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Effect of salinity on the biochemical composition of the alga *Botryococcus braunii* Kütz IPPAS H-252

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Abstract The effect of 0.3 and 0.7 M NaCl on biomass yield, total nitrogen content, intracellular lipid content, and fatty acid profile of the lipids of the alga *Botryococcus braunii* IPPAS H-252 in different phases of the culture cycle was studied. The presence of sodium chloride in the medium inhibited the growth of algal cells for the first 3 days of the experiment, causing a decrease in total nitrogen, enhanced synthesis of triacylglycerols, and considerable changes in the lipid fatty acid profile: decreases in polyenoic acid contents (from 68.34% to 29.38% and 12.8%) and proportions of long-chain saturated acids (from 0.53% to 5.3% and 14.13% of the total fatty acids) at 0.3 M NaCl and 0.7 M NaCl, respectively. In later phases of the culture, at 0.3 M NaCl, the content of polyenoic acids rose to the values characteristic of the active growth phase of this alga. At 0.7 M NaCl, the proportion of polyenoic acids grew less significantly, but biomass concentration and total nitrogen increased, similarly to the experiment with 0.3 M NaCl.

Keywords Botryococcus · Salinity · Fatty acid composition · Lipid content

Introduction

The green unicellular colonial microalga *Botryococcus braunii* Kützing is considered to be a potential producer of biofuel—liquid hydrocarbons, whose composition depends on the race of the alga: A, B, or L. Hydrocarbons as a fraction of total lipids can amount to 2–86% of dry biomass; their content is determined by strain specificity and culture conditions (Metzger and Largeau 1999). Moreover, lipid content, fatty acid profile, and acid value are also important parameters of biofuel (Gouveia et al. 2009). It is known that lipid production by microalgae is regulated by environmental factors (Harwood and Jones 1989). One of the factors influencing lipid content of algae and causing its increase is salinity. Thus, in *Isochrysis* sp. and *Nannochloropsis oculata*, elevated salinity resulted in an increase in lipid proportion (Renaud and Parry 1994). However, the data on the effect of sodium chloride on *B. braunii* are scarce and contradictory, probably due to strain specificity of the alga. Ben-Amotz et al. (1985) showed in their study that *B. braunii* cells grown in 0.5 M NaCl contained lower levels of protein, carbohydrates, and pigments than the cells cultured in the absence of sodium chloride, but the content of lipids increased. Cells of *B. braunii* LB 572 cultured in the presence of low NaCl concentrations (0.17–0.85 mM) also contained enhanced amounts of lipids, including hydrocarbons (Rao et al. 2007). However, Vazquez-Duhalt and Arredondo-Vega (1991) reported similar content of lipids in cells of two *B. braunii* strains (Austin & Göttingen) cultured in the presence and absence of NaCl.

Data on the effect of salt on fatty acid composition of the alga are scarce too. An increase in salt concentration of the medium caused a decrease in the proportion of linoleic acid and a rise in oleic and palmitoleic acids in *B. braunii* LB 572 cells. In addition to that, the fatty acid profile indicated the presence of long-chain fatty acids (22:0 and 24:0) (Rao et al. 2007). However, the fatty acid profile in acylglycerols was practically unaffected by the presence of sodium chloride in two strains of *B. braunii* (Austin & Göttingen) (Vazquez-Duhalt and Arredondo-Vega 1991).

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Previously, we demonstrated that nitrogen deficiency and elevated or low temperatures influence biochemical composition (mainly the fatty acid spectrum of lipids) of *B. braunii* IPPAS H-252. However, no significant increase in lipid content was found (Kalacheva et al. 2002b; Zhila et al. 2005). Thus, the purpose of this study was to examine the effect of sodium chloride on variations in the lipid and fatty acid profile of the alga *B. braunii* IPPAS H-252.

