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Chapter

The Colonial Microalgae *Botryococcus braunii* as Biorefinery

Edmundo Lozoya-Gloria, Xochitl Morales-de la Cruz and Takehiro A. Ozawa-Uyeda

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Abstract

The growing shortage of fossil fuels caused an increase in the demand for alternative and renewable fuels. Biofuels, like bioethanol and biodiesel, have received more attention as a sustainable replacement of fossil fuels. However, these have a poor oxidative stability, little energy content by volume, and many oxygenated compounds, which may cause corrosion and damage to the engines. Therefore, they are used as a mixture with standard fuels. Some species of microalgae are candidates to produce oils as triglycerides (TGA) to produce biodiesel by transesterification; however, the problem will remain. The colonial microalgae *Botryococcus braunii* produces and accumulates a high amount of long-chain nonoxygenated hydrocarbons, similar to those obtained from the fractionated distillation of crude petroleum. This is one of the few organisms reported to have a direct contribution in the formation of the oil reserves currently in use. Additionally, *B. braunii* produces pigments and long-chain carbohydrates that have interesting properties for various industries. There are still problems to be solved in order to consider it as economically viable and profitable, but important progress is being made. Therefore, this microalga is very attractive for the synthesis of hydrocarbons and other value-added compounds, making it an interesting biorefinery organism.

Keywords: biorefinery, *Botryococcus*, exopolysaccharides, hydrocarbons, lipids, pigments

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1. Introduction

Botryococcus braunii is a colonial microalga Trebouxiophyceae, distributed in brackish and sweet water [1]. It reaches densities of 1.4×10^6 colonies/L [2], and its geochemistry significance is important. Paleobotanical studies suggest that it is one of the largest sources of hydrocarbons in oil-rich deposits dating back to the Ordovician period [1, 3–5]. It is the only colonial microalga that accumulates and secrets liquid hydrocarbons (**Figure 1**), and depending on the strain and growing conditions, race B can accumulate hydrocarbons up to 85% and race A up to 61% of their dry weight.

B. braunii is related with *Characium vaculatum and Dunaliella parva* [1]. Due to the hydrocarbons and the molecular phylogeny of *B. braunii* [6], it is classified in three races (A, B, and L). Race A produces *n*-alkadienes and alkatrienes of C_{23}–C_{33}

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[7], although two unusual hydrocarbons have been characterized, the triene $C_{27}H_{51}$ and tetraene $C_{27}H_{48}$ [1]. Race A hydrocarbon dry weight varies from 0.4 to 61% [7, 8]. Race B produces triterpenoids hydrocarbons known as botryococcenes (C_nH_{2n-10}, n = 30-37) [9] and methylsqualenes C_{31}–C_{34} [10, 11]. The botryococcenes can be from 27 to 86% of the dry weight [12]. Race L produces a tetraterpene C_{40} known as lycopadiene and constitutes from 0.1 to 8% of the dry weight [13, 14]. This race contains 5% of lycopatriene, lycopatetraene, lycopapentaene, and lycopahexaene [15]. In addition, a race S is proposed, which synthesizes saturated *n*-alkanes C_{18} and C_{20}, and epoxy-alkanes; however, its existence is not yet fully accepted [6].

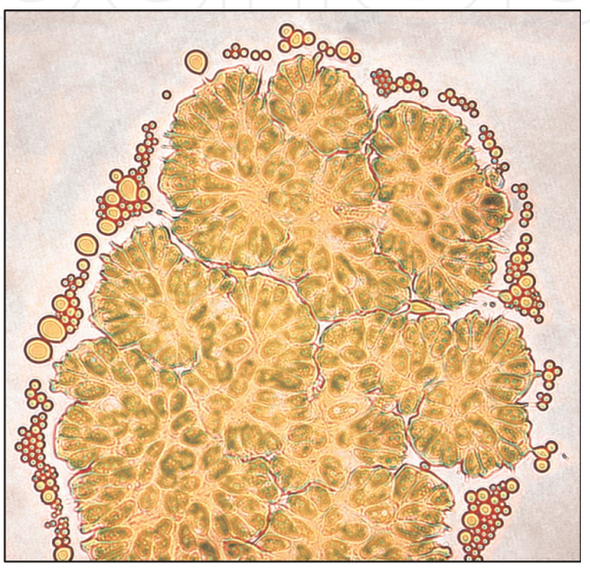

Figure 1.
B. braunii race B colony secreting liquid hydrocarbons.

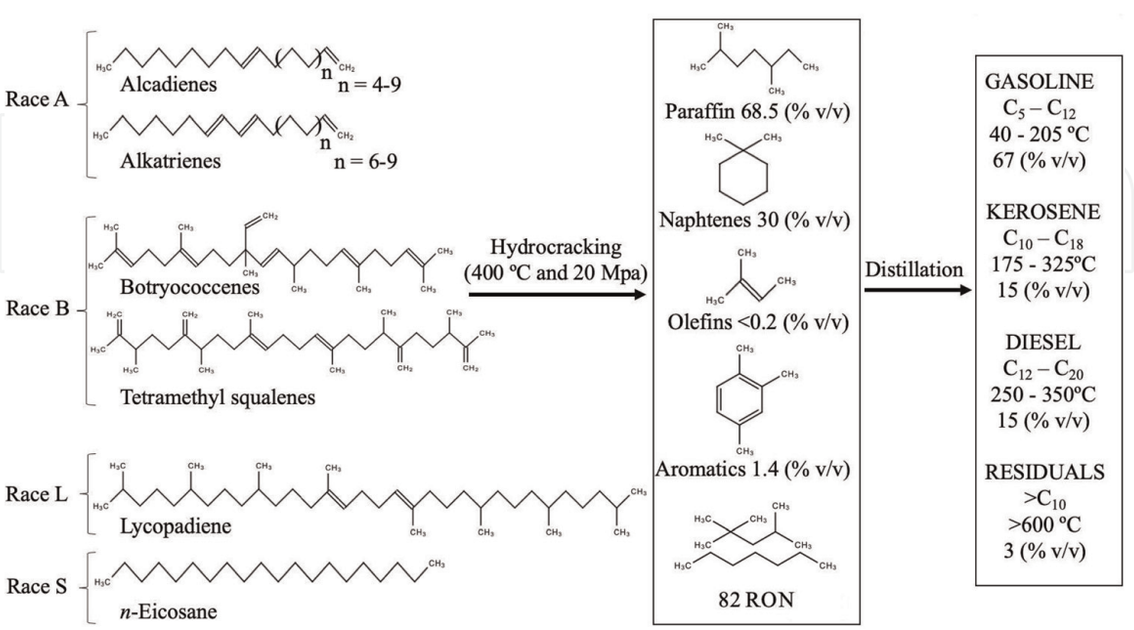

Figure 2.
Hydrocarbons produced by the B. braunii races. Biofuels derived from race B are shown. RON, research octane number = 92–98, this is a measure of autoignition resistance in a spark-ignition engine. In the USA: regular (97 RON) and premium (95 RON). Adapted from [16–18].

After the hydrocracking process and subsequent distillation, race B hydrocarbons become biofuels currently used in internal combustion engines [16] as shown in **Figure 2**.

2. Physiology and biochemistry of *Botryococcus braunii*

B. braunii races differ also by its morphological and physiological characteristics. Cells from A and B races are of 13 μm × 7–9 μm, and those of L race are 8–9 μm × 5 μm [19].

