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Separation and identification of hydrocarbons and other organic compounds from *Scenedesmus obliquus* and three cyanobacterial species

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ABSTRACT

Four algal species named *Scenedesmus obliquus* (green algae), *Oscillatoria agardhii*, *Oscilatoria limnetica*, and *Anabaena sphaerica* (blue green algae) were isolated from Ismailia Canal water (Port Said, Egypt). The fat of the samples was Soxhlet extracted using hexane for 8 h. The organic fractions were purified using silica columns. Organic compounds such as *n*-alkanes, polycyclic aromatic hydrocarbons (PAHs), and other hydrocarbons that can be produced from these strains were determined, separated, and identified by GC and GC/MS. The identification and quantification of the aliphatic hydrocarbons as well as PAHs have been carried out using gas chromatography with flame ionization detector. The total hydrocarbon content was between 11.7 and 30.4 mg/g dry weight, while the concentrations of PAHs and *n*-alkanes varied between 145.1–238.7 μg/L and 106.4–120.6 μg/L, respectively. On the other hand, unknown compounds were verified by Varian 4500 GC/MS.

Keywords: Algae; Hydrocarbons; Organics; Separation; Identification

1. Introduction

Hydrocarbons produced from biomass resources or directly from solar energy and carbon dioxide through photosynthetic biological systems are becoming a significant goal of research and development in both academia and industry. Cyanobacteria, which are capable of the photosynthetic production of hydrocarbons and exhibit multiple adaptive morphological, biochemical, and metabolic properties, have garnered particular attention because of their huge potential for renewable energies [1–3].

Cyanobacteria are a diverse group of photosynthetic microorganisms that have evolved a remarkable array of adaptive traits including oxygenic photosynthesis, N_2 fixation, a wide morphological diversity, extensive secondary metabolite biosynthetic capacity, and a range of symbiotic relationships with other organisms. Cyanobacteria are estimated to contribute 30% of Earth's annual oxygen production and play a major role in biogeochemical cycles [4].

Early research in the 1960s showed that hydrocarbons could be produced in a variety of cyanobacterial strains (e.g. *Nostoc* sp., *Anacystis* sp., *Anacystis nidulans*, *Trichodesmium erythaeum*, *Microcoleus chthonoplastes*, *Plectonema terebrans*, *Oscillatoria williamsii*, and *Lyngbya lagerhaimii*). This research also revealed that

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cyanobacteria mainly produced hydrocarbons with carbon chain lengths varying from C15 to C18 with a predominance of *n*-C17 compounds [5–7]. Eukaryotic microalgae are an important resource for biofuel production [8–10]. Unlike microalgae, which accumulate large amounts of triacylglycerols (TAGs) through lipid metabolism involving several different cellular compartments, cyanobacteria contain few or no TAGs. Fatty acid metabolism in cyanobacteria is associated with a type II fatty acid synthesis (FASII) and is conducted by a multienzyme system in the cytoplasm. Fatty acid molecules must be activated to fatty acyl-thioesters by fatty acyl-CoA synthetase (ACS) or fatty acyl-ACP synthetase (AAS) before hydrocarbons can be synthesized. Free fatty acids exist mainly in the form of C18 with a small amount of C16 in cyanobacteria, and thus, the predominant hydrocarbon is C17. One precursor of hydrocarbon biosynthesis in cyanobacteria is thought to be fatty acyl–acyl carrier proteins (ACPs), which are essential metabolites for the production of lipid-based biofuels [3,11–13].

The unicellular photosynthetic microalga *Botryococcus braunii* is member of the chlorophyceae (chlorophyta). *B. braunii* is regarded as a potential source of renewable fuel because of its ability to produce large amounts of hydrocarbons. Depending on the strain and growth conditions, up to 75% of algal dry mass can be hydrocarbons. The chemical nature of hydrocarbons varies with the producer strain. Three races of *B. braunii* have been documented, and these can be differentiated on the basis of the characteristic hydrocarbon they produce. The A race produces odd numbered C25 to C31, *n*-alkadienes, and trienes. The B race produces triterpenoid hydrocarbons known as botryococcenes (C_nH_{2n-10}, $n = 30–37$), apparently of isoprenoid origin. The L race produces lycopadiene, a C40 tetraterpene [14,15].

