound metabolic changes, which resulted in a partial restoration of the vital activity of the alga. However, by the 20th day, the content of polyenoic acids was considerably less than at the stage of active growth.

It can be concluded that, under nitrogen limitation, *B. braunii* Kütz IPPAS H-252 cells accumulated lipids in the form of oleic acid-rich triacylglycerols. In this strain, there was no evidence for the effect of nitrogen limitation on hydrocarbon synthesis. The changes in the FA composition of the alga manifested themselves in both triacylglycerol accumulation and a change in the FA composition of polar lipids.

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