Materials and methods

The strain was obtained from the Culture Collection of Unicellular Algae at the K.A. Timiryazev Institute of Plant Physiology RAS (IPPAS), where it is registered as *B. braunii* Kützing IPPAS H-252. The Institute had in turn obtained it from the Cambridge Culture Collection of Algae and Protozoa (now Culture Collection of Algae and Protozoa, Dunstaffnage, Scotland) as *B. braunii* No. LB 807/1 Droop 1950 H-252 (the green variety). However, previously, we proved that the key parameters of this strain (hydrocarbon and fatty acid composition) are closer to those of another *Botryococcus* species, namely, *Botryococcus sudeticus* Lemmermann (Kalacheva et al. 2002a).

The alga was cultured in 1.0-L conical flasks at 25°C. The light flux density was 20 Wm^{-2}; the light–dark cycle was 14 h/10 h. The culture was continuously aerated with 1% (*v*/*v*) CO_2–air at a rate of 1 $Lmin^{-1}$ using a membrane compressor. A modified Prate medium (our modification) was used (Kalacheva et al. 2002a) and the tested sodium chloride concentrations were 0.3 and 0.7 M.

Measurements and analysis

Aliquots of the culture were taken periodically for analysis. Biomass concentration was determined by filtration of the aliquots on the previously weighed 0.85–0.95-μm-pore-size Vladipor filters (Vladimir, Russia). The filters with cells were dried to constant weight at 70°C and weighed. To extract lipids, an aliquot of suspension was centrifuged, and the pellet was washed with a 0.2% NaCl solution. Then, boiling isopropanol was added, and three sequential extractions were carried out with a 1:1 (*v*/*v*) mixture of chloroform and isopropanol (Kates 1975). Extraction of lipids and separation of lipids by thin-layer chromatography were carried out as previously reported (Kalacheva et al. 2002a).

Methanolysis of fatty acids was performed in a 50:1 (*v*/*v*) mixture of methanol and sulfuric acid for 2 h at 90°C. Fatty acid methyl esters were analyzed with a GCD Plus gas chromatograph–mass spectrometer (Hewlett Packard, USA), using a 30-m-long HP-5 capillary column with an inner diameter of 0.25 mm (Hewlett Packard, USA). Chromatographic conditions were carrier gas, helium; flow rate, 1 mL min^{-1}; sample input temperature, 230°C; initial temperature, 100°C, programmed to 230°C at the rate of 8°C min^{-1}; and detector temperature, 230°C. Samples were injected with the flow split at a ratio of 1:50. Fatty acids were identified by mass spectra and were compared for retention times with those of authentic standards ("Serva" and "Sigma"). The position of double bonds of monoenoic acids was determined by mass spectra of dimethyl disulfide adducts of the respective fatty acid methyl esters (Christie 1989). Fatty acid contents are given as mol%.

Experiments were done in three replicates. Arithmetic means of the results and their standard errors are presented.

Results

Biomass yields of *B. braunii* grown in medium containing NaCl were almost 2.5 times lower than those of the control culture after 12 days of the experiment (Fig. 1). Moreover, the alga cultured in the presence of NaCl passed through a lag phase (3 days), and concentration of nitrogen-containing compounds in the cells decreased 1.5-fold and 1.3-fold at 0.3 M NaCl and 0.7 M NaCl, respectively, versus the control (Table 1). In later phases of the experiment, concentrations of nitrogen-containing compounds in the algal cells grown in the media with sodium chloride rose to the levels comparable with those in the control.