Each colony is constituted by a group of 50–100 piriform cells embedded in a hydrocarbon network and the extracellular matrix (ECM). This ECM contains three main components: (1) a fibrous cell wall surrounding each cell and having β-1,4- and/or β-1,3-glucans including cellulose; (2) the intracolonial space constituted by a network of liquid hydrocarbons; and (3) a fibrillary sheath composed mainly of arabinose and galactose polysaccharides, holding the liquid hydrocarbons [20].

B. braunii may have a hetero-, mixo-, or phototrophic grow and the morphology will depend on the C source and the amount of light [21]. The hydrocarbon production is associated with the cell division [22], likely due to the localization of the enzymes involved in the alkadienes, alkatrienes (race A), and botryococcenes (race B) biosynthesis [23].

Other difference among the races is the keto-carotenoid accumulation in the stationary phase of cultures. Races B and L change color from green-brown to orange, and race A changes from green to yellow-orange [1]. The production of carotenoids is also a stress response by environmental factors. The *DAD1* gene expression, a suppressor of programmed cell death, was reported in race B, under stress conditions at 10–60 min [24]. *B. braunii* is tolerant to desiccation and extreme temperatures, which allows its global dispersion in different environments [25]. The reproduction mechanism of *B. braunii* seems to be autosporic [26].

Symbiotic bacteria have been reported after microscopic observations, and an ectosymbiont α-proteobacteria (BOTRYCO-2) that promotes the productivity of biomass and hydrocarbons was described [2, 27].

2.1 Biosynthesis of alkadienes and alkatrienes

Characteristic alkadienes and alkatrienes of race A have double links and similar stereochemistry as oleic acid. Experiments with labeled fatty acids have shown that this one is the main precursor by the long-chain fatty acids (LCFAs) pathway, followed by a decarboxylation process [1, 17, 28, 29]. The first step is the elongation of oleic acid (18:1 *cis*-Δ9) and its isomer elaidic acid (18:1 *trans*-Δ9). The acyl-CoA reductase and decarbonylase enzymes in race A microsomes suggest an alternative mechanism where the LCFAs are reduced to aldehydes and decarbonylated to produce alkadienes and alkatrienes [17, 30]. Race A transcriptome allowed the identification of six candidate genes potentially involved in this biosynthesis [31].

2.2 Biosynthesis of botryococcenes

The analysis of race B transcriptome and other evidences suggests that the biosynthesis of isoprenoids comes from the deoxyxylulose phosphate/methylerythritol phosphate (DXP/MEP) pathway [32–34]. Expressed sequence tag (EST) markers for enzymes of the DXP/MEP pathway [34], as well as multiple isoforms of

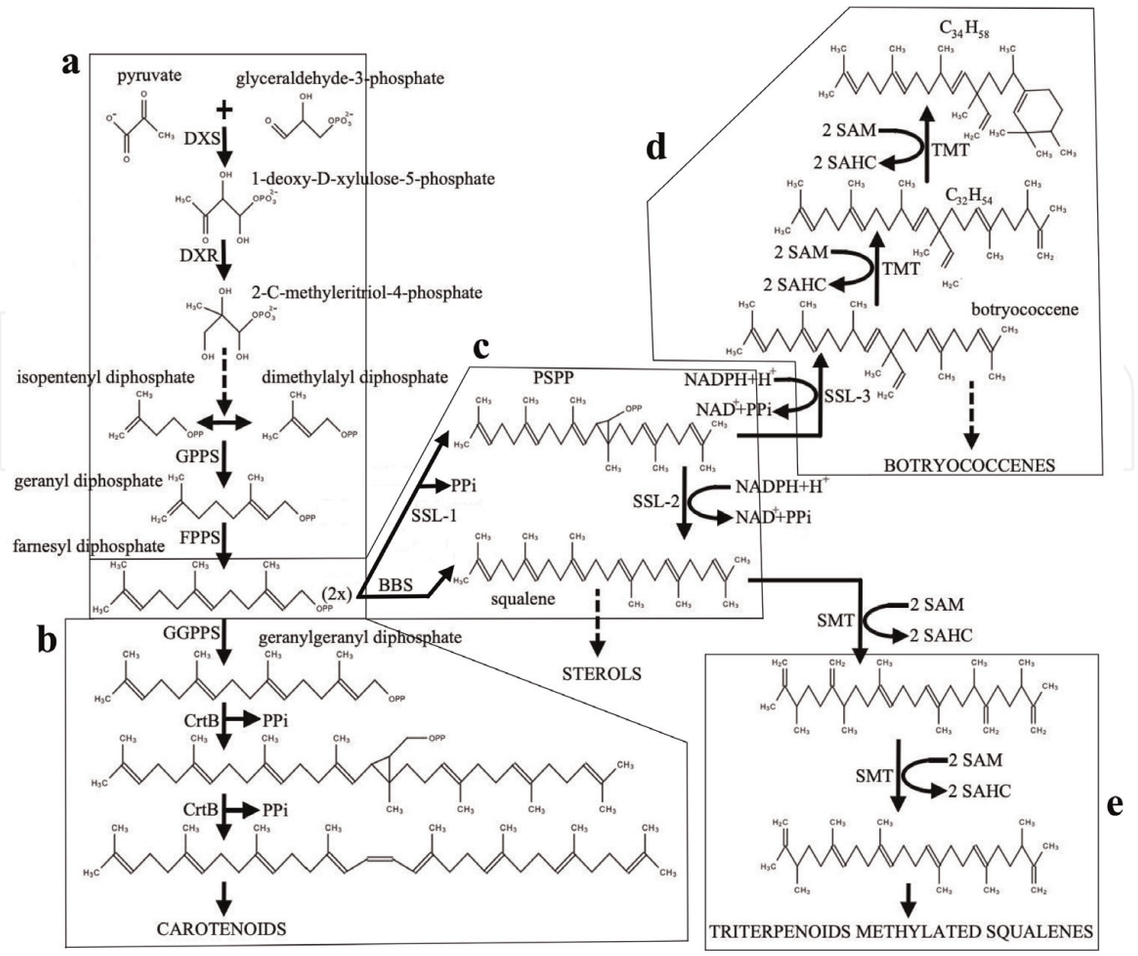

Figure 3.
Biosynthesis of tri- and tetraterpenes in B. braunii race B. (a) FPP production; (b) carotenoid production from GGPP; (c) squalene production from FPP; (d) methylated botryococcene production; (e) methylated squalene production. BSS, Botryococcus squalene synthase; CtrB, phytoene-synthase; DXR, 1-deoxy-D-xylulose-5-phosphate reductase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; FPPS, farnesyl diphosphate synthase; GPPS, geranyl diphosphate synthase; NADPH+ and NADP+, nicotinamide adenine dinucleotide phosphate (reduced and oxidized); PPi, inorganic pyrophosphate; PSPP, cyclopropyl presqualene diphosphate; SAM, S-adenosyl methionine; SAH, S-adenosyl-L-homocysteine; SSL, squalene synthase-like; SMT, squalene methyltransferase; TMT, triterpene methyltransferase. Adapted from [17, 34].

enzymes for the 3-phospho-D-glycerate biosynthesis from D-glyceraldehyde-3-phosphate and pyruvate as precursors, were identified. Some of the respective transcripts are present in high abundance (>250 reads/Kb), suggesting a high metabolic flow in *B. braunii* [31].

