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Hydrocarbons in Green and Blue-Green Algae

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ABSTRACT. Liquid column chromatography and thin-layer chromatography were used to determine the total content of hydrocarbons and gas chromatography was used to evaluate composition of hydrocarbons in green algae (*Chlorella kessleri, C. vulgaris, Chlorella* sp., *Scenedesmus acutus, S. acuminatus, S. obliquus*) and the blue-green alga (*Spirulina platensis*) cultivated under autotrophic or heterotrophic conditions. In *C. kessleri* cultivated under heterotrophic conditions the content of hydrocarbons was found to be about 10^{-2} % (per dry mass), whereas under autotrophic conditions it was about 10^{-3} % (per dry mass). The highest content of hydrocarbons was detected in species of the genus *Scenedesmus* cultivated autotrophically (10^{-1} %). Heptadecane and hexacosane were found as major alkanes, 1-heptadecene was detected among alkenes.

The occurrence of hydrocarbons in green and blue-green algae has only rarely been studied (Han *et al.* 1968; Paoletti *et al.* 1976). In most of the papers the occurrence of hydrocarbons was studied in collections containing green algae and other microorganisms.

Due to the economical significance of cultivated green algae the results obtained when studying the occurrence of hydrocarbons in the industrially important collection of green algae of the genera *Chlorella* and *Scenedesmus* and blue-green alga *S. platensis* are referred to in the present communication.

MATERIALS AND METHODS

Hexane (International Enzymes Limited, England) and chloroform (Lachema, Czechoslovakia) were rectified on a column with 50 theoretical plates. Silica gel 100—250 (Lachema) was used for column chromatography, silica gel G according to Stahl (Merck, GFR) for thin-layer chromatography. Green algae *Chlorella* sp. and *S. acutus* were kindly supplied by the Institute of Algology, Bulgarian Academy of Sciences, Bulgaria. Other green and blue-green algae were obtained from the Department of Autotrophic Microorganisms, Institute of Microbiology, Czechoslovak Academy of Sciences, Třeboň, in the form of lyophilized or drum-dried powder. Green algae *C. kessleri* and *C. vulgaris* were cultivated also heterotrophically, other algae only autotrophically (see Table II).

A sample of algal biomass (100 g) was extracted for 16 h in a Soxhlet extractor. Hexane was evaporated to dryness and the black-green oil was dissolved in 100 mL chloroform. The chloroform solution was evaporated to dryness with 20 g silica gel and applied to a column (30 × 500 mm) packed with 100 g of silica gel. Elution with

TABLE I. Percent representation of hydrocarbons

Organism	Cultivation[a]	%[b]
C. kessleri	A	0.002
C. kessleri	H	0.01
C. vulgaris	H	0.02
Chlorella sp.	A	0.001
S. obliquus	A	0.25
S. acuminatus	A	0.31
S. acutus	A	0.28
S. platensis	A	0.07

[a] A autotrophic, H heterotrophic.
[b] Referred to dry mass of cells.

hexane yielded a yellow eluate which was evaporated and dried to constant mass (see Table I). It was then chromatographed using preparative TLC (200 × 200 mm plates) in hexane. Hydrocarbons (R_F 0.85—0.95) were detected with 0.2 % ethanolic morin (Lachema) under UV light. After extraction from silica gel a mixture was obtained which was further separated by gas chromatography, using a Varian Aerograph, model 2740 with flame-ionization detector, stainless steel column (0.25 mm ID × 1820 mm), packed with 1 % OV-1 on Gas Chrom P HMDS (80—100 mesh). Working regime: Temperature of injector 290 °C, temperature of detector 310 °C, temperature was programmed from 100 to 300 °C at a rate of 4 K/min, carrier gas nitrogen, gas flow 30 mL/min. Individual hydrocarbons were identified on the basis of identical retention intervals with standards of hydrocarbons (decane to triacontane, 1-dodecene to 1-nonadecene (Merck)).

RESULTS AND DISCUSSION

The content of hydrocarbons varies significantly, depending on cultivation conditions and on the algal species used (Table I). It was found that *C. kessleri* cultivated heterotrophically produces about 10 times more hydrocarbons than when cultivated under autotrophic conditions.

The genus *Scenedesmus* cultivated autotrophically was found to produce about 10^{-1} % hydrocarbons, *i.e.* 10—100 times more than the genus *Chlorella*. In *S. platensis* 0.07 % hydrocarbons were detected. The detected hydrocarbon contents are thus in agreement with literature data for the genus *Scenedesmus* (Paoletti *et al.* 1976). On the contrary, the results obtained in the *Chlorella* sp. and in *S. platensis* differ from those described in the literature. Han *et al.* (1968) found 0.032 % hydrocarbons in the autotrophically cultivated *Chlorella* sp., which is comparable with results referred to here and obtained in the heterotrophic cultivation. The occurrence of hydrocarbons in the heterotrophic culture described in the present communication cannot be compared with literature data. In the paper of Patterson (1967) the measured values obtained in *C. vulgaris* were demonstrated only by means of a graphic record without numerical data. In the blue-green alga *S. platensis* Paoletti *et al.* (1976) found about a 2.5-fold higher content of hydrocarbons as compared with the data presented here.