At day 3, the percentages of intracellular lipids were similar in the control and in the cells grown in the presence of sodium chloride: 14–20% dry weight (Table 1). By the end of the experiment, lipid contents had decreased both in the control (to 9%) and in the cells grown in the presence of sodium chloride—to 10% and 15% at 0.3 M NaCl and 0.7 M NaCl, respectively. However, triacylglycerol (TAG) content varied significantly. In cells grown in the presence

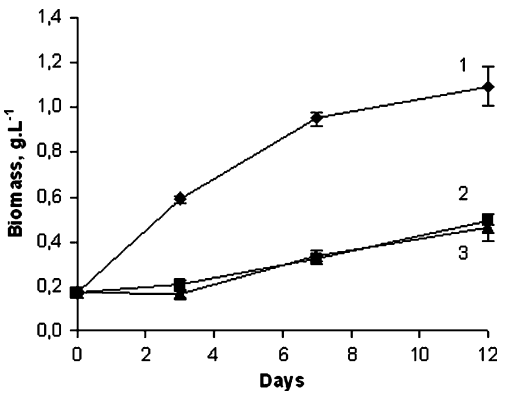

Fig. 1 Biomass accumulation by *Botryococcus braunii* IPPAS H-252 at 0.0 M NaCl (*1*), 0.3 M NaCl (*2*), and 0.7 M NaCl (*3*). Error bar is the standard error of the mean

Table 1 The content of total nitrogen (% of dry biomass), intracellular lipids (% of dry biomass), and triacylglycerols (% of lipids amount) of *Botryococcus braunii* IPPAS H-252 grown under different NaCl concentrations (mean±standard error, *N*=3)

NaCl, M	Total nitrogen			Intracellular lipids			Triacylglycerols		
	Day 3	Day 7	Day 13	Day 3	Day 7	Day 13	Day 3	Day 7	Day 13
0	2.7±0.1	2.6±0.2	2.6±0.2	19.5±0.6	16.0±2.7	9.2±2.4	3.8±0.2	5.5±0.2	4.5±0.2
0.3	1.8±0.1	2.7±0.1	2.7±0.1	14.3±1.4	10.20±0.4	9.2±0.3	19.0±0.9	24.6±1.6	27.7±1.3
0.7	2.1±0.1	2.5±0.1	2.6±0.1	19.9±1.4	13.0±0.6	14.9±1.3	27.9 ±1.4	28.9±2.1	31.0±1.8

of NaCl (0.3 and 0.7 M), TAG content increased considerably at day 3 and remained high throughout the experiment (Table 1). No TAG increase was shown in the control (Table 1); polar lipids dominated throughout the experiment (amounting to 50% of the total lipids). No significant effect of NaCl on the synthesis of intracellular hydrocarbons was observed in the study strain.

The fatty acid profiles of intracellular lipids of *B. braunii* IPPAS H-252 cultured under different sodium chloride concentrations are shown in Table 2. The results of two-way analysis of variance of intracellular lipid fatty acids of *B. braunii* IPPAS H-252 grown at different NaCl concentrations are shown in Table 3. Two factors (salinity and physiological state of algae) could influence fatty acid composition. Therefore, we took this into account while analyzing obtained results.

The *B. braunii* fatty acid profile during the active growth phase contained high proportions of C16 and C18 polyenoic acids (62% of total fatty acids), with monoenoic acids amounting to just 8.4%, and that was consistent with our previous data (Kalacheva et al. 2001). The ratios of monoenoic to polyenoic fatty acids and monoenoic to dienoic ones were very low—0.15 and 0.45, respectively. Later on, the proportion of trienoic acids decreased significantly (from 42.7% to 11.8–19.7%), while the proportion of oleic acid grew (from 3.6% to 25.8–32.1%). The percent of linoleic acid remained unchanged, but the proportion of another dienoic acid (C16:2) dropped almost twofold. The ratio of monoenoic to polyenoic fatty acids increased 6.3–9.5-fold and that of monoenoic to dienoic ones, 5.2–5.6-fold.