The first step is the formation of 1-deoxy-D-xylulose-5-phosphate (DOXP) by the DOXP synthase (DXS) (**Figure 3**).

The characterization of three DXS isoenzymes in race B shows that they are active and have similar kinetic parameters, which increases the metabolic flow for the production of terpenoids [35]. The DOXP is reduced by the DXP reductoisomerase (DXR) to 2-C-methylerythritol-4-phosphate (MEP), and converted to isopentenyl diphosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). In the *B. braunii* transcriptome, only one DXR has been found [34]. The next step involves condensation of IPP and DMAPP to form geranyl diphosphate (GPP), and the addition of other IPP produces farnesyl diphosphate (FPP) [17] (**Figure 3a**). Two *B. braunii* genes code for farnesyl diphosphate synthase isoenzymes (FPPS) with an amino acid identity of 72% [34].

Addition of another IPP forms the geranylgeranyl diphosphate (GGPP), precursor of the tetraterpenoid carotenoids (**Figure 3b**). This begins with the formation of a *trans*-isoprenyl diphosphate by the phytoene synthase (CtrB) enzyme, condensing two GGPP molecules in two steps with the release of pyrophosphate. In the first step, (1R, 2R, 3R)-prephytoene diphosphate is produced from half cyclopropyl (C1′-2-3) reordered to provide 15-*cis*-phytoene, which can be converted into a wide variety of carotenoids [34, 36–38]. All are important antioxidant photoprotectors and modulators of the function of membrane proteins for photosynthetic complexes [39].

The squalene production [40] starts with the *Botryococcus* squalene synthase (BSS) enzyme, using two FPP molecules. Botryococcenes production uses also two FPP molecules but the product is the intermediary cyclopropyl presqualene diphosphate (PSPP) (**Figure 3c**). With NADPH, the PSPP has two options; one forms the botryococcene with a C3-C1 connection between the FPP molecules (**Figure 3d**). The other option forms a C1-C1′ between two FPP molecules producing squalene

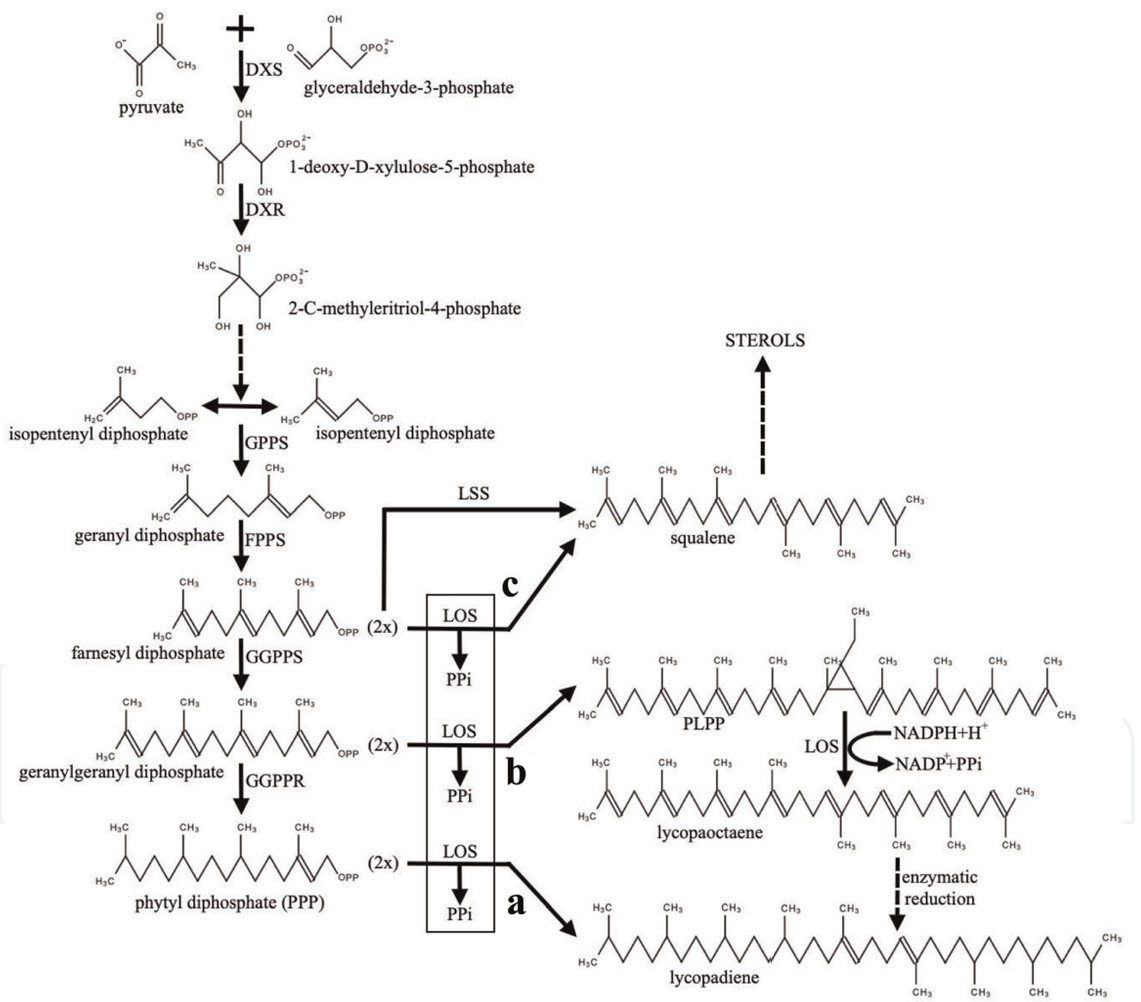

Figure 4.
Lycopadiene biosynthetic pathway. (a) Reduction of GGPP to PPP and condensation by LOS. (b) LOS condensation of GGPP to form phytyl diphosphate and reduction to lycopaoctaene. (c) FPP use by LSS or LOS for squalene production. DXR, 1-deoxy-D-xylulose-5-phosphate reductase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; FPP, farnesyl diphosphate; FPPS, farnesyl diphosphate synthase; GGPP, geranylgeranyl diphosphate; GPPS, geranyl diphosphate synthase; GPPR, geranyl diphosphate reductase; NADPH+ and NADP+, nicotinamide adenine dinucleotide phosphate (reduced and oxidized); PPi, inorganic pyrophosphate; PPP, phytyl diphosphate; PLPP, prelycopaoctaene diphosphate; LOS, lycopaoctaene synthase; LSS, B. braunii race L squalene synthase. Adapted from [15].

that will be methylated (**Figure 3e**) further on. These reactions are catalyzed by squalene synthase-like (SSL) enzymes. Three SSL genes have been identified but none is directly related with the botryococcene biosynthesis [41]. However, when the 3 SSLs enzymes were mixed *in vivo* and *in vitro*, botryococcene (SSL-1 + SSL-3) or squalene (SSL1 + SSL-2) was synthesized. SSL-1 condenses two FPP molecules to produce PSPP [42], demonstrating the versatility and potential for metabolic engineering of botryococcene biosynthesis.

Most botryococcenes are excreted to the ECM where they are methylated. The di- and tetramethyl forms are related to six genes coding for triterpene and squalene methyltransferases (TMT, SMT) [43] (**Figures 3d** and **3e**). The botryococcenes are methylated to produce C_{31}–C_{37} hydrocarbons, C_{34} being the main in race B. Three cyclic botryococcene C_{33} molecules and a trimethylsqualene isomer were recently found [44]. Also, two squalene epoxidase (*BbSQE-I* and *-II*) enzymes converting squalene into membrane sterols were identified [45]. Data of the *B. braunii* race B nuclear genome will allow the search for possible regulatory routes of this singular metabolism [46].

