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**EXPERIMENTAL ARTICLES**

# Lipids of the Green Alga *Botryococcus* Cultured in a Batch Mode

**G. S. Kalacheva, N. O. Zhila, and T. G. Volova**

*Institute of Biophysics, Siberian Division, Russian Academy of Sciences, Krasnoyarsk, 660036 Russia*

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**Abstract**—The lipid fraction of the green alga *Botryococcus* cultured in a batch mode was found to contain polar lipids (more than 50% of the total lipids), di- and triacylglycerols, sterols and their esters, free fatty acids, and hydrocarbons. In aging culture, the content of polar lipids somewhat decreased and that of triacylglycerols increased by more than four times. The content of hydrocarbons in the algal biomass did not exceed 0.9% and depended little on the culture age. Intracellular lipids contained saturated and unsaturated (mono-, di-, and trienoic) fatty acids. The maximum content of $C_{16:3}$ and $\alpha$-$C_{18:3}$ fatty acids (up to 35% of the total fatty acids) was detected in the phase of active growth. The extracellular and intracellular lipids of the alga differed in the proportion of particular lipids and in the fatty acid pattern.

*Key words*: *Botryococcus braunii*, batch culture, lipids, fatty acids.

The green colonial alga *Botryococcus* is able to synthesize liquid hydrocarbons, whose yield and composition depend on the strain and cultivation conditions. The content of hydrocarbons in different strains of this alga varies from 3 to 75% and is maximum in the stage of active growth [1, 2]. There is evidence that the synthesis of hydrocarbons is closely related to the fatty acid pattern of the alga [3, 4]. It should be noted that data concerning the fatty acids of the alga are contradictory [5–8] and that the investigations of *B. braunii* are mainly concerned with the structure of its hydrocarbons and lipids. At the same time, data on the influence of environmental factors and the physiological state of *B. braunii* on its lipid metabolism are virtually absent, although they are very important for understanding the mechanism of the algal synthesis of hydrocarbons and the optimization of their production.

The aim of the present work was to study the extracellular and intracellular lipids of the alga *Botryococcus* in the course of its cultivation in a batch mode.

## 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 [9].

The lipids of algal cells were studied as follows. Cells from 50–100 ml of algal culture were harvested by centrifugation, washed in 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 overnight at room temperature. The extract was then 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 evaporator, the lipids were dried further in a desiccator, and they were quantified by weighing them together with the flask.

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

Lipid extracts were separated by thin-layer microchromatography 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) [10, 11]. Polar lipids were separated by two-dimensional chromatography using a chloroform–acetone–methanol–85% formic acid–water (150 : 43 : 43 : 8.5 : 1) solvent system in the first dimension and an acetone–benzene–85% formic acid–water (200 : 27 : 2.7 : 8.5) solvent system in the second dimension. The separated lipids were identified by comparing their $R_f$ values with those of the respective authentic lipid samples and by spraying the developed plates with specific reagents: ninhydrin, anthrone, Vaskovsky reagent, Dragendorf reagent, and sulfuric acid [12–14]. The lipids were quantified by the dichromate method, measuring absorbance at

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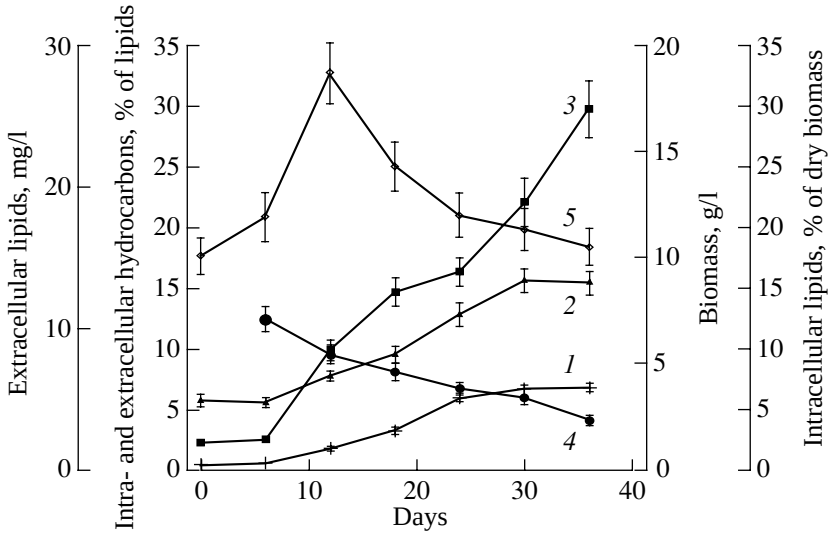