The fatty acid profile of the alga grown in the medium containing 0.3 M NaCl varied as follows: At day 3 (during the lag phase) the proportions of all dienoic and trienoic acids dropped dramatically, from 64% to 29% (of the total fatty acids), while the concentration of monoenoic acids rose (from 8.5% to 24.85%), mainly due to an increase in the content of oleic acid (C18:1ω9). The total ratios of monoenoic to polyenoic acids and monoenoic to dienoic ones increased sixfold and 5.6-fold, respectively, versus the control. However, at day 7 of the experiment, the proportion of polyenoic acids grew to 43%, mainly due to the doubling of linolenic acid content, which reached 25.7%. At the same time, the proportion of oleic acid decreased to 13.64%, and thus, total lipid unsaturation did not change and remained at the same level throughout the rest of the experiment.

Similar, but more pronounced, changes were demonstrated in the fatty acid profile of the alga cultured in the medium containing 0.7 M NaCl. During the lag phase, the percentage of polyenoic acids dropped even more dramatically than at 0.3 M NaCl (more than twofold, to 12.8% of the total fatty acids). The degree of lipid unsaturation decreased to 0.67. Concentrations of trienoic acids (C16:3 and C18:3) decreased significantly, reaching 2.1% and 3.4%, respectively. Then, concentration of polyenoic acids grew to 36.02%, and the degree of lipid unsaturation increased to 1.54. That was lower than the control value, but comparable with the unsaturation in the 0.3 M NaCl experiment.

The proportion of monoenoic acids, which were mainly represented by oleic acid, similarly to the experiment with 0.3 M NaCl, remained practically unchanged throughout the experiment, amounting to 20.57–21.38%. During culture, the ratio of monoenoic to polyenoic acids decreased almost threefold, from 2.12 to 0.72, as the proportion of the latter increased (from 12.8% to 36%).

Considerable variations were found in the content of long-chain saturated fatty acids. At 0.3 M NaCl, during the lag phase (at day 3), concentrations of C20:0, C22:0, and C24:0 increased eight- to tenfold versus the control, amounting to 0.6–1%, 0.5–0.8%, and 2–3%, respectively, of the total fatty acids; at 0.7 M NaCl, their total percentage increased to 14.1, and that was 25 times larger than the proportion of these acids in the control. As the culture got adapted to the salt stress, the percentage of these acids decreased to the level registered in the control. In addition to that, among fatty acids of intracellular lipids of the alga grown in the presence of sodium chloride, we detected C26:0, whose proportion decreased toward the end of the experiment too.

Discussion

This study addressed the effect of NaCl on growth and chemical composition of the cells of *B. braunii* IPPAS H-

Table 2 Total fatty acid profile of lipids of *Botryococcus braunii* IPPAS H-252 grown under different NaCl concentrations of the medium (% of the total fatty acids, mean±standard error, $N=3$)