Table II summarizes the data obtained here and literature data concerned with the composition of hydrocarbons. Alkanes and 1-alkenes that could be identified with the used standards were included. Quality of chromatographic records made it possible to classify saturated hydrocarbons (up to hentriacontane) and unsaturated

TABLE II. Composition of hydrocarbons (%) in green and blue-green algae (literature data and data obtained here)

Hydrocarbons[a]	Organism[b, c]						
	1	2	3	4	5	6	7
n-12	—	0.70	—	—	—	—	1.95
Δ-12	—	tr	—	—	—	—	—
n-13	—	0.40	—	—	—	—	—
Δ-13	—	—	0.50	0.30	—	—	—
n-14	0.65	0.50	0.35	0.25	0.2	—	tr 2
Δ-14	—	0.60	1.00	1.20	—	—	—
n-15	4.70	0.30	0.10	tr	2.0	0.12	—
Δ-15	1.65	0.50	1.25	0.40	—	—	—
n-16	17.80	1.10	tr	9.40	1.0	0.07	0.90
Δ-16	0.60	5.70	68.25	11.15	—	—	—
n-17	15.25	1.70	2.65	3.10	25.24	18.16	tr
Δ-17	0.55	7.05	3.15	45.20	35.08	81.63	8.80
n-18	10.75	0.95	tr	0.20	tr	0.02	tr
Δ-18	0.90	tr	0.05	0.05	—	—	—
n-19	1.15	tr	—	—	—	0.01	tr
Δ-19	tr	1.50	tr	0.10	—	—	tr
n-20	2.40	0.45	—	—	tr	—	tr
Δ-20	3.05	tr	—	—	—	—	—
n-21	0.60	0.80	—	—	—	—	0.85
n-22	2.40	1.55	2.15	0.85	2.0	—	tr
n-23	tr	0.55	—	—	—	—	0.15
Δ-23	—	—	—	—	0.3	—	—
n-24	0.80	0.40	10.10	2.15	—	—	21.95
n-25	tr	2.85	0.85	1.30	5.0	—	—
Δ-25	—	—	—	—	—	—	—
n-26	tr	9.15	0.40	0.20	1.2	—	44.90
Δ-26	—	—	—	—	—	—	—
n-27	0.45	0.70	0.35	0.05	—	—	1.80
Δ-27	—	—	—	—	0.4	—	—
n-28	0.50	5.10	0.10	tr	—	—	—
n-29	0.50	2.35	0.10	—	—	—	tr
n-30	1.35	tr	tr	—	—	—	—
n-31	0.25	0.80	—	—	—	—	—

[a] *n* designates alkanes, Δ 1-alkenes, number indicates the number of carbon atoms.
[b] 1 *C. kessleri*, 2 *C. vulgaris*, 3 *C. kessleri*, 4. *Chlorella* sp., 5 *Chlorella* sp. (Paoletti *et al.* 1976), 6 *Chlorella* sp. (Han *et al.* 1968), 7 *S. obliquus*, 8 *C. acutus*, 9 *S. acuminatus*, 10 *S. acutus* (Zolotovitch *et al.* 1974), 11 *S. quadricauda* (Paoletti *et al.* 1976), 12 *S. platensis*, 13 *S. platensis* (Paoletti *et al.* 1976), 14 *S. platensis* (Paoletti *et al.* 1976), 15 *Spirulina* sp. (Paoletti *et al.* 1976), 16 *S. platensis* (Gelpi *et al.* 1970).
[c] Cultivation: heterotrophic for algae 1 and 2, autotrophic for algae 3—16.

hydrocarbons (up to 1-cosene) on the stationary phase OV-1 comparable with the phase SE-30 used by Paoletti *et al.* (1976). The representation of the identified hydrocarbons varies from 46 % (alga 2) up to almost 100 % (alga 12).

Cultivation conditions (heterotrophic, autotrophic) influence significantly the representation of the hydrocarbons produced. Hexadecane, heptadecane and octadecane are most frequent in the heterotrophic cultures of *C. kessleri*. Autotrophically cultivated *C. kessleri* contains 1-hexadecene as the dominant hydrocarbon. Content of higher hydrocarbons in *C. kessleri* cultivated heterotrophically is negligible but tetracosane is significantly represented in the autotrophically cultivated alga. As compared with *C. kessleri*, *C. vulgaris* cultivated under heterotrophic conditions