2.3 Biosynthesis of lycopadiene

The formation of lycopadiene of race L is similar to the squalene. In the *B. braunii* transcriptome, there are two homologous contigs to squalene synthase (SS) [31]. One encodes a squalene synthase (LSS) and the other for a lycopaoctaene synthase (LOS). LOS uses preferentially *in vivo* GGPP, and C_{15} and C_{20} prenyl diphosphates as substrates [15] (**Figure 4**).

There are two biosynthetic mechanisms for lycopadiene from C_{20} prenyl diphosphate intermediates. In one, the GGPP reduction by a GGPP-reductase produces phytyl diphosphate (PPP), and LOS condenses two PPP molecules producing lycopadiene (**Figure 4a**). In the other one, LOS condenses two GGPP molecules producing prelycopaoctaene diphosphate (PLPP), which rearranges into lycopaoctaene. Finally, lycopadiene seems to be produced by enzymatic reductions not yet identified (**Figure 4b**).

LOS may also form squalene from FPP (**Figure 4c**). These results show the plasticity of L race to synthesize squalene and lycopadiene.

2.4 Extracellular matrix (ECM) polymers

ECM contains long chains of polymerized polyacetal hydrocarbons joined to specific hydrocarbons of each race. There is a fibrillary sheath that envelops the entire colony, formed mainly by arabinose (42%) and galactose (39%). The cell wall contains β-1,4 and/or β-1,3 glucans making a cellulose-like polymer [20].

Also, there's a biopolymer resistant to nonoxidative chemical degradation as acetolysis. This biopolymer resembles sporopollenins [1] of the outer walls of pollen grains and spores of microorganisms [47]. It seems to be formed by oxidized carotenoid polymers and phenolic compounds that absorb UV-B light as *p*-coumaric and *p*-ferulic acids [48].

3. Profitability of *B. braunii* derivatives

3.1 Hydrocarbons

Both bioethanol and biodiesel have a poor oxidative stability, low energy content by volume, and high content of oxygenated compounds, which damage combustion

engines and cause corrosion, erosion, and accumulation of deposits in the nozzles; because of these reasons, they are mixed with standard fuels [49, 50]. *B. braunii* accumulates hydrocarbons similar to those of the crude oil, and their direct contribution in the formation of oil reserves currently in use has been reported [3–5]. The *B. braunii* oils showed almost equal values in density and surface tension than the diesel, but with higher kinematic viscosity and distillation temperature [50]. The *B. braunii* race B oil was already converted into diesel with an 85% performance, using a simple conversion process under mild conditions of 260°C and 1 atm. The physical properties are relatively close to the specification for diesel, with 40 as estimated cetane (CN) number [51].

The limitation to use *B. braunii* as biorefinery is the slow growth rate of days in comparison with hours in other algae [49, 52]. Other factors affecting the growth

St	Culture conditions				SCGR	Dt	T_{HC}	Ref.
	°C	PAR	Php	CO_2				
Showa (B)	30	850	14:10	1	0.5	1.40	NIA	[54]
Showa (B)	25, 30	85–398	14:10	1.0–10.0	0.19–0.44	1.60–3.60	30–39	[54]
Showa (B)	23–25	250	24	0.3	0.42	1.70	24–29	[52]
Showa (B)	23	150	16:8	2	0.17	4.08[d]	25	[55]
Yayoi (B)	25	240	12:12	2	0.2	3.50	40.5	[38]
AC759 (B)	23	150	16:8	2	0.07	9.90[d]	21	[55]
AC761 (B)	23	150	16:8	2	0.11	6.30[d]	45	[55]
IPE001 (B)	25	35	16:8	1	0.15[c]	4.50[c]	64.3	[61]
BOT-144 (B)	25	60[a]	24	0	0.16	4.33[d]	50	[62]
LB-572 (A)	26	12 Klux	24	2	0.07[c]	10.60[c]	28	[53]
Gottingen 807/1 (A)	25	26[b]	14:10	1	0.3	2.30	40.5	[67]
AC755 (A)	23	150	16:8	2	0.05	13.86[d]	16	[55]
CCALA777 (A)	23	150	16:8	2	0.06	11.55[d]	10	[55]
CCALA778 (A)	23	150	16:8	2	0.17	4.08[d]	0	[55]
CCAp807/2 (A)	23	150	16:8	2	0.11	6.30[d]	7	[55]
765	25	150	24	20	0.13[c]	5.50[c]	24	[64]
765	25	120	24	ASLW	NIA	NIA	23.8	[65]
GUBIOTJTBB1	25	35	16:8	0	0.112	6.19	52.6	[66]
AP 103	23	30	16:8	0	NIA	NIA	13	[67]

ASLW, aerated swine lagoon wastewaters (not sterile); °C, temperature; CO_2, % v/v; Dt, doubling time (days); NIA, no information available; PAR*, photosynthetic active radiation (μmols of photons/m^2 s); Php, photoperiod (light/dark hours); SCGR, specific cell growth rate (μ/day); μ, specific velocity of growth rate; St, strain (race); T_{HC}, total hydrocarbons (% DW, dry weight).*
[a]Blue light λ = 470 nm.
[b]W/m^2.
[c]Estimated values [54].
[d]Calculated values from μ, using Ln(2)/μ equation.

Table 1.
Comparison of culture conditions and productivity of hydrocarbons between B. braunii strains at laboratory scale.

and hydrocarbon production are the strain, CO_2, light, water, nutrients, temperature, pH, and salinity [53–55, 60] (**Table 1**). A JET PASTER treatment was used to do a mechanical cell disruption and removal of the polysaccharides of the *B. braunii* colonies, increasing the hydrocarbon extraction up to 82.8%. This treatment did not affect the photosynthetic function of the cells [56]. On the other hand, a repetitive nondestructive extraction with heptane was reported as having some advantages [57]. Also, a continuous growth and extraction column of *n*-dodecane was reported recently as an efficient hydrocarbon extraction method without significant loss of the viability of the cells [58]. Considering these milking procedures and achieving a 10% rate of return, a minimum sales price (MSP) of US$3.20 per liter was calculated, and a reduction down to US$1.45 per liter was proposed, if hydrocarbon content increases and extraction procedures become more efficient [59].

There are different open and closed culture systems in photobioreactors (PBR) [63, 64], but more studies are required at pilot and industrial scale, to reduce problems by contamination and low yield of biomass and hydrocarbon production [49]. **Table 2** summarizes some data about cell growth and hydrocarbon productivity using different culture systems.