Fatty acid	Day 0	Day 3			Day 7			Day 12		
		0M NaCl	0.3M NaCl	0.7M NaCl	0M NaCl	0.3M NaCl	0.7M NaCl	0M NaCl	0.3M NaCl	0.7M NaCl
12:0	0.73	0.82±0.03	0.37±0.06	0.33±0.19	0.10±0.05	0.29±0.09	0.29±0.17	0.15±0.04	0.41±0.09	0.11±0.04
14:0	0.8	1.60±0.16	1.82±0.02	1.99±0.57	0.63±0.22	2.10±0.42	1.96±0.20	0.84±0.06	2.34±0.46	0.91±0.08
15:0	0.45	0.54±0.11	1.14±0.11	0.55±0.05	0.23±0.07	1.01±0.04	1.28±0.10	0.40±0.04	0.99±0.11	0.26±0.07
16:0	21.0	23.07±4.09	29.84±0.99	35.68±2.68	24.80±2.11	27.47±2.04	32.64±1.87	26.50±0.47	28.97±2.50	30.84±0.95
16:1ω7	1.31	1.35±0.69	1.64±0.13	1.83±0.30	1.51±0.25	1.82±0.23	2.18±0.25	1.09±0.15	1.11±0.12	1.96±0.17
16:1ω6	0.19	0.21±0.11	0.09±0.09	0.40±0.01	0.20±0.06	0.42±0.05	0.43±0.11	0.20±0.04	0.46±0.16	0.16±0.04
16:1ω13tr	0.45	0.72±0.11	–	–	0.02±0.02	0.11±0.07	–	–	0.15±0.08	–
16:2	6.53	6.62±0.33	3.03±0.06	2.11±0.27	3.47±0.41	1.70±0.07	1.72±0.10	3.65±0.52	1.98±0.42	3.49±0.12
16:3	15.22	13.06±0.66	6.11±1.16	2.24±0.24	5.33±1.14	6.11±0.19	3.07±0.21	5.71±0.53	7.80±1.91	5.64±0.37
17:0	0.11	0.13±0.10	0.38±0.11	0.44±0.12	0.31±0.05	0.22±0.07	0.43±0.27	0.29±0.09	0.32±0.04	0.28±0.10
18:0	2.89	3.03±0.54	7.01±0.62	6.94±0.27	5.94±0.26	7.14±0.23	8.11±0.55	5.21±0.19	6.00±0.80	5.52±0.04
18:1ω9	1.97	3.61±3.05	20.04±2.08	21.49±1.65	32.07±5.49	9.34±1.50	20.40±0.27	25.78±1.21	13.64±2.94	20.57±2.84
18:1ω7	1.23	2.50±0.20	3.00±0.35	3.42±0.24	3.57±1.08	3.72±0.45	3.79±0.56	4.55±0.66	3.98±0.45	1.83±0.16
18:2	13.61	12.53±0.91	6.86±0.14	5.09±0.28	13.01±2.12	9.44±2.17	7.58±0.70	10.45±1.97	7.87±0.45	11.29±2.91
18:3ω3	32.98	29.67±5.84	13.38±2.70	3.36±0.32	6.46±1.10	25.65±1.57	11.47±1.28	13.97±2.03	20.25±4.14	15.60±1.99
20:0	0.19	0.20±0.04	0.95±0.10	0.95±0.11	0.95±0.10	0.72±0.06	0.74±0.09	0.50±0.17	0.57±0.11	0.74±0.07
22:0	0.12	0.09±0.04	0.80±0.12	1.22±0.25	0.44±0.07	0.64±0.15	0.84±0.22	0.24±0.07	0.53±0.21	0.31±0.06
24:0	0.22	0.26±0.03	3.01±0.39	9.38±4.04	0.96±0.45	1.97±0.69	2.62±0.84	0.47±0.21	2.44±1.64	0.43±0.14
26:0	–	–	0.55±0.55	2.57±0.84	–	0.13±0.13	0.44±0.44	–	0.20±0.11	0.05±0.05
ΣUnsaturat/Σsaturat	2.77	2.51±0.49	1.19±0.11	0.67±0.07	1.95±0.24	1.41±0.11	1.03±0.07	1.89±0.01	1.39±0.26	1.54±0.06
ΣMonoen/Σpolyen	0.08	0.15±0.06	0.89±0.20	2.13±0.10	1.41±0.36	0.36±0.01	1.14±0.12	0.94±0.07	0.57±0.19	0.72±0.16
ΣMonoen/Σdien	0.26	0.45±0.15	2.51±0.25	3.78±0.15	2.51±0.81	1.45±0.19	2.93±0.29	2.29±0.26	1.98±0.33	1.83±0.46
ΣDien/Σtrien	0.42	0.47±0.06	0.55±0.10	1.29±0.03	1.46±0.30	0.36±0.09	0.64±0.02	0.75±0.16	0.39±0.10	0.70±0.12
ΣLong-chain acids	0.53	0.55±0.06	5.30±0.88	14.13±4.86	2.35±0.56	3.45±0.81	4.64±1.57	1.21±0.45	3.74±1.86	1.54±0.26

Table 3 The results of two-way analysis of variance of intracellular lipid fatty acids of *Botryococcus braunii* IPPAS H-252 grown at different concentrations of NaCl