**Fig. 1.** Dynamics of the (*1*) biomass, (*2*) intracellular lipids, (*3*) extracellular lipids, (*4*) intracellular hydrocarbons, and (*5*) extracellular hydrocarbons in a batch culture of *B. braunii*.

350 nm against distilled water in 1-cm-path-length cuvettes [14].

Fatty acid methyl esters were analyzed with a Chrom-5 gas–liquid chromatograph (Laboratorni Pristroje, Czech Republic) and identified by comparing their retention times with those of the respective authentic fatty acids (saturated and monoenoic $C_{10}$–$C_{20}$ acids, $C_{18:2}$, $\alpha$-$C_{18:3}$, and $\gamma$-$C_{18:3}$) purchased from Serva (Germany) and Sigma (the United States). Fatty acids were also identified by mass-chromatography using a GCD Plus spectrometer (Hewlett Packard). The position of double bonds in monoenoic acids was determined after the dimethyl sulfide derivatization of the respective fatty acid methyl esters [15].

**Table 1.** The content of various fatty acids in the *B. braunii* lipids (% of the total fatty acids)

| Fatty acid | Cultivation time, days | | | | |
|---|---|---|---|---|---|
| | 0 | 6 | 13 | 24 | 38 |
| C12 : 0 | 0.9 | Traces | 0.1 ± 0.0 | 0.4 ± 0.1 | 0.2 |
| C14 : 0 | 2.8 | 0.1 ± 0.0 | 0.8 ± 0.2 | 1.5 ± 0.3 | 0.8 |
| C14 : 1 | 0.3 | 0.1 ± 0.0 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.6 |
| C15 : 0 | 2.5 | 0.4 ± 0.2 | 0.5 ± 0.2 | 0.3 ± 0.1 | 0.5 |
| C16 : 0 | 42.8 | 21.0 ± 2.2 | 16.6 ± 1.1 | 14.8 ± 1.2 | 27.9 |
| C16 : 1* | 9.0 | 1.4 ± 0.3 | 2.4 ± 0.4 | 2.8 ± 0.6 | 1.8 |
| C17 : 0 | Traces | 3.1 ± 1.0 | 1.7 ± 0.9 | 1.5 ± 1.1 | 1.6 |
| C16 : 2 | 0.8 | Traces | Traces | Traces | Traces |
| C16 : 3 | 1.3 | 4.6 ± 1.3 | 6.2 ± 1.1 | 3.4 ± 0.8 | 2.1 |
| C18 : 0 | 10.3 | 2.7 ± 0.6 | 4.3 ± 0.9 | 4.1 ± 0.4 | 2.0 |
| C18 : 1** | 16.4 | 21.8 ± 1.8 | 23.9 ± 1.4 | 29.8 ± 1.6 | 46.0 |
| C18 : 2 | 5.5 | 13.6 ± 1.4 | 11.9 ± 1.6 | 14.6 ± 1.3 | 7.4 |
| $\alpha$-C18 : 3 | 3.5 | 30.0 ± 2.5 | 28.8 ± 2.2 | 25.0 ± 0.9 | 9.1 |
| C20 : 1 | 1.6 | 0.8 ± 0.3 | 0.6 ± 0.2 | 0.6 ± 0.2 | Traces |
| C20 : 0 | 2.3 | 0.4 ± 0.2 | 0.2 ± 0.1 | 0.3 ± 0.1 | " |
| C22 : 0 | Traces | Traces | 0.5 ± 0.3 | 0.6 ± 0.3 | " |
| C24 : 0 | " | " | 1.1 ± 0.4 | 0.5 ± 0.2 | " |
| $\frac{\text{Saturated}}{\text{Unsaturated}}$ | 1.6 | 0.33 | 0.32 | 0.31 | 0.49 |

* The sum of the $\Delta$7-$C_{16:1}$ and $\Delta$10-$C_{16:1}$ isomers and an unidentified $C_{16:1}$ isomer.
** The sum of the $\Delta$9-$C_{18:1}$ and $\Delta$11-$C_{18:1}$ isomers.