The isolated species of green algae *B. braunii*, collected from Bear Shola Falls at Kodaikanal, India, were cultured and examined for its hydrocarbon content by Dayananda et al. [16]. The type of hydrocarbons produced by the Kodaikanal isolates was analyzed and identified as saturated hydrocarbons in the range of C21–C33 by GC/MS. Tetracosane and octacosane were found as the major components among the saturated hydrocarbons. Meanwhile, Audino et al. [17] confirmed the presence of a highly aliphatic, non-hydrolysable, and insoluble biomacromolecule (algaenan) found in their outer cell walls.

This study aimed to identify the quantity and composition of hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and *n*-alkanes from the four algal species.

2. Materials and methods

2.1. Isolation, purification, and culture condition of algal species

Four algal species were isolated from phytoplankton blooms along the River Nile in Ismailia canal in front of Port Said water plant intake. The algal species transferred and recultivated in a fresh algal nutrient medium BG11 [18]. Three species were from cyanobacterial division, which are *Oscillatoria agardhii*, *Oscilatoria limnetica*, *Anabaena sphaerica*, and one specie from green algae group (*Scenedesmus obliquus*). *O. agardhii* and *Scendesmus obliquus* isolated in 100% $NaNO_3$ (1.5 g/L). Meanwhile, *Oscillatoria limnetica* was isolated by diluting the concentration of sodium nitrate ($NaNO_3$) to 1/5th the original concentration (0.3 mg/L $NaNO_3$), where *A. sphaerica* was isolated after excluding $NaNO_3$ concentration completely [19].

The algal species were in the maximum growth when introduced to the standard media. The cultures were incubated at 24 ± 2 and 30 ± 2°C for *S. obliquus* and all cyanobacterial strains under continuous white fluorescent illumination (33.3 $E/m^2/s$). Cultures were shaken once daily to prevent clumping of algal cells and adherence to the flasks.

2.2. Preparation of biomass for organic extraction

At optimal growth, the algae were harvested by centrifugation at 5,000 rpm for 15 min. Then, after removal of clear liquid, the pellet of algae was washed several times by distilled water till the effluents became almost transparent. The washed biomass was then dried in an oven at 40°C until a constant weight was reached. The dried biomass was then ground into fractions.

The extraction of organic metabolites produced by algae was carried out by packing of 5 g of dried powder algae into extraction thimbles (pre-extracted with *n*-hexane), and a layer of anhydrous Na_2SO_4 has been added over the sample to ensure that the *n*-hexane extract remains dry. The sample was then exhaustively extracted with 125 ml of *n*-hexane in a Soxhelt apparatus for not less than 8 h, at roughly 20 cycles per hour. Once cool, the sample extract is quantitatively transferred to a 100-ml volumetric flask and the volume adjusted to exactly 100 ml, then stored in fridge for further analysis.

2.3. Clean up procedure

The analysis of hydrocarbons by gas chromatography requires the removal of polar lipids and other

co-extracted materials from the *n*-hexane extract. This was carried out by column chromatography. The chromatography column used has an effective length of 20 cm and 10-mm pore size. The bottom end of the column was plugged with *n*-hexane-washed glass wool or cotton. The column was slurry-packed, and the slurries of 5% deactivated alumina being prepared in *n*-hexane. The packed column has 10 cm of alumina and 2 cm of anhydrous Na_2SO_4 on the top to ensure that the extract is completely dry and to maintain the correct level of deactivation of both absorbents. The column was then washed with 20 ml *n*-hexane. The sample extract was concentrated to about 1–2 ml using rotary evaporator. The concentrated sample was quantitatively transferred to the top of the column using a Pasteur pipette. The collected cleaned extract was stored in clean glass containers coated with aluminum foil for further analysis.

2.4. GC–MS analysis

GC–MS analysis of the volatiles was performed on a Hewlett–Packard gas chromatograph 6890 equipped with ECD detector. A HP5-MS capillary column was used (30 m/0.25 mm, 0.25 mm film thickness). The temperature was programmed from 40 to 280°C at a rate of 6°C/min. Helium was used as a carrier.

Also, the extracted metabolites were analyzed by GC/MS, Varian 4000 equipped with MS detector Varian 4500.

2.5. Identification of compounds

Identification was accomplished using computer searches on a NIST11 MS data library. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the basis of its mass-spectral fragmentation. If available, reference compounds were co-chromatographed to confirm GC retention times.