St	System	Cultures			Biomass			HCs		Ref
		°C	PAR	CO_2	SCGR	X_{max}	Px	CNT	W_{HC}	
GUBIOT JTBB1	Plain (3 L)	25	35 (16 h)	0%	0.112	NIA	13	52.6	6.8	[62]
765	Column (3 L)	25	150 (24 h)	20%	0.13[g]	NIA	92.4	24.45[g]	22.6	[64]
Showa (B)	PBR[a]	25-28	282 (15 h)	5–7%	NIA	20	1500	22.5	225-340	[68]
NIA	PBR[b]	25	270 (24 h)	Mixo-trophic	NIA	4.55	234	29.7	71.1	[69]
UTEX-LB 572 (A)	Circular (50 L)	rT	Sol r	0%	NIA	NIA	77.8	19	13.2	[70]
N-836 (B)	Rcwy (80 L)	rT	Sol r	0%	NIA	NIA	40	24	10.8	[70]
LB572 (B)	PBR[c]	20	Sol r	0%	0.04	0.3	15	NIA	2.4	[71]
AP103	Rcwy (1800 L)	29	Sol r 5 kWh/m^2.day	0%	0.38	NIA	114	11	12.5	[67]
UTEX-LB 572 (A)	PBR[d]	25	55 (24 h)	1%	NIA	96.4	0.71[i]	NIA	NIA	[72]
FACHB 357 (B)	Attchd[e]	25	500 (24 h)	1%	NIA	62[h]	5.5–6.5[i]	19.43	1.06[i]	[73]
TN101	Rcwy sc[f]	rT	Sol r	0%	NIA	NIA	33.8[i]	22.6	8.2-13[i]	[74]

°C, temperature; CNT, content (% DW dry weight); CO_2, % v/v; HCs, hydrocarbons; PAR*, photosynthetic active radiation (μmols of photons/m^2 s); PBR, photobioreactor; Php, photoperiod (light/dark hours); P_x, biomass productivity (mg/L day); NIA, no information available; Rcwy, raceway; rT, room temperature; SCGR, specific cell growth rate (μ/day); μ, specific velocity of growth rate; Sol r, solar radiation; St, strain (race); W_{HC}, weight of hydrocarbons (mg/L day); X_{max}, maximum cellular concentration (g/L).*

[a]*"Tickle film" (30.5 × 16.5 in) continuous.*

[b]*"Airlift" (10 L).*

[c]*Panel (1000 L) outdoor and semicontinuous.*

[d]*"Biofilm" (0.275 m^2 or 600 mL).*

[e]*"Attached" bioreactor (0.08 m^2 or 240 mL).*

[f]*(25 m^2 or 5000 L) semicontinuous.*

[g]*Estimated values [64].*

[h]*g/m^2.*

[i]*g/m^2/day; shadow area indicates the highest reported values up to now.*

Table 2.
Comparison of culture conditions and productivity of hydrocarbons between strains of B. braunii in bioreactors.

3.2 Lipids

B. braunii also produces saturated and monounsaturated fatty acids, especially palmitic (16:0) and oleic (18:1), as well as triacylglycerols (TAGs). The percentages of total lipids as saturated, monounsaturated, and polyunsaturated fatty acids in dry biomass are around 44.97, 9.85, 79.61, and 10.54%, respectively [64, 75]. Studies *in vitro* and *in vivo* showed that these fatty acids effectively improve the absorption of lipophilic drugs like flurbiprofen, through the skin [76].

B. braunii stores TAGs and saturated fatty acids in the lag phase as an adaptation to stress conditions but most are synthesized during the stationary phase. Although highest content of these acids is intracellular, *B. braunii* secretes oily drops in small quantities observed on the surface of the cell apex [64].

The yield and lipid composition depends on the strain, the culture system used, growth conditions and cell aging, as well as nitrogen, phosphorus, and micronutrient concentrations (**Table 3**).

St	System	TRT	Biomass			Lipids			Ref.
			SCGR	X_{Max}	P_x	CNT	Yld.	Prod.	
UTEX 572 (A)	EF (125 mL)	0.04 mM NO_3	0.09	0.16	NIA	63	NIA	0.009	[77]
		0.37 mM NO_3	0.185	0.38	NIA	36	0.19	0.019	
KMITL 2 (n.d.)	EF (1 L)	86 mg/L NO_3	NIA	0.48	NIA	39.42	0.19	NIA	[78]
		222 mg/L PO_4	NIA	0.86	NIA	54.69	0.47	NIA	
		444 mg/L PO_4	NIA	1.91	NIA	23.23[a]	0.45	NIA	
		27 mg/L Fe	NIA	0.22	NIA	34.93	0.08	NIA	
KMITL 2 (n.d.)	Outdoor oval pond (150 L)	0.17 g/L NO_3	0.045	4.84	NIA	35.24	NIA	0.016	[79]
		2.5 g/L NO_3	0.049	5.62	NIA	38.60	NIA	0.0189	
LB572 (A)	FBR column (625 mL)	0083 g/L PO_4 and 0.1 g/L SO_4	NIA	NIA	0.296	64.96	NIA	0.19	[80]
		0058 g/L PO_4 and 0.09 g/L SO_4	NIA	NIA	0.304	59.56	NIA	0.18	
TRG	EF (250 mL)	Photoaut. (CO_2)	0.093	1.14	NIA	25.1	NIA	0.0241	[81]
		Heterot. (gluc 5 g/L)	0.115	1.75	NIA	29.3	NIA	0.0467	
		Mixot. (gluc 5 g/L + CO_2)	0.195	2.46	NIA	37.5	NIA	0.0645	
IBL-C117	EF (1 L)	Chu (0.75×)	0.13	0.9	0.12	47.1	NIA	NIA	[82]
		Chu (1.0×)	0.13	0.7	0.1	46	NIA	NIA	
		Chu (2.0×)	0.11	1	0.15	41.3	NIA	NIA	
LB572 (A)	EF (1 L)	Chu (0.75×)	0.15	1.3	0.18	20.2	NIA	NIA	[82]
		Chu (1.0×)	0.16	1.4	0.2	22.5	NIA	NIA	
		Chu (2.0×)	0.17	1.5	0.22	11	NIA	NIA	
2441 (A)	FBR Airlift (2 L)	(N:P = 1:1) in Chu	NIA	4.963	0.173	33.7	NIA	NIA	[83]
		(N:P = 3:3) in Chu	NIA	3.857	0.215	34.6	NIA	NIA	
		(N:P = 6:6) in Chu	NIA	3.987	0.223	32.1	NIA	NIA	

St	System	TRT	Biomass			Lipids			Ref.
			SCGR	X_{Max}	P_x	CNT	Yld.	Prod.	
BOT22 (B)	Biofilm bioreac.	Nitrocell. Memb. (diam. 25 mm and pore size 0.45 μm)	NIA	3.12[a]	0.42[b]	NIA	0.83[a]	NIA	[84]
BOT84 (L)			NIA	10.04[a]	3.8[b]	NIA	1.11[a]	NIA	
BOT7 (S)			NIA	13.6[a]	0.99[b]	NIA	0.83[a]	NIA	

CNT, content (% DW dry weight); Chu, Chu media for microalgae [8]; EF, Erlenmeyer flask; FBR, photobioreactor; N:P, proportion of nitrogen: phosphate; P_x, biomass productivity (g/L day); NIA, no information available; Prod., productivity (g/L day); Rcwy, raceway; SCGR, specific cell growth rate (μ/day); μ, specific velocity of growth rate; St, strain (race); TRT, treatment; Yld., yield (g/L); X_{max}, maximum cellular concentration (g/L).
[a] *mg/cm^2.*
[b] *mg/cm^2/day.*

Table 3.
Comparison of crop conditions and lipid productivity in B. braunii.

3.3 Pigments

Algae pigments have been reported to have antioxidant, anticancer, anti-inflammatory, antiobesity, and antiangiogenic properties and function as neuroprotectives [85]. So, they could replace synthetic dyes in food, cosmetic, nutraceutical, and pharmaceutical products [86].