Fatty acid	F_a	F_b
12:0	1.32	8.00
14:0	1.56	9.09
15:0	9.63	47.27
16:0	0.30	10.11
16:2	21.55	54.91
16:3	5.08	18.66
18:0	10.14	20.65
18:1ω9	3.74	6.36
18:2	1.24	6.14
18:3ω3	0.41	9.04
20:0	2.85	5.24
22:0	4.32	10.12
24:0	3.44	4.21
26:0	5.98	6.05

F_a, calculated F test for algae physiological state factor; F_b, calculated F test for salinity factor

F=6.01−table value, $P \leq 0.01$

252. In preliminary experiments, we found that this strain was not able to grow in a medium containing 0.75 M NaCl. In cultures containing NaCl (0.3 and 0.7 M), the growth of the algae cells was inhibited. It was a lag phase (3 days), and the content of nitrogen-containing compounds decreased. The decrease of specific growth rate at 0.5 M NaCl was demonstrated for two strains of *B. braunii* (CHN 357 and UK 807-2; Li and Qin 2005). Lower protein contents at 0.5 M NaCl were also reported for other *B. braunii* strains (Austin & Göttingen) (Ben-Amotz et al. 1985; Vazquez-Duhalt and Arredondo-Vega 1991). Hagemann et al. (1990) showed that immediately after NaCl was added to the medium, total protein synthesis in *Synechocystis* sp. PCC 6803 was almost completely repressed; repression of the synthesis of some proteins in *Anabaena* sp. L-31 and *Anabaena torulosa* was shown even under low sodium chloride concentrations (Fernandes et al. 1993).

No significant differences were found in the contents of intracellular lipids between the control and the cells grown in the medium containing NaCl (F test for control and 0.3-M NaCl is 4.26; for control and 0.7-M NaCl, 2.46; F=6.03− table value, $P \leq 0.01$), and this is consistent with the data reported for other *B. braunii* strains (Austin & Göttingen) (Vazquez-Duhalt and Arredondo-Vega 1991). However, the data on the effect of sodium chloride on intracellular lipid content in some algal species are quite contradictory. Elevated salinity of the medium caused lipids to decrease in *Nitzschia frustulum* (Renaud and Parry 1994), *Cladophora vagabunda* (Elenkov et al. 1996), and the halophilic alga *Dunaliella salina* (Al-Hasan et al. 1987), which, like *B. braunii*, can synthesize hydrocarbons. In contrast, in *Isochrysis* sp. and *N. oculata*, elevated salinity resulted in the increase in lipid proportion (Renaud and Parry 1994).

Many algae species, in response to unfavorable environmental conditions, synthesize large amounts of storage lipids such as triacylglycerols (Harwood and Jones 1989). In the culture with NaCl, increasing of TAGs was observed at day 3 of the experiment (Table 1).

The chief role of fatty acids in algae is related to functions of cell membrane and metabolic processes (Guschina and Harwood 2006). The degree of unsaturation of membrane fatty acids is also a significant parameter in adaptation of algae to environmental conditions. Changes in the lipid fatty acid profile in response to elevated salinity of the medium are necessary to keep the membrane fluid and prevent its destruction.

However, the data on the effect of sodium chloride on the fatty acid composition of algae lipids are scarce and contradictory. Cells of *Isochrysis* sp. grown under elevated NaCl concentrations in the medium contained increased proportions of polyunsaturated fatty acids—C18 and C22 (Ben-Amotz et al. 1985). In contrast, Renaud and Parry (1994) reported a decrease in C18:5 and C22:6 in *Isochrysis* sp. grown under elevated salinity. A lower degree of unsaturation of lipids at elevated NaCl concentrations was reported for the algae *Dunaliella* sp., *Nannochloropsis* sp., and *N. frustulum* (Renaud and Parry 1994; Xu and Beardall 1997; Hu and Gao 2006).