3. Results and discussion

Numerous biogenic compounds from phytoplankton species are well documented. A series of volatile and non volatile metabolites have been identified by GC and GC/MS in the extract of green and/or blue green algae [20].

The composition of the hydrocarbons and volatile organics is presented in Table 1. More than 31 hydrocarbons and other organic compounds were identified from green alga *Scenedosmus obliquus* including 12 alkanes, 14 PAHs and 5 cyclopentane, cyclohexane and cyclooctane derivatives, which are considered volatile compounds. On the other hand, the other three cyano bacteria (*O. agardhii*, *O. limnetica*, and *A. sphaerica*) gave 28 hydrocarbons and other metabolites including 12 alkanes, 4 PAHs, and 12 volatile compounds such as 7 cyclohexane derivatives and 5 other derivatives.

Separation of compounds isolated from algal species was conducted with two coupled columns (30 m). The first column allows reasonable retention of the low volatile compounds, but other components are not separated and pass into the second column while the volatile compounds are detected [21].

In 60s and 70s of the last century, it was stated that cyanobacteria contain only three alkanes. The content of C7-and 8-methyl-C17 is dominating and varies from 60 up to 97% of the total hydrocarbons [21].

From Table 2, we can conclude that, the mycobiont synthesizes alkane hydrocarbons from C20 up to C30, with dominating C24 and C30.

Hydrocarbons (alkanes and alkenes) are abundantly present in the volatiles. All hydrocarbons have formerly been found in the cells of *Scenedesmus* by extraction with organic solvents. The proportion of hydrocarbons found in volatiles and those found in solvent extracts are rather different. Hydrocarbons C15, C17, and C27 predominate in lipophilic extracts, while in volatiles hydrocarbons C11–C32 are evenly distributed. All membranes contain hydrocarbons as previously established. Solvent extraction leads to isolation of the total hydrocarbons from all of the membranes together [22]. The observed differences in hydrocarbon composition might be explained by the assumption that steam distillation yields substances from the plasma membrane only but not from the intracellular membranes. This suggestion could be supported by the absence of methyl esters of long-chain fatty acids in the volatiles of the studied algal species. Probably, they are localized in inner membranes and do not leave the cell if it is not disintegrated as reported by Kambourova et al. [23].

The concentration of PAHs was varied between green and blue green algal species as shown in Table 3 and has higher concentration than *n*-alkanes as shown in Fig. (1). Since, *Scenedosmus obliquus* (from green algae) has total PAHs concentration (238.66 μg/g) much higher than that detected with *O. agardhii* (174.28 μg/g), *O. limnetica* (163.9 μg/g), and *A. sphaerica* (145.12 μg/g) (blue green algae). The only detected PAHs with blue green algal species were fluorine, pyrene, benzo (a) anthracene and chryzene. On the other hand, 14 PAHs were detected with *S. obliquus* as green algae.

Table 1
Hydrocarbons and other organics identified from cultivated green and blue green algae