Carotenoid pigments are unsaturated hydrocarbons, while xanthophylls have one or more functional groups containing oxygen such as lutein, canthaxanthin, and astaxanthin [85–87].

Carotenoids abound in races B and L, lutein being the main pigment (22–29%), followed by others as β-carotene, echinenone, 3-OH echinenone, canthaxanthin, violaxanthin, loroxanthin, and neoxanthin. Transition to stationary phase causes a color change in *B. braunii* from green to brown, reddish orange, and pale yellow by accumulation of carotenoids and a decrease of intracellular pigments [88]. Canthaxanthin (46%) and echinenone (20–28%) are predominant in the stationary phase in response to nitrogen deficiency [36]. The BOT-20 strain showed a dark red color during growth because of the accumulated echinenone of about 30.5% dry weight and 630 mg/L production, but with few hydrocarbons (8%) [89].

Adonixanthin was detected in race L during the stationary phase [90], and botryoxanthin A, botryoxanthin B, and braunixanthin 1 and 2 were detected in race B [37, 38, 91]. The 2-azahypoxanthine (AHX) similar to the phytohormone induced the accumulation of secondary carotenoids like botryoxanthin A and braunixanthin 1 and decreased the content of botryococcenes during the stationary phase [92], imitating a lack of nitrogen condition without inhibiting the growth.

In race A, lutein (79–84%) is the main carotenoid followed by β-carotene (1.75–2.14%), violaxanthin (6–9%), astaxanthin (3–8%), and zeaxanthin (0.32–0.78%). In salinity and high light intensity conditions, the lutein increases [53, 93]. All of these compounds shown antioxidant properties and inhibitory effect against lipid peroxidation *in vitro* and *in vivo* and activated antioxidant enzymes such as catalase [94, 95].

3.4 Polysaccharides

The aqueous extracts of *B. braunii* (strain LB 572) reduce the skin dehydration, stimulate collagen synthesis, promote the differentiation of adipocytes, and

promote antioxidant and anti-inflammatory activities [96]. The extracellular polysaccharides (exopolysaccharides, EPS) constitute most of the organic material of high molecular weight released to the environment by microalgae and other microorganisms. They have antioxidant, immunomodulatory, antibacterial, antiviral, anticarcinogenic, and antihypocholesterolemic effects [97]. They are used as thickeners, emulsifiers, bioflocculants, stabilizers, and gelling agents in foods and cosmetics; are soluble in water; and modify the rheological properties of solutions increasing their viscosity to form gels [1, 98].

The ECM and the fibrillar pod are composed of mucilaginous polysaccharides [20], and other detected EPS are fucose, glucose, mannose, rhamnose, uronic acids, and unusual sugars such as 3-O-methyl fucose, 3-O-methyl rhamnose, and 6-O-methyl hexose [1]. Galactose is involved in the innate and adaptive immune system [99]. L-Arabinose is used as food additive for its sweet taste and poor absorption in humans [100] and is an antiglycemic agent by selective inhibition of invertases, reducing the glycemic response after sucrose ingestion [101]. Uronic acid is a chelating agent to remove metal ions. Fucose has high commercial value for its anticancer properties and for chemical synthesis of flavoring agents [1, 55].

Some *B. braunii* (UC 58) strains produce 4.0–4.5 g/L EPS with few hydrocarbons (5%). The EPS amount varies with the strain, race, physiological conditions, and culture. Strains of A and B races can produce up to 250 mg/L EPS, and race L up to 1 g/L plus glucose [1].

Greater EPS production correlates with minor growth by N deficiency. Urea and ammonia decrease the pH, as well as EPS production. Optimal conditions for EPS production were nitrate (8 mM) and between 25 and 30°C. Out of these temperatures, the EPS polymerization decreased significantly [1, 102]. Light/dark (16:8) photoperiod produced more hydrocarbons, but continuous light with agitation increased EPS until 1.6 and 0.7 g/L in LB 572 and SAG-30 strains, respectively [103]. EPS production increased (2–3 g/L) in low salinity levels (17–85 mM) as osmoprotectants [53]. High salinity and low N content in D medium induced EPS production (0.549 ± 0.044 g/L) in comparison to the BG11 medium (0.336 ± 0.009 g/L), but biomass was higher in BG11 (1.019 ± 0.051 g/L) than in D (0.953 ± 0.056 g/L) [104]. Modification of culture conditions could be used to increase EPS production, to facilitate the removal, and to increase hydrocarbon recovery. With *Botryococcus braunii* CCALA 778 (race A), a light:dark cycle at 26°C resulted in an increased production of EPS, and a milking procedure for these polysaccharides has been proposed [105, 106]. EPS can be used as thickening or gelling agents [107].

3.5 Other biopolymers

Algenanes are aliphatic, nonhydrolyzable, and insoluble biopolymers found in the ECM at 9 and 10% dry weight of race A and B, respectively. Due to their high resistance to degradation, they are attributed to the good preservation of colonies in sedimentary rocks [108].

Another reported biopolymer was the polyhydroxybutyrate (PHB), a biodegradable plastic with a yield of about 20% of the dry weight [109]. PHB is a polyester with thermoplastic and biodegradable properties, and it's a carbon and energy storage compound. For its similar physical properties to polypropylene and polystyrene, it is of commercial interest [110]. Under pH 7.5, 40°C, and with 60% wastewater as culture medium, a maximum yield of 247 ± 0.42 mg/L PHB was reported [111].

B. braunii (UTEX 572) was used to produce intra- and extracellular Ag nanoparticles (AgNPs) with antimicrobial properties, and analysis suggested that the exopolysaccharides were the possible reducing and capping agents [112].

4. Conclusions

Although *B. braunii* has been considered mainly as a good source of biofuels by the possibility to convert its hydrocarbons into currently used fuels, without the necessity of engine modifications, it produces many other high-value derivatives that can be exploited for their promising attractive profits. Besides, along the photosynthetic process, this alga converts 3% of solar energy into hydrocarbons [1] and can reduce CO_2 emissions up to 1.5×10^5 tons/year [113]. There are several reports about modifications of the culture conditions through vitamin addition, affecting the yield of several derivatives like biomass, hydrocarbon, and carbohydrate in *Botryococcus braunii* KMITL 5 [114]; however, those are from not clearly recognized strains and should be carefully taken. With *B. braunii* race A, B, or L, the main challenge is to accelerate the doubling rate because, depending on the race, it varies between 2 and 10 days. This results in easy contamination with faster growing microorganisms in open ponds used for industrial production, or a high cost of sterile conditions in closed bioreactors. In spite of these disadvantages, we consider that *B. braunii* is an excellent model of biorefinery. Other strategies to use *B. braunii* as biorefinery and bioreactor are being developed like the immobilization in polyester [115] or bioharvesting with *Aspergillus* sp. [116].