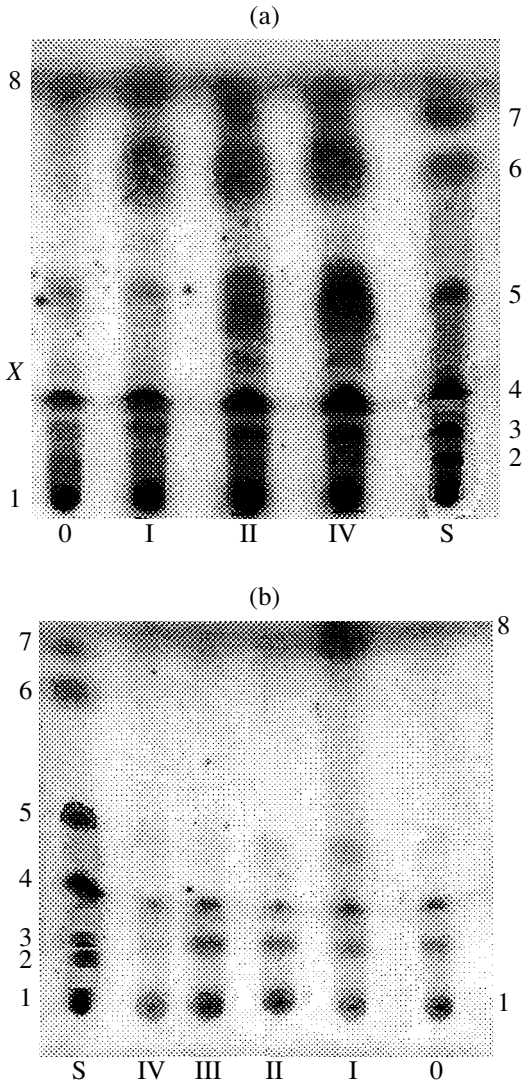


**Fig. 2.** Thin-layer chromatography in a hexane–diethyl ether–acetic acid (85 : 15 : 1) solvent system of the (a) intracellular and (b) extracellular lipids of *B. braunii.* Lanes: S, reference standard; 0, zero cultivation time; I, 6 days of growth; II, 13 days of growth; III, 24 days of growth; and IV, 38 days of growth. 1, Polar lipids; 2, diacylglycerols; 3, sterols; 4, free fatty acids; 5, triacylglycerols; 6, unidentified compound present in the sample and fatty acid methyl esters in standards; 7, sterol esters; 8, hydrocarbons; and *X*, unidentified compound detected in sample.

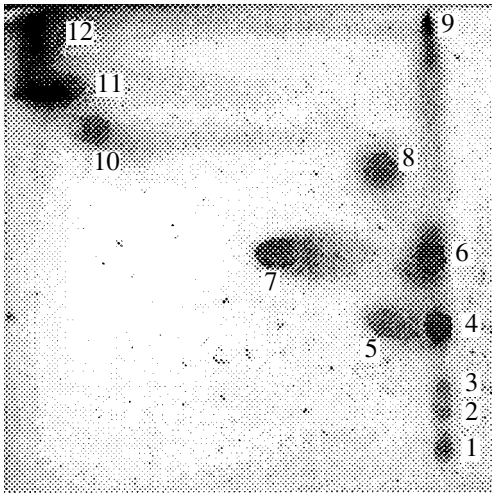


**Fig. 3.** Two-dimensional chromatography of the polar lipids of *B. braunii.* Chloroform–acetone–methanol–85% formic acid–water (150 : 43 : 43 : 8.5 : 1) in the first dimension and acetone–benzene–85% formic acid–water (200 : 27 : 2.7 : 8.5) in the second dimension. 1, Start; 2, 3, and 10, unidentified; 4, phosphatidylcholine; 5, sulfolipid; 6, phosphatidylethanolamine; 7, digalactosyldiacylglycerol; 8, phosphatidylglycerol; 9, free fatty acids; 11, monogalactosyldiacylglycerol; and 12, neutral lipids.