Scenedosmus obliquus (green algae)			*Anabaena sphaerica* (blue green algae)			*Oscillatoria agardhii* (blue green algae)			*Oscilatoria limnetica* (blue green algae)		
Compound	Molecular formula	Molecular weight	Compound	Molecular formula	Molecular weight	Compound	Molecular formula	Molecular weight	Compound	Molecular formula	Molecular weight
Nonane	C_9H_{20}	128	Nonane	C_9H_{20}	128	Nonane	C_9H_{20}	128	Nonane	C_9H_{20}	128
Dodecane	$C_{12}H_{26}$	146	Dodecane	$C_{12}H_{26}$	146	Dodecane	$C_{12}H_{26}$	146	Dodecane	$C_{12}H_{26}$	146
Tetradecane	$C_{14}H_{30}$	198	Tetradecane	$C_{14}H_{30}$	198	Tetradecane	$C_{14}H_{30}$	198	Tetradecane	$C_{14}H_{30}$	198
Hexadecane	$C_{16}H_{34}$	226	Hexadecane	$C_{16}H_{34}$	226	Hexadecane	$C_{16}H_{34}$	226	Hexadecane	$C_{16}H_{34}$	226
Octadecane	$C_{18}H_{38}$	254	Octadecane	$C_{18}H_{38}$	254	Octadecane	$C_{18}H_{38}$	254	Octadecane	$C_{18}H_{38}$	254
Eicosane	$C_{20}H_{42}$	282	Eicosane	$C_{20}H_{42}$	282	Eicosane	$C_{20}H_{42}$	282	Eicosane	$C_{20}H_{42}$	282
Docoane	$C_{22}H_{46}$	310	Docoane	$C_{22}H_{46}$	310	Docoane	$C_{22}H_{46}$	310	Docoane	$C_{22}H_{46}$	310
Tetracosane	$C_{24}H_{50}$	338	Tetracosane	$C_{24}H_{50}$	338	Tetracosane	$C_{24}H_{50}$	338	Tetracosane	$C_{24}H_{50}$	338
Hexacosane	$C_{28}H_{58}$	366	Hexacosane	$C_{28}H_{58}$	366	Hexacosane	$C_{28}H_{58}$	366	Hexacosane	$C_{28}H_{58}$	366
Octacosane	$C_{29}H_{60}$	394	Octacosane	$C_{29}H_{60}$	394	Octacosane	$C_{29}H_{60}$	394	Octacosane	$C_{29}H_{60}$	394
Triacontane	$C_{30}H_{62}$	422	Triacontane	$C_{30}H_{62}$	422	Triacontane	$C_{30}H_{62}$	422	Triacontane	$C_{30}H_{62}$	422
Dotriacontane	$C_{32}H_{66}$	450	Dotriacontane	$C_{32}H_{66}$	450	Dotriacontane	$C_{32}H_{66}$	450	Dotriacontane	$C_{32}H_{66}$	450
Naphthlaene	$C_{10}H_{12}$	132	Cyclohexane, 1-methyl-4-(1-methylethyl)	C_{10} H_{20}	140	Cyclohexane, 1-methyl-4-(1-methylethyl)	C_{10} H_{20}	140	Cyclohexane, 1-methyl-4-(1-methylethyl)	C_{10} H_{20}	140
1-methylnapthalene	$C_{11}H_{10}$	142	Cyclohexane, 1-methyl-3-(1-methylethyl)	C_{10} H_{20}	140	Cyclohexane, 1-methyl-3-(1-methylethyl)	C_{10} H_{20}	140	Cyclohexane, 1-methyl-3-(1-methylethyl)	C_{10} H_{20}	140
2-methylnaphthalene	$C_{11}H_{10}$	142	Cyclohexane, diethyl	C_{10} H_{20}	140	Cyclohexane, diethyl	C_{10} H_{20}	140	Cyclohexane, diethyl	C_{10} H_{20}	140
Acenaphthalene	$C_{12}H_8$	152	Cyclohexane, 1-ethyl-1,3-dimethyl	C_{10} H_{20}	140	Cyclohexane, 1-ethyl-1,3-dimethyl	C_{10} H_{20}	140	Cyclohexane, 1-ethyl-1,3-dimethyl	C_{10} H_{20}	140
Acenaphthylene	$C_{12}H_8$	152	Cyclohexane, 1-methyl-2-propyl	$C_{10}H_{20}$	140	Cyclohexane, 1-methyl-2-propyl	$C_{10}H_{20}$	140	Cyclohexane, 1-methyl-2-propyl	$C_{10}H_{20}$	140
Fluorene	$C_{12}H_8$	152	Cyclohexane, 1-methyl-3-propyl	$C_{10}H_{20}$	140	Cyclohexane, 1-methyl-3-propyl	$C_{10}H_{20}$	140	Cyclohexane, 1-methyl-3-propyl	$C_{10}H_{20}$	140
Anthracene	$C_{14}H_{10}$	178	Cyclopentane pentyl	$C_{10}H_{20}$	140	Cyclopentane pentyl	$C_{10}H_{20}$	140	Cyclopentane pentyl	$C_{10}H_{20}$	140
Fluoranthene	$C_{16}H_{10}$	202	Cyclohexane-1-propyl	C_9H_{18}	126	Cyclohexane-1-propyl	C_9H_{18}	126	Cyclohexane-1-propyl	C_9H_{18}	126
pyrene	$C_{16}H_{10}$	202	2,4,7-tridecatrien-1-al	$C_{13}H_{20}O$	192	2,4,7-tridecatrien-1-al	$C_{13}H_{20}O$	192	2,4,7-tridecatrien-1-al	$C_{13}H_{20}O$	192
Benzo (a)anthracene	$C_{18}H_{12}$	228	4,7,10,13,16,19-docosahexaenoic acid, methyl ester	$C_{23}H_{34}O_2$	342	4,7,10,13,16,19-docosahexaenoic acid, methyl ester	$C_{23}H_{34}O_2$	342	4,7,10,13,16,19-docosahexaenoic acid, methyl ester	$C_{23}H_{34}O_2$	342
Chyrsene	$C_{18}H_{12}$	228	Fluorene	$C_{13}H_{10}$	166	Fluorene	$C_{13}H_{10}$	166	Fluorene	$C_{13}H_{10}$	166
Benzo (b) anthracene	$C_{16}H_{10}$	202	Pyrene	$C_{16}H_{10}$	202	pyrene	$C_{16}H_{10}$	202	pyrene	$C_{16}H_{10}$	202
Benzo (k) Fluoranthene	$C_{20}H_{12}$	252	Benzo (a) Anthracene	$C_{18}H_{12}$	228	Benzo(a)anth	$C_{18}H_{12}$	228	Benzo(a)anth	$C_{18}H_{12}$	228
Benzo (a) Pyrene	$C_{20}H_{12}$	252	Chyrsene	$C_{18}H_{12}$	228	Chyrsene	$C_{18}H_{12}$	228	Chyrsene	$C_{18}H_{12}$	228
nonadecatetraene	$C_{19}H_{32}$	260	Heptanedecatrien-1-ol	$C_{17}H_{30}O$	250	Heptanedecatrien-1-ol	$C_{17}H_{30}O$	250	Heptanedecatrien-1-ol	$C_{17}H_{30}O$	250
	C_8H_{10}	106		$C_{18}H_{24}O$	272		$C_{18}H_{24}O$	272		$C_{18}H_{24}O$	272