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Banerjee A, Sharma R, Chisti Y, Banerjee UC. *Botryococcus braunii*: A renewable source of hydrocarbons and other chemicals. Critical Reviews in Biotechnology. 2002;**22**:245-279. DOI: 10.1080/07388550290789513

[2] Tanabe Y, Okazaki Y, Yoshida M, Matsuura H, Kai A, Shiratori T, et al. A novel alphaproteobacterial ectosymbiont promotes the growth of the hydrocarbon-rich green alga *Botryococcus braunii*. Scientific Reports. 2015;**5**:10467. DOI: 10.1038/srep10467

[3] Moldowan JM, Seifert WK. First discovery of botryococcane in petroleum. Journal of the Chemical Society, Chemical Communications. 1980;**19**:912-914. DOI: 10.1039/C39800000912

[4] McKirdy DM, Cox RE, Volkman JK, Howell VJ. Botryococcane in a new class of Australian non-marine crude oils. Nature. 1986;**320**:57-59. DOI: 10.1038/320057a0

[5] Summons RE, Metzger P, Largeau C, Murray AP, Hope JM. Polymethylsqualanes from *Botryococcus braunii* in lacustrine sediments and crude oils. Organic Geochemistry. 2002;**33**:99-109. DOI: 10.1016/S0146-6380(01)00147-4

[6] Kawachi M, Tanoi T, Damura M, Kaya K, Watanabe MM. Relationship between hydrocarbons and molecular phylogeny of *Botryococcus braunii*. Algal Research. 2012;**1**:114-119. DOI: 10.1016/j.algal.2012.05.003

[7] Metzger P, Berkaloff C, Casadevall E, Coute A. Alkadiene and botryococcene-producing races of wild strains of *Botryococcus braunii*. Phytochemistry. 1985;**24**:2305-2312. DOI: 10.1016/S0031-9422(00)83032-0

[8] Metzger P, Largeau C. *Botryococcus braunii*: A rich source for hydrocarbons and related ether lipids. Applied Microbiology and Biotechnology. 2005;**66**:486-496. DOI: 10.1007/s00253-004-1779-z

[9] Metzger P, Casadevall E, Pouet MJ, Pouet Y. Structures of some botryococcenes: Branched hydrocarbons from the B-race of the green alga *Botryococcus braunii*. Phytochemistry. 1985;**24**:2995-3002. DOI: 10.1016/0031-9422(85)80043-1

[10] Huang Z, Poulter CD. Tetramethylsqualene, a triterpene from *Botryococcus braunii* var. Showa. Phytochemistry. 1989;**28**:1467-1470. DOI: 10.1016/S0031-9422(00)97766-5

[11] Achitouv E, Metzger P, Rager MN, Largeau C. C31-C34 methylated squalenes from a Bolivian strain of *Botryococcus braunii*. Phytochemistry. 2004;**65**:3159-3165. DOI: 10.1016/j.phytochem.2004.09.015

[12] Brown C, Knights BA, Conway E. Hydrocarbon content and its relationship to physiological state in the green alga *Botryococcus braunii*. Phytochemistry. 1969;**8**:543-547. DOI: 10.1016/S0031-9422(00)85397-2

[13] Metzger P, Allard B, Casadevall E, Berkaloff C, Couté A. Structure and chemistry of a new chemical race of *Botryococcus braunii* Chlorophyceae that produces lycopadiene, a tetraterpenoid hydrocarbon. Journal of Phycology. 1990;**26**:258-266. DOI: 10.1111/j.0022-3646.1990.00258.x

[14] Metzger P, Pouet Y, Summons R. Chemotaxonomic evidence for the similarity between *Botryococcus braunii* L race and *Botryococcus neglectus*. Phytochemistry. 1997;**44**:1071-1075. DOI: 10.1016/S0031-9422(96)00698-X

[15] Thapa HR, Naik MT, Okada S, Takada K, Molnár I, Xu Y, et al. A squalene synthase-like enzyme initiates production of tetraterpenoid hydrocarbons in *Botryococcus braunii* Race L. Nature Communications. 2016; **7**:11198. DOI: 10.1038/ncomms11198

[16] Hillen LW, Pollard G, Wake LV, White N. Hydrocracking of the oils of *Botryococcus braunii* to transport fuels. Biotechnology and Bioengineering. 1982;**24**:193-205. DOI: 10.1002/bit.260240116

[17] Cornejo-Corona I, Thapa HR, Devarenne TP, Lozoya-Gloria E. The biofuel potential of the green colonial microalga *Botryococcus braunii*. In: Torres-Bustillos LG, editor. Microalgae and Other Phototrophic Bacteria. Culture, Processing, Recovery and New Products. 1st ed. New York, USA: Nova Science Publishers, Inc.; 2015. pp. 41-58

[18] Griehl C, Kleinert C, Griehl C, Bieler S. Design of a continuous milking bioreactor for non-destructive hydrocarbon extraction from *Botryococcus braunii*. Journal of Applied Phycology. 2015;**27**:1833-1843. DOI: 10.1007/s10811-014-0472-6

[19] Metzger P, Casadevall E, Coute A. Botryococcene distribution in strains of green alga *Botryococcus braunii*. Phytochemistry. 1988;**27**:1383-1388. DOI: 10.1016/0031-9422(88)80199-7

[20] Tatli M, Ishihara M, Heiss C, Browne DR, Dangott LJ, Vitha S, et al. Polysaccharide associated protein PSAP from the green microalga *Botryococcus braunii* is a unique extracellular matrix hydroxyproline-rich glycoprotein. Algal Research. 2018;**29**:92-103. DOI: 10.1016/j.algal.2017.11.018

[21] Tanoi T, Kawachi M, Watanabe MM. Effects of carbon source on growth and morphology of *Botryococcus braunii*. Journal of Applied Phycology. 2011;**23**:25-33. DOI: 10.1007/s10811-010-9528-4

[22] Hirose M, Mukaida F, Okada S, Noguchi T. Active hydrocarbon biosynthesis and accumulation in a green alga, *Botryococcus braunii* race A. Eukaryotic Cell. 2013;**12**:1132-1141. DOI: 10.1128/EC.00088-13

[23] Suzuki R, Ito N, Uno Y, Nishii I, Kagiwada S, Okada S, et al. Transformation of lipid bodies related to hydrocarbon accumulation in a green alga, *Botryococcus braunii* Race B. PLoS One. 2013;**8**:e81626. DOI: 10.1371/journal.pone.0081626

[24] Cornejo-Corona I, Thapa HR, Browne DR, Devarenne TP, Lozoya-Gloria E. Stress responses of the oil-producing green microalga *Botryococcus braunii* Race B. Peer J. 2016;**4**:e2748. DOI: 10.7717/peerj.2748

[25] Demura M, Ioki M, Kawachi M, Nakajima N, Watanabe MM. Desiccation tolerance of *Botryococcus braunii* Trebouxiophyceae, Chlorophyta and extreme temperature tolerance of dehydrated cells. Journal of Applied Phycology. 2014;**26**:49-53. DOI: 10.1007/s10811-013-0059-7

[26] Senousy H, Beakes GW, Hack E. Phylogenetic placement of *Botryococcus braunii* Trebouxiophyceae and *Botryococcus sudeticus* isolate UTEX 2629 Chlorophyceae. Journal of Phycology. 2004;**40**:412-423. DOI: 10.1046/j.1529-8817.2004.03173.x

[27] Gouveia D, Lian J, Steinert G, Smidt H, Sipkema D, Wijffels RH, et al. Associated bacteria of *Botryococcus braunii* Chlorophyta. Peer J. 2019;**7**:e6610. DOI: 10.7717/peerj.6610

[28] Templar J, Largeau C, Casadevall E. Mechanism of non-isoprenoid hydrocarbon biosynthesis in *Botryococcus braunii*. Phytochemistry.