8	9	10	11	12	13	14	15	16
—	tr	1.1	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
0.05	0.35	2.2	—	—	—	—	—	—
—	tr	—	—	1.25	—	—	—	—
1.15	0.20	3.6	1.0	—	0.2 .	0.2	—	—
—	tr	—	—	6.20	—	—	—	—
2.10	0.20	4.7	2.3	tr	2.7	3.7	2.9	10
—	—	—	—	4.10	—	—	—	—
3.20	1.25	6.7	2.5	—	3.7	3.8	2.8	20
—	0.10	—	—	—	—	—	—	—
1.25	15.75	33.8	41.4	3.40	66.9	84.0	71.7	70
12.15	32.50	—	tr	84.90	3.4	3.1	5.2	1
0.25	0.25	6.8	2.0	tr	0.6	0.5	0.7	—
—	—	—	—	—	—	—	—	—
1.15	1.00	5.1	2.5	—	0.9	1.3	1.9	—
—	—	—	—	—	—	—	—	—
0.15	—	4.6	—	tr	0.3	—	—	—
—	—	—	2.3	tr	—	tr	—	—
tr	0.45	3.4	—	—	—	—	—	—
—	tr	2.5	2.0	tr	3.2	0.5	1.7	—
—	0.45	2.2	—	—	—	—	—	—
—	—	—	1.1	—	0.3	tr	tr	—
0.25	1.10	2.2	—	tr	—	—	—	—
—	7.20	5.7	11.4	—	1.8	0.3	1.2	—
—	—	—	—	—	—	—	—	—
2.42	22.30	3.0	1.4	tr	1.3	0.2	0.6	—
—	—	—	—	—	—	—	—	—
35.20	12.15	2.1	—	—	tr	—	—	—
—	—	—	—	—	—	—	—	—
1.40	0.25	7.3	—	—	—	—	—	—
0.35	tr	—	—	—	—	—	—	—
tr	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—

produces more alkenes, 1-hexadecene and 1-heptadecene in particular. Hexacosane is a higher hydrocarbon represented in higher amounts. *Chlorella* sp. used in the present experiments contains pronounced quantities of lower hydrocarbons (1-heptadecene and 1-hexadecene) and their reaction products (heptadecane and hexadecane), whereas higher hydrocarbons are represented only negligibly.

For *C. vulgaris* cultivated autotrophically and heterotrophically Patterson (1967) presented a chromatographic record of the representation of individual hydrocarbons, again without numerical values; however, it follows from the record that 1-pentacosene and 1-heptacosene are the major hydrocarbons. In the species *Chlorella* sp. Paoletti *et al.* (1976) described heptadecane, heptacosane and 1-heptadecane as dominant hydrocarbons and Han *et al.* (1968) heptadecane and 1-heptadecene, the latter representing more than 80 % of the hydrocarbon mixture.

The genus *Scenedesmus* exhibits two maxima in the hydrocarbon content: The first is represented by hydrocarbons with 17 carbon atoms, 1-heptadecene in particular, the second by hexacosane and heptacosane. Of higher hydrocarbons hexacosane and tetracosane are significantly represented in *S. obliquus*. In *S. acutus* heptacosane and 1-heptadecene are dominantly represented. *S. acuminatus* contains again 1-heptadecene as the main hydrocarbon, but heptadecane, hexacosane and heptacosane are also significantly represented.

Two maxima in the content of hydrocarbons in the genus *Scenedesmus* were observed by Stránský *et al.* (1967), Gelpi *et al.* (1970) and Paoletti *et al.* (1976). However, as compared with the data referred to here, they detected heptadecane as the major hydrocarbon, in spite of the fact that the value 41.4 % (see Table II, alga 11) for heptadecane holds for *S. quadricauda*. The value of heptadecane in *S. acutus* obtained by Zolotovitch *et al.* (1974) is particularly interesting as it was detected during analysis of the same alga cultivated under identical conditions in 1973.

1-Heptadecene was detected as the main hydrocarbon in the blue-green alga *S. platensis*. As also other alkenes were found it may be assumed that the last reaction step — reduction of alkene to alkane is blocked in the strain used. Paoletti *et al.* (1976) and Gelpi *et al.* (1970) found only alkenes in *S. platensis* but this fact may be explained on the basis of biosynthetic relationships (heptadecane is formed by reduction of 1-heptadecene; Kolattukudy 1970).

A similar phenomenon was also detected in *C. kessleri* where the autotrophically cultivated alga contains 1-hexadecene whereas the heterotrophically cultivated alga contains hexadecane. However, Patterson (1967) detected just the opposite in *C. vulgaris*, *i.e.* the occurrence of 1-pentacosene and 1-heptacosene in the heterotrophically cultivated alga. Relations between the occurrence of alkanes and alkenes in the genus *Scenedesmus* can be explained in a similar way. In this genus alkenes are apparently formed due to a lower effectivity of the last biosynthetic step—hydrogenation of the double bond to alkane. Substantial interspecies and intergeneric differences in the content of hydrocarbons were detected as demonstrated in the genera *Chlorella* and *Scenedesmus*. The genus *Spirulina* differs completely from the genera *Chlorella* and *Scenedesmus* which can be explained by the fact that these organisms belong to different taxons (*Prokaryota* and *Eukaryota*).

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