All the experiments were performed in triplicate. Data are presented as arithmetic mean ± standard error.

## RESULTS AND DISCUSSION

Earlier [9], we showed that actively growing *B. braunii* cells from the exponential and early linear growth phases contain maximum amounts of chlorophylls *a* and *b* (6.03 and 3.5 mg/g dry biomass, respectively) and total nitrogen (6–8% of the dry biomass). In aging culture from the stationary growth phase, the content of chlorophylls and nitrogen-containing cellular compounds decreased by 2–3 times, the content of potassium and magnesium in cells increased by about 2 times and that of phosphorus by 1.5 times, whereas the content of sodium somewhat decreased. In this case, the cellular content of carbohydrates increased by an order of magnitude and that of the total lipids by 2.5–3 times. Cellular hydrocarbons were detected in all growth phases in approximately equal amounts. At the same time, the concentration of extracellular hydrocarbons in the culture liquid was maximum in the late exponential and early linear growth phases (about 10 mg/l medium).

The content of cellular lipids in the actively growing *B. braunii* cells was low (6–8% of the dry biomass), increasing to 15–17% in the stationary growth phase (Fig. 1). The lipid fraction of cells was found to contain polar lipids, di- and triacylglycerols, sterols and their esters, free fatty acids (FFAs), hydrocarbons, as well as some unidentified compounds (Fig. 2a). Polar lipids were dominant in all growth phases, showing a maximum (50% or more of the total lipids) in the early linear growth phase. Among these lipids, we identified galacto- and phospholipids, including mono- and digalactosyldiacylglycerols, sulfolipids, phosphatidylglycerols, phosphatidylethanolamine, and phosphatidylcholine; three phospholipids remained unidentified (Fig. 3). The cellular content of sterols and their esters was low (less than 5% of the total lipids) and was almost independent of the growth phase (Fig. 4a). With the transition to the stationary phase, the content of diacylglycerols increased from 3.4 to 5.8% of the total lip-

ids and that of FFA increased from 8 to 11.3%. In this case, the cellular content of triacylglycerols, which serves as reserve compounds in eukaryotic algae [16], increased by more than four times. The relatively high content of FFA is probably typical not only of the *B. braunii* strain under study, but also of other *B. braunii* strains [8].

Cellular hydrocarbons were detected in the all growth phases of *B. braunii*, exhibiting a maximum (12–13% of the total lipids) in the physiologically active cells from the late exponential and early linear growth phases and a minimum (4–5%) in the stationary phase. With respect to the algal biomass, the cellular content of hydrocarbons did not exceed 0.9%.

The dynamics and the pattern of extracellular lipids (Fig. 2b) differed from those of intracellular lipids (Fig. 4b). The relative content of extracellular polar lipids (about 25% of the total lipids) was lower than that of intracellular polar lipids and depended weakly on the growth phase. The contents of extracellular FFAs, triacylglycerols, and sterols were close to that of polar lipids. The content of extracellular hydrocarbons was about three times higher than that of intracellular hydrocarbons, exhibiting a maximum in the actively growing *B. braunii* culture (Fig. 1).