One of the reasons why these results are so contradictory can be that fatty acid composition was analyzed in algal cells of different growth phases. There seems to be only one available paper reporting lipid fatty acid compositions of algal cells grown under different NaCl concentrations that were taken for analysis during the exponential and the stationary growth phases. During the exponential growth phase, 18:3 increased and C16:0 decreased at 1.0 M NaCl and higher concentrations, and C20:4 increased at 1.5 M. During the stationary growth phase, C18:0 was demonstrated in the fatty acid profile, and there was a decrease in C20:4 that was not related to sodium chloride concentration of the medium (Lee et al. 1989).

In this study, we analyzed variations in lipid fatty acids in the course of growth of the alga and its adaptation to salinity. In our previous study, we showed that under optimal conditions, during active growth of the alga, the major lipid fatty acids are C16 and C18 polyenoic acids (their total proportion being higher than 50% of the total fatty acids). As the alga enters the stationary growth phase, the saturated, monoenoic (mostly oleic) acids increase while the polyunsaturated ones decrease (Kalacheva et al. 2001). So, the increase in oleic acid content found in the fatty acid profile of the control culture at day 7 of the experiment and a simultaneous decrease in the proportion of polyenoic acids, mainly α-linolenic one, can be indicative of the culture entering the stationary growth phase.

The decrease in the content of polyenoic acids in the lipids of the alga in the presence of NaCl, especially noticeable at 0.7 M, was first found at day 3 of the

experiment. It may be related to partial degradation of chloroplast membranes.

Destruction of chloroplast membranes causes termination of synthesis of certain galactolipids—the main lipids of photosynthesizing membranes (Harwood and Jones 1989). For instance, cells of *Synechococcus* 6311 grown under elevated salinity contained lower proportions of monogalactosyl diacylglycerols, but higher proportions of digalactosyl diacylglycerols (Huflejt et al. 1990; Khoumutov et al. 1990).

Larger proportions of long-chain fatty acids in cells grown in the presence of NaCl can be due to accumulation of these acids in TAG. In our previous study, we already reported the presence of saturated fatty acids in the fraction of triacylglycerols (Kalacheva et al. 2001). Larger proportions of long-chain fatty acids (22:0 and 24:0) in the presence of sodium chloride were reported for *B. braunii* LB 572 (Rao et al. 2007). *Synechococcus* 6311 was also shown to contain increased percentages of longer-chain fatty acids under elevated NaCl concentrations (Huflejt et al. 1990; Khoumutov et al. 1990).

Generally, fatty acid composition undergoes alterations in response to salinity change. However, the physiological state of cells could influence fatty acid composition. Two-way analysis of variance allowed us to determine the degree to which the duration of alga culturing and salinity influenced the fatty acids of *B. braunii* IPPAS H-252 lipids. It was found that the influence of salinity is more important and statistically significant in comparison with the physiological state of algae. So, variations in the fatty acid composition of the alga were mainly due to the salinity of the medium (Table 3).

Thus, the presence of 0.3 M NaCl and 0.7 M NaCl in the medium inhibited the growth of algal cells for the first 3 days of the experiment, causing considerable changes in the lipid fatty acid profile: a decrease in polyenoic acid contents and an increase in the proportions of oleic acid and saturated long-chain fatty acids. In later phases of the culture, algal biomass increased, and the degree of lipid unsaturation rose, mainly due to a rise in the content of polyenoic acids.