(*Continued*)

Table 1 (*Continued*)

Scenedosmus obliquus (green algae)			*Anabaena sphaerica* (blue green algae)			*Oscillatoria agardhii* (blue green algae)			*Oscilatoria limnetica* (blue green algae)		
Compound	Molecular formula	Molecular weight	Compound	Molecular formula	Molecular weight	Compound	Molecular formula	Molecular weight	Compound	Molecular formula	Molecular weight
7-methyl-1,3,5-cyclo heptatrien			Heptane decatrienoic acid-methyl ester			Heptane decatrienoic acid-methyl ester			Heptane decatrienoic acid-methyl ester		
Octadecadiynoic acid	$C_{19}H_{30}O_2$	290									
Cyclohexadiene-1,4-dione 2,6-bis (1,1-methyl)	$C_{14}H_{20}O_2$	220									
Cyclohexane-1-propanol	$C_9H_{18}O$	142									
Octadecanoic acid, 2-phenyl-1,3-dioxolane-4,4,5 methyl ester	$C_{28}H_{46}O_4$	446									

Table 2
Concentration of *n*-alkanes from cultivated green and blue green algae (μg/g)

	Scenedosmus obliquus (green algae)	*Anabaena sphaerica* (blue green algae)	*Oscillatoria agardhii* (blue green algae)	*Oscilatoria limnetica* (blue green algae)
C9	0.271	0.307	1.040	0.771
C12	1.763	1.998	3.774	2.784
C14	1.135	1.285	3.149	2.663
C16	1.520	1.722	2.774	2.224
C18	2.222	2.517	4.012	3.158
C20	9.093	10.301	12.441	11.570
C22	9.997	11.326	14.112	12.957
C24	20.860	23.633	27.880	25.554
C26	4.327	4.902	7.099	5.748
C28	6.398	7.248	10.224	8.841
C30	46.595	52.790	60.439	54.358
C32	2.244	2.542	4.135	3.478
Total	106.423	120.572	150.039	133.335

Table 3
Concentration of PAHs from cultivated green and blue green algae (μg/g)

	Scenedosmus obliquus (green algae)	*Anabaena sphaerica* (blue green algae)	*Oscillatoria agardhii* (blue green algae)	*Oscilatoria limnetica* (blue green algae)
Naphthlaene	5.78	N.D	N.D	N.D
1-methylnaphthalene	11.57	N.D	N.D	N.D
2-methylnaphthalene	0.09	N.D	N.D	N.D
Acenaphthalene	0.80	N.D	N.D	N.D
Acenaphthylene	3.54	N.D	N.D	N.D
Fluorene	5.67	131.33	155.65	147.98
Anthracene	49.48	N.D	N.D	N.D
Phenanthrene	N.D	N.D	N.D	N.D
Fluoranthene	11.40	N.D	N.D	N.D
Pyrene	15.86	4.54	6.74	5.79
Benzo (a) anthracene	27.87	8.97	10.88	9.36
Chyrsene	15.78	0.28	1.01	0.77
Benzo (b) anthracene	13.19	N.D	N.D	N.D
Benzo (k) fluoranthene	14.72	N.D	N.D	N.D
Benzo (a) Pyrene	62.91	N.D	N.D	N.D
Total Hydrocarbons (mg/g)	238.66	145.12	174.28	163.90