It is believed that the set of fatty acids, which are precursors of algal hydrocarbons, in *B. braunii* is strain-specific [1]. Saturated fatty acids were dominated by palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids, whose relative contents in the course of the culture growth varied from 14.8 to 42.8% and from 2 to 10.3%, respectively (Table 1). Monoenoic acids were represented by three isomers of hexadecenoic acid and two isomers of octadecenoic acid. The isomers were identified by the mass spectrometry of the dimethyl sulfide addicts of fatty acids: the dominant isomer of $C_{16:1}$ gave rise to two ionic fragments with *m/z* 217 and 145, suggesting that the double bond is located at the carbon atom 9, as enumerated from the carbonyl terminus of the molecule. The second isomer of $C_{16:1}$ gave rise to two ionic fragments with *m/z* = 231 and 131, which corresponded to the position of the double bond at the carbon atom 10. We failed to identify the third isomer of $C_{16:1}$, but we assume that it is *trans*-3-hexadecenoic acid. The major isomer of octadecenoic acids was found to be oleic acid (it gave rise to two ionic fragments with *m/z* 217 and 173), whereas the fraction of *cis*-vaccenic acid, which gave rise to ionic fragments with *m/z* 245 and 145, did not exceed 3% of the total fatty acids. Polyenoic fatty acids were represented by dienoic and trienoic $C_{16}$ and $C_{18}$ acids. In the exponential-phase algal cells, lipids were more saturated than in the other growth phases due to the high relative content of $C_{16:0}$ and $C_{18:0}$ fatty acids (42.8 and 10.3%, respectively). In the linear growth phase, the degree of the lipid saturation decreased due to the high content of trienoic acids: in comparison with the exponential phase, the content of $\alpha$-$C_{18:3}$ and $C_{16:3}$ fatty acids

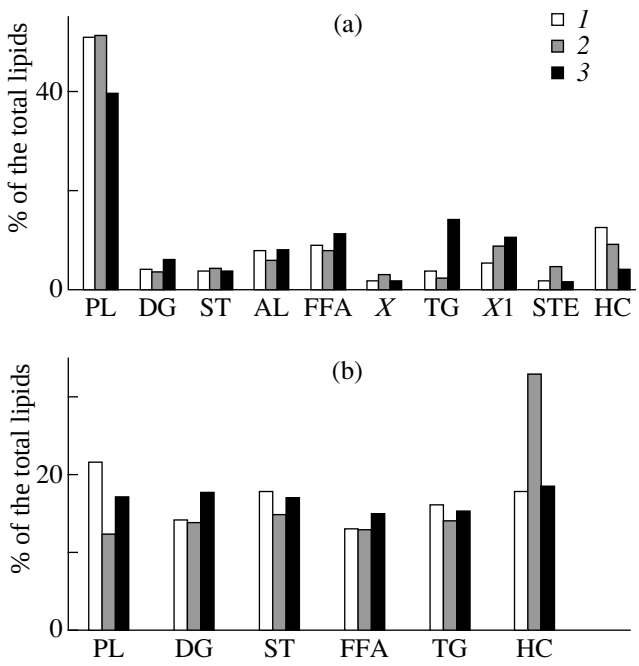


**Fig. 4.** (a) Intra- and (b) extracellular lipids in a batch culture of *B. braunii*: *1*, zero cultivation time; *2*, 6 days of growth; and *3*, 38 days of growth. Designations: PL, polar lipids; DG, diacylglycerols; ST, sterols; AL, alcohols; FFA, free fatty acids; TG, triacylglycerols; STE, sterol esters; HC, hydrocarbons; *X* and *X1*, unidentified.

increased from 1.6 to 25–30% and from 1.3 to 3.4–6.2%, respectively. By the beginning of the stationary growth phase, the relative content of polyenoic acids decreased and that of oleic acid increased, presumably due to the enhanced synthesis of triacylglycerols.

The major fatty acids of polar lipids were $C_{16:0}$ and $C_{18:1}$ (up to 16% of the total fatty acids in the period of the active algal growth), as well as the trienoic $C_{16:3}$ and $\alpha$-$C_{18:3}$ acids (up to 30%). The dienoic $C_{16:2}$ and $C_{18:2}$ acids were also detected in sufficient amounts (Table 2). The physiologically active algal cells from the exponential and early linear growth phases synthesized polar lipid by the so-called prokaryotic way, which is characterized by a high level of $C_{16:3}$ (more than 16% of the total fatty acids) and a relatively low level of another trienoic acid, $\alpha$-$C_{18:3}$ (about 13%). In the course of their growth, algal cells began to synthesize polar lipids by the eukaryotic way: the high relative content of $\alpha$-$C_{18:3}$ observed in the exponential phase (about 30% of the total fatty acids) remained at this level until the beginning of the stationary phase, whereas the content of all other polyenoic acids decreased. Since the content of monoenoic fatty acids did not change in this case, the decrease in the degree of the lipid unsaturation was obviously due to the increased content of the saturated $C_{16:0}$ fatty acid. In this case, polar lipids were insignificantly acylated by long-chain fatty acids.