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References

Al-Hasan RH, Ghannoum MA, Sallal A-K, Abu-Elteen KH, Radwan SS (1987) Correlative changes of growth, pigmentation and lipid composition of *Dunaliella salina* in response to halostress. J Gen Microbiol 133:2607–2616

Ben-Amotz A, Tornabene TG, Thomas WH (1985) Chemical profile of selected species of microalgae with emphasis on lipids. J Phycol 21:72–81

Christie WW (1989) Gas chromatography and lipids. A practical guide. The Oily Press, Ayr, p 230

Elenkov I, Stefanov K, Dimitrova-Konaklieva S, Popov S (1996) Effect of salinity on lipid composition of *Cladophora vagabunda*. Phytochemistry 42:39–44

Fernandes TA, Iyer V, Apte SK (1993) Differential responses of nitrogen-fixing cyanobacteria to salinity and osmotic stresses. Appl Environ Microbiol 59:899–904

Gouveia L, Marquez AE, da Silva TL, Reis A (2009) *Neochloris oleabundans* UTEX1185: a suitable renewable lipid source for biofuel production. J Ind Microbiol Biotech 36:821–826

Guschina IA, Harwood JL (2006) Lipids and lipid metabolism in eukaryotic algae. Prog Lipid Res 45:160–186

Hagemann M, Wolfel L, Kruger B (1990) Alterations of protein synthesis in the cyanobacterium *Synechocystis* sp. PCC 6803 after a salt shock. J Gen Microbiol 136:1393–1399

Harwood JL, Jones AL (1989) Lipid metabolism in algae. Adv Bot Res 10:1–53

Hu H, Gao K (2006) Response of growth and fatty acid compositions of *Nannochloropsis* sp. to environmental factors under elevated CO_2 concentration. Biotechnol Lett 28:987–992

Huflejt ME, Tremolieres A, Pineau B, Lang JK, Hatheway J, Packer L (1990) Changes in membrane lipid composition during saline growth of the fresh water cyanobacterium *Synechococcus* 6311. Plant Physiol 94:1512–1521

Kalacheva GS, Zhila NO, Volova TG (2001) Lipids of the green alga *Botryococcus* cultured in a batch mode. Microbiology (Mikrobiologiya) 70:256–262

Kalacheva GS, Zhila NO, Volova TG (2002a) Lipid and hydrocarbon compositions of a collection strain and a wild sample of the green microalga *Botryococcus*. Aquat Ecol 36:317–330

Kalacheva GS, Zhila NO, Volova TG, Gladyshev MI (2002b) The effect of temperature on the lipid composition of the green alga *Botryococcus*. Microbiology (Mikrobiologiya) 71:286–293

Kates M (1975) Techniques of lipidology. Isolation, analysis and identification of lipids. Mir, Moscow, p 305

Khoumutov G, Fry IV, Huflejt ME, Packer L (1990) Membrane lipid composition, fluidity, and surface charge changes in response to growth of the fresh water cyanobacterium *Synechococcus* 6311 under high salinity. Arch Biochem Biophys 277:263–267

Lee Y-K, Tan H-M, Low C-S (1989) Effect of salinity of medium on cellular fatty acid composition of marine alga *Porphyridium cruentum* (Rhodophyceae). J Appl Phycol 1:19–23

Li Y, Qin JG (2005) Comparison of growth and lipid content in three *Botryococcus braunii* strains. J Appl Phycol 17:551–556

Metzger P, Largeau C (1999) Chemicals of *Botryococcus braunii*. In: Cohen Z (ed) Chemicals from microalgae. Taylor & Francis, London, pp 205–260

Rao RA, Dayananda C, Sarada R, Shamala TR, Ravishankar GA (2007) Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. Bioresour Technol 98:560–564

Renaud SM, Parry DL (1994) Microalgae for use in tropical aquaculture II: effect of salinity on growth, gross chemical composition and fatty acid composition of three species of marine microalgae. J Appl Phycol 6:347–356

Vazquez-Duhalt R, Arredondo-Vega BO (1991) Haloadaptation of the green alga *Botryococcus braunii* (race A). Phytochemistry 30:2919–2925

Xu X-Q, Beardall J (1997) Effect of salinity on fatty acid composition of a green microalga from an Antarctic hypersaline lake. Phytochemistry 45:655–658

Zhila NO, Kalacheva GS, Volova TG (2005) Influence of nitrogen deficiency on biochemical composition of the green alga *Botryococcus*. J Appl Phycol 17:309–315