Fluorene has the highest concentration (131.33–155.65 μg/g) among the detected 4 PAHs in blue green algal species compared with pyrene, benzo(a)anthracene, while chryzene showed the lowest concentration (0.28–1.01 μg/g). On the other hand, benzo(a)pyrene had the highest concentration (62.91 μg/g) among PAHs detected from the metabolites of *S. obliquus* followed by anthracene, while 2-methyl naphthalene showed the lowest concentration (0.09 μg/g).

These compounds can be a source of biofuel that can be produced from algae depending on the technique and the part of the cells used. The lipid or oily part of the algae biomass can be extracted and converted into biodiesel or converted in a refinery into "drop-in" replacements for petroleum-based fuels. Also, the carbohydrate content of algae can be fermented into bioethanol or biobutanol.

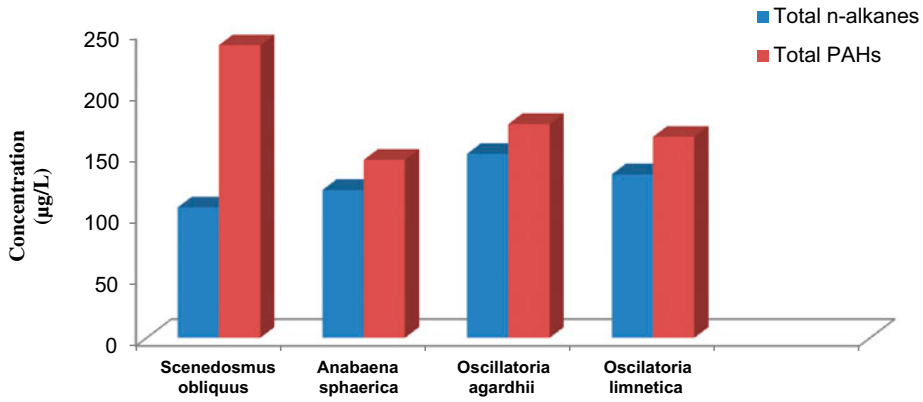

Fig. 1. Comparison of total concentration of PAHs and *n*-alkanes in the metabolites of the studied algal species.

4. Conclusion

In this study, we described the successful separation of the hydrocarbons and other organic compounds using GC and GC/Ms.

14 PAHs were identified from the extraction of *S. obliquus* (green algae), which are much more than that identified by extraction from each of *O. agardhii*, *O. limnetica*, and *A. sphaerica* (blue green algae) that give only 4 PAHs. On the other hand, the number of volatile organic compounds (five compounds) produced by *S. obliquus* was much lower than that produced from the extraction of *O. agardhii*, *O. limnetica*, and *A. sphaerica* (12 compounds). Meanwhile, the number of alkanes was 12 compounds in all extracted algal species.

The studied green algal species gave higher concentrations of PAHs compared with any algal species from the studied blue green algae. On the other hand, the variation in alkanes concentration was not significant. These compounds can be a source of biofuel that can be produced from algae