**Table 2.** The content of various fatty acids in *B. braunii* polar lipids and triacylglycerols (% of total fatty acids)

| Fatty acid | Cultivation time, days | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | | 6 | 13 | | 24 | | 38 | |
| | polar lipids | triacyl-glycerols | polar lipids | polar lipids | triacyl-glycerols | polar lipids | triacyl-glycerols | polar lipids | triacyl-glycerols |
| C12 : 0 | 1.2 | 1.3 | 0.3 ± 0.1 | 0.4 ± 0.2 | 0.9 ± 0.3 | 0.3 ± 0.1 | Traces | 1.1 | 0.2 |
| C14 : 0 | 3.2 | 2.8 | 1.0 ± 0.3 | 1.1 ± 0.4 | 1.8 ± 0.4 | 0.8 ± 0.4 | 0.2 ± 0.1 | 0.9 | 1.0 |
| C14 : 1 | 0.5 | 0.4 | 0.6 ± 0.4 | 0.3 ± 0.2 | 0.6 ± 0.2 | 0.1 ± 0.1 | Traces | 0.8 | 0.6 |
| C15 : 0 | 3.2 | 1.6 | 0.3 ± 0.2 | 0.5 ± 0.4 | 2.8 ± 0.6 | 0.2 ± 0.2 | 0.1 ± 0.0 | 1.0 | 0.4 |
| C16 : 0 | 23.4 | 16.1 | 25.4 ± 1.4 | 24.6 ± 2.3 | 22.7 ± 1.9 | 22.6 ± 1.3 | 17.6 ± 2.6 | 38.7 | 21.9 |
| C16 : 1* | 3.3 | 3.2 | 1.7 ± 0.5 | 3.1 ± 0.8 | 1.2 ± 0.4 | 2.2 ± 0.5 | 0.9 ± 0.3 | 2.6 | 1.8 |
| C17 : 0 | 4.9 | 2.1 | 0.4 ± 0.2 | 0.9 ± 0.4 | 1.4 ± 0.6 | 1.5 ± 0.3 | 3.1 ± 1.1 | 2.4 | 3.5 |
| C16 : 2 | 0.3 | 0.1 | 0.1 ± 0.0 | 0.1 ± 0.0 | Traces | 0.9 ± 0.3 | 0.2 ± 0.1 | 0.4 | 0.2 |
| C16 : 3 | 16.0 | 1.2 | 3.4 ± 0.5 | 5.0 ± 1.0 | " | 3.0 ± 0.3 | 3.4 ± 0.6 | 2.9 | 2.2 |
| C18 : 0 | 1.8 | 13.0 | 1.2 ± 0.4 | 3.7 ± 0.5 | 7.1 ± 1.1 | 0.6 ± 0.2 | 3.8 ± 2.1 | 1.9 | 5.9 |
| C18 : 1** | 15.4 | 32.4 | 30.2 ± 1.6 | 26.1 ± 3.2 | 45.4 ± 2.3 | 33.1 ± 2.2 | 41.8 ± 3.3 | 27.8 | 43.7 |
| C18 : 2 | 8.6 | 10.0 | 5.5 ± 0.8 | 6.3 ± 1.4 | 0.9 ± 0.3 | 5.8 ± 1.1 | 13.1 ± 2.2 | 4.6 | 7.8 |
| α-C18 : 3 | 18.9 | 8.0 | 29.7 ± 1.3 | 27.9 ± 2.2 | 2.3 ± 1.2 | 28.3 ± 1.4 | 13.2 ± 2.5 | 12.7 | 5.8 |
| C20 : 0 | Traces | 0.7 | Traces | – | 0.7 ± 0.1 | 0.4 ± 0.2 | 0.6 ± 0.1 | Traces | 0.8 |
| C22 : 0 | " | 1.8 | " | – | 4.6 ± 0.5 | 0.1 ± 0.0 | 1.4 ± 0.3 | " | 0.9 |
| C24 : 0 | " | 5.3 | " | – | 7.6 ± 0.8 | 0.2 ± 0.1 | 0.6 ± 0.2 | 2.2 | 3.3 |
| $\frac{\text{Saturated}}{\text{Unsaturated}}$ | 0.59 | 0.55 | 0.38 | 0.45 | 0.52 | 0.36 | 0.32 | 0.85 | 0.49 |

The fatty acids of triacylglycerols, which serve as reserve substances in algae, were dominated by the $C_{16:0}$ and $C_{18:1}$ acids (up to 50–70% of the total fatty acids). Unlike polar lipids, triacylglycerols contained less polyenoic $C_{18}$ acids and almost no $C_{16:3}$ and $C_{16:2}$ acids (Table 2). Long-chain saturated fatty acids were also detected. The proportion between saturated and unsaturated fatty acids in triacylglycerols was almost constant in all growth phases.

The free fatty acids of the alga contained from 12 to 24 carbon atoms per molecule (Table 3) and were dominated by the $C_{16:0}$ (60–80% of the total FFA) and $C_{18:0}$ (5–15% of the total FFA) acids, which are the primary products of the biosynthesis of fatty acids. Polyenoic acids were represented only by $C_{18:2}$ (1–6% of the total FFA). The composition of FFA almost did not change during the algal growth.

The fatty acid composition of the *B. braunii* strain under study differed from that of other strains of this alga in the higher content of trienoic acids and in the absence of eicosapentaenoic acid and long-chain monoenoic acids [5–8]. In this respect, as well as in the low content of hydrocarbons, the strain at hand is closer to *Botryococcus sudeticus* than to *B. braunii*. It should be noted that Vazquez-Duhalt and Greppin, who studied the lipids, fatty acids, and hydrocarbons of the same strain *B. braunii* Kutz No LB 807/1 Droop 1950, arrived at a similar conclusion and even reclassified this strain to *B. sudeticus*. The strain is presently deposited in the culture collection of algae at the University of Texas in Austin as *B. sudeticus* Lemm. In our opinion, for the conclusive determination of the taxonomic position of this strain, it is necessary to perform thorough analysis of the relative content and structure of its hydrocarbons.

The major extracellular fatty acids of the alga were $C_{14:0}$ (4–7.5% of the total fatty acids), $C_{15:0}$ (5–8%), $C_{16:0}$ (38–44.5%), $C_{18:0}$ (14–18.9%), and $C_{18:1}$ (11–14%). Extracellular lipids contained only one polyenoic acid, linoleic acid (1–2% of the total), although the content of polyenoic acids in intracellular lipids was as high as 12 to 47% in different growth phases. Extracellular FFA contained more $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, and $C_{18:0}$ acids and less $C_{16:0}$ acid than intracellular FFA (Table 3). The extracellular FFA pattern almost did not change during the algal growth.

The difference in the composition of extracellular and intracellular lipids and their dynamics during the growth of *Botryococcus* suggest that they occur in the medium due to the process of excretion and not of cell lysis. The data presented indicate that the process of lipid excretion does not depend on the growth phase of the alga.

**Table 3.** The content of various fatty acids in the intracellular and extracellular FFA and extracellular lipids of *B. braunii* (% of the total fatty acids)