References

[1] A. Schirmer, M.A. Rude, X. Li, E. Popova, S.B. del Cardayre, Microbial biosynthesis of alkanes, Science 329 (2010) 559–562.
[2] X.F. Lu, A perspective: Photosynthetic production of fatty acid-based biofuels in genetically engineered cyanobacteria, Biotechnol. Adv. 28 (2010) 742–746.
[3] C. Jansson, Metabolic engineering of cyanobacteria for direct conversion of CO_2 to hydrocarbon biofuels, in: U. Lüttge, W. Beyschlag, B. Büdel, D. Francis (Eds.), Progress in Botany, vol. 73, 2012, pp. 81–93.
[4] N. Sharma, S. Tiwari, K. Tripathi, A. Rai, Sustainability and cyanobacteria (bluegreen algae): Facts and challenges, J. Appl. Phycol. 23 (2010) 1–23.
[5] K. Winters, P.L. Parker, C. Van Baalen, Hydrocarbons of blue-green algae: Geochemical significance, Science 163 (1969) 467–468.
[6] V.M. Dembitsky, T. Řezanka, Metabolites produced by nitrogen-fixing *Nostoc* species, Folia Microbiol. 50 (2005) 363–391.
[7] J. Han, E.D. McCarthy, W.V. Hoeven, M. Calvin, W.H. Bradley, Organic geochemical studies, ii. A preliminary report on the distribution of aliphatic hydrocarbons in algae, in bacteria, and in a recent lake sediment, Proc. Nat. Acad. Sci. USA 59 (1968) 29–33.
[8] B. Taylor, N. Xiao, J. Sikorski, M. Yong, T. Harris, T. Helme, Techno-economic assessment of carbon-negative algal biodiesel for transport solutions, Appl. Energy 106 (2013) 262–274.
[9] M. Daroch, S. Geng, G.Y. Wang, Recent advances in liquid biofuel production from algal feedstocks, Appl. Energy 102 (2013) 1371–1381.
[10] I. Rawat, R. Ranjith Kumar, T. Mutanda, F. Bux, Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production, Appl. Energy 88 (2011) 3411–3424.
[11] E. Gelpi, H. Schneider, J. Mann, J. Oró, Hydrocarbons of geochemical significance in microscopic algae, Phytochemistry 9 (1970) 603–612.
[12] X.Y. Liu, J. Sheng, R. Curtiss III, Fatty acid production in genetically modified cyanobacteria, Proc. Nat. Acad. Sci. USA 108 (2011) 6899–6904.
[13] P. Hu, S. Borglin, N.A. Kamennaya, L. Chen, H. Park, L. Mahoney, Metabolic phenotyping of the cyanobacterium *Synechocystis* 6803 engineered for production of alkanes and free fatty acids, Appl. Energy 102 (2013) 850–859.
[14] A. Banerjee, R. Sharma, Y. Chisti, U.C. Banerjee, *Botryococcus braunii:* A renewable source of hydrocarbons and other chemicals, Crit. Rev. Biotechnol. 22 (September) (2002) 245 –279.
[15] P. Metzger, C. Largeau, *Botryococcus braunii*: A rich source for hydrocarbons and related ether lipids, Appl. Microbiol. Biotechnol. 66(February) (2005) 486–496.
[16] C. Dayananda, R. Sarada, V. Kumar, G.A. Ravishankar, Isolation and characterization of hydrocarbon producing green alga *Botryococcus braunii* from Indian freshwater bodies, Electron. J. Biotechnol. 10 (2007) 15–20.

[17] M. Audino, K. Grice, R. Alexander, R.I. Kagi, Macrocyclic alkanes in crude oils from the algaenan of *Botryococcus braunii*, Org. Geochem. 33 (2002) 979–984.
[18] W.W. Carmichael, Isolation, culture and toxicity testing of toxic fresh water cyanobacteria (blue-green algae), in: V. Shilo (Ed.), Fundamental Research in Homogeneous Catalysis, vol. 3, Gordon & Breach, New York, NY, 1986, pp. 1249–1262.
[19] S. Abdo, M. Hetta, M.H. El Din, R.A. Salah, G.H. Ali, Growth evaluation and bioproduct characteristics of certain freshwater algae isolated from river Nile, Egypt. J. Appl. Sci. Res. 6 (2010) 642–652.
[20] G. Ali, Identification of volatile organic compounds by algae, Egypt. J. Ohyscol. 5 (2004) 71–81.
[21] V.M. Dembitsky, I. Shkrob, I. Dor, Separation and identification of hydrocarbons and other volatile compounds from cultured blue-green alga *Nostoc* sp. by gas chromatography–mass spectrometry using serially coupled capillary columns with consecutive nonpolar and semipolar stationary phases, J. Chromatogr. A 862 (1999) 221–229.
[22] G. Petkov, Lipids of photoautotrophic cultivated microalgae, Thesis Doct., Institute of Plant Physiology – Bulgarian Academy of Sciences, 1990, 119 p.
[23] R. Kambourova, V. Bankova, G. Petkova, Volatile Substances of the Green Alga *Scenedesmus incrassatulus*, Verlag der Zeitschrift für Naturforschung., D. 2003 (2003) 187–190.