| Fatty acid | Cultivation time, days | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 6 | | | 13 | | | 24 | | |
| | intracellular FFA | intracellular FFA | extracellular lipids | extracellular FFA | intracellular FFA | extracellular lipids | extracellular FFA | intracellular FFA | extracellular lipids | extracellular FFA |
| C12 : 0 | 0.2 | 0.1 ± 0.0 | 1.3 ± 0.3 | 1.7 ± 0.3 | 0.1 ± 0.0 | 2.3 ± 0.2 | 1.5 ± 0.5 | 0.5 ± 0.2 | 2.0 | 1.0 |
| C14 : 0 | 1.2 | 0.1 ± 0.2 | 5.5 ± 0.5 | 6.4 ± 1.2 | 0.7 ± 0.3 | 7.6 ± 0.4 | 6.6 ± 0.5 | 2.2 ± 0.4 | 6.6 | 8.9 |
| C14 : 1 | – | – | – | – | – | 0.3 ± 0.3 | – | – | – | – |
| *ai*-C15 : 0 | – | – | – | – | – | 0.2 ± 0.2 | 0.2 ± 0.2 | – | – | 1.2 |
| *i*-C15 : 0 | – | – | – | – | – | 0.6 ± 0.4 | 0.2 ± 0.2 | – | – | 1.4 |
| C15 : 0 | 0.5 | 0.6 ± 0.2 | 4.1 ± 1.5 | 3.4 ± 0.4 | 0.5 ± 0.1 | 5.1 ± 1.0 | 3.4 ± 0.4 | 1.3 ± 0.4 | 5.4 | 6.4 |
| C16 : 0 | 56.7 | 70.1 ± 3.4 | 46.6 ± 3.2 | 62.1 ± 2.3 | 62.0 ± 5.2 | 46.5 ± 3.1 | 52.0 ± 1.7 | 80.5 ± 4.2 | 44.8 | 60.6 |
| C16 : 1Δ10 | – | 1.6 ± 0.3 | – | – | – | 1.0 ± 1.0 | – | – | – | – |
| C16 : 1Δ7 | 1.2 | 2.1 ± 0.4 | 0.9 ± 0.5 | 0.4 ± 0.2 | – | 4.7 ± 0.9 | 1.7 ± 1.5 | 0.7 ± 0.3 | – | 2.1 |
| C17 : 0 | 0.3 | 0.4 ± 0.1 | 0.6 ± 0.6 | 0.4 ± 0.4 | 0.7 ± 0.2 | 0.4 ± 0.4 | 0.3 ± 0.2 | 0.8 ± 0.2 | 1.2 | 2.4 |
| C16 : 2 | 4.0 | – | 0.5 ± 0.5 | – | – | – | – | – | – | – |
| C16 : 3 | 2.1 | – | 0.8 ± 0.5 | – | – | – | – | – | – | – |
| C18 : 0 | 3.9 | 3.5 ± 1.0 | 17.7 ± 1.9 | 17.7 ± 2.4 | 15.9 ± 2.5 | 13.8 ± 0.8 | 23.8 ± 4.0 | 5.1 ± 1.4 | 16.5 | 13.6 |
| C18 : 1Δ11 | 7.6 | 3.1 ± 0.3 | 0.6 ± 0.3 | 1.7 ± 1.0 | 4.2 ± 0.5 | 4.7 ± 1.1 | 2.7 ± 0.8 | 2.4 ± 0.6 | 2.8 | – |
| C18 : 1Δ9 | 9.8 | 10.2 ± 2.2 | 11.7 ± 1.4 | 1.8 ± 0.2 | 9.5 ± 1.5 | 8.9 ± 1.8 | 4.5 ± 1.8 | 4.0 ± 1.8 | 14.1 | 1.6 |
| C18 : 2 | 12.1 | 6.2 ± 2.2 | 1.9 ± 0.8 | 1.1 ± 1.0 | 1.3 ± 0.3 | 1.1 ± 0.5 | 1.0 ± 0.4 | 1.0 ± 0.4 | 2.1 | – |
| α-C18 : 3 | – | – | 2.2 ± 1.7 | – | – | – | – | – | – | – |
| C20 : 0 | 0.2 | 0.4 ± 0.1 | 2.5 ± 1.1 | 1.6 ± 0.1 | 2.7 ± 0.3 | 1.4 ± 0.6 | 1.2 ± 0.1 | 0.5 ± 0.2 | 1.5 | 0.8 |
| C22 : 0 | 0.1 | 0.4 ± 0.2 | 1.9 ± 0.8 | 0.8 ± 0.1 | 1.6 ± 0.5 | 0.7 ± 0.3 | 0.5 ± 0.2 | 0.5 ± 0.2 | 1.3 | – |
| C24 : 0 | 0.1 | 0.2 ± 0.1 | 1.2 ± 0.6 | 0.9 ± 0.4 | 0.9 ± 0.3 | 0.7 ± 0.5 | 0.4 ± 0.1 | 0.5 ± 0.2 | 1.7 | – |

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