1984;**23**:1017-1028. DOI: 10.1016/S0031-9422(00)82602-3

[29] Templier J, Largeau C, Casadevall E. Effect of various inhibitors on biosynthesis of non-isoprenoid hydrocarbons in *Botryococcus braunii*. Phytochemistry. 1987;**26**:377-383. DOI: 10.1016/S0031-9422(00)81418-1

[30] Dennis MW, Kolattukud PE. Alkane biosynthesis by decarbonylation of aldehyde catalyzed by a microsomal preparation from *Botryococcus braunii*. Archives of Biochemistry and Biophysics. 1991;**287**:268-275. DOI: 10.1016/0003-9861(91)90478-2

[31] Unkefer CJ, Sayre RT, Magnuson JK, Anderson DB, Baxter I, Blaby IK, et al. Review of the algal biology program within the national alliance for advanced biofuels and bioproducts. Algal Research. 2017;**22**:187-215. DOI: 10.1016/j.algal.2016.06.002

[32] Sato Y, Ito Y, Okada S, Murakami M, Abe H. Biosynthesis of the triterpenoids, botryococcenes and tetramethylsqualene in the B race of *Botryococcus braunii* via the non-mevalonate pathway. Tetrahedron Letters. 2003;**44**: 7035-7037. DOI: 10.1016/S0040-4039(03)01784-2

[33] Lohr M, Schwender J, Polle JE. Isoprenoid biosynthesis in eukaryotic phototrophs: A spotlight on algae. Plant Science. 2012;**185**:9-22. DOI: 10.1016/j.plantsci.2011.07.018

[34] Molnár I, Lopez D, Wisecaver JH, Devarenne TP, Weiss TL, Pellegrini M, et al. Bio-crude transcriptomics: Gene discovery and metabolic network reconstruction for the biosynthesis of the terpenome of the hydrocarbon oil-producing green alga, *Botryococcus braunii* race B Showa. BMC Genomics. 2012;**13**:576. DOI: 10.1186/1471-2164-13-576

[35] Matsushima D, Jenke-Kodama H, Sato Y, Fukunaga Y, Sumimoto K, Kuzuyama T, et al. The single cellular green microalga *Botryococcus braunii*, race B possesses three distinct 1-deoxy-D-xylulose 5-phosphate synthases. Plant Science. 2012;**185**:309-320. DOI: 10.1016/j.plantsci.2012.01.002

[36] Grung M, Metzger P, Liaaen-Jensen S. Primary and secondary carotenoids in two races of the green alga *Botryococcus braunii*. Biochemical Systematics and Ecology. 1989;**17**:263-269. DOI: 10.1016/0305-1978(89)90001-X

[37] Okada S, Matsuda H, Murakami M, Yamaguchi K. Botryoxanthin A, a member of a new class of carotenoids from the green microalga *Botryococcus braunii* Berkeley. Tetrahedron Letters. 1996;**37**:1065-1068. DOI: 10.1016/0040-4039(95)02349-6

[38] Okada S, Tonegawa I, Matsuda H, Murakami M, Yamaguchi K. Braunixanthins 1 and 2, new carotenoids from the green microalga *Botryococcus braunii*. Tetrahedron. 1997;**53**: 11307-11316. DOI: 10.1016/S0040-4020(97)00705-9

[39] Takaichi S. Carotenoids in algae: Distributions, biosynthesis and functions. Marine Drugs. 2011;**9**: 1101-1118. DOI: 10.3390/md9061101

[40] Okada S, Devarenne TP, Chappell J. Molecular characterization of squalene synthase from the green microalga *Botryococcus braunii*, race B. Archives of Biochemistry and Biophysics. 2000; **373**:307-317. DOI: 10.1006/abbi.1999.1568

[41] Niehaus TD, Okada S, Devarenne TP, Watt DS, Sviripa V, Chappell J. Identification of unique mechanisms for triterpene biosynthesis in *Botryococcus braunii*. PNAS. 2011;**108**: 12260-12265. DOI: 10.1073/pnas.1106222108

[42] Okada S, Devarenne TP, Murakami M, Abe H, Chappell J. Characterization of botryococcene synthase enzyme activity, a squalene synthase-like activity from the green microalga *Botryococcus braunii*, Race B. Archives of Biochemistry and Biophysics. 2004;**422**:110-118. DOI: 10.1016/j.abb.2003.12.004

[43] Niehaus TD, Kinis S, Okada S, Yeo Y, Bell SA, Cui P, et al. Functional identification of triterpene methyltransferases from *Botryococcus braunii* Race B. The Journal of Biological Chemistry. 2012;**287**:8163-8173. DOI: 10.1074/jbc.M111.316059

[44] Tatli M, Naik M, Okada S, Dangott LJ, Devarenne TP. Isolation and characterization of cyclic C_{33} botryococcenes and a trimethylsqualene isomer from *Botryococcus braunii* race B. Journal of Natural Products. 2017;**80**: 953-958. DOI: 10.1021/acs.jnatprod.6b00934

[45] Uchida H, Sumimoto K, Ferriols VME, Imou K, Saga K, Furuhashi K, et al. Isolation and characterization of two squalene epoxidase genes from *Botryococcus braunii*, Race B. PLoS One. 2015;**10**: e0122649. DOI: 10.1371/journal.pone.0122649

[46] Browne DR, Jenkins J, Schmutz J, Shu S, Barry K, Grimwood J, et al. Draft nuclear genome sequence of the liquid hydrocarbon–accumulating green microalga *Botryococcus braunii* race B Showa. Genome Announcements. 2017; **5**:e00215-e00217. DOI: 10.1128/genomeA.00215-17

[47] Brooks J, Shaw G. Sporopollenin: A review of its chemistry, palaeochemistry and geochemistry. Grain. 1978;**17**: 91-97. DOI: 10.1080/00173137809428858

[48] Rozema J, Blokker P, Fuertes MM, Broekman R. UV-B absorbing compounds in present-day and fossil pollen, spores, cuticles, seed coats and wood: Evaluation of a proxy for solar UV radiation. Photochemical & Photobiological Sciences. 2009;**8**:1233-1243. DOI: 10.1039/B904515E

[49] Jin J, Dupré C, Yoneda K, Watanabe MM, Legrand J, Grizeau D. Characteristics of extracellular hydrocarbon-rich microalga *Botryococcus braunii* for biofuels production: Recent advances and opportunities. Process Biochemistry. 2016;**51**:1866-1875. DOI: 10.1016/j.procbio.2015.11.026

[50] Nagano S, Yamamoto S, Nagakubo M, Atsumi K, Watanabe MM. Physical properties of hydrocarbon oils produced by *Botryococcus braunii*: Density, kinematic viscosity, surface tension, and distillation properties. Procedia Environmental Sciences. 2012; **15**:73-79. DOI: 10.1016/j.proenv.2012.05.012

[51] Yamamoto S, Mandokoro Y, Nagano S, Nagakubo M, Atsumi K, Watanabe MM. Catalytic conversion of *Botryococcus braunii* oil to diesel fuel under mild reaction conditions. Journal of Applied Phycology. 2014;**26**:55-64. DOI: 10.1007/s10811-013-0065-9

[52] Wolf FR, Nemethy EK, Blanding JH, Bassham JA. Biosynthesis of unusual acyclic isoprenoids in the alga *Botryococcus braunii*. Phytochemistry. 1985;**24**:733-737. DOI: 10.1016/S0031-9422(00)84886-4

[53] Rao AR, Dayananda C, Sarada R, Shamala TR, Ravishankar GA. Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. Bioresource Technology. 2007;**98**: 560-564. DOI: 10.1016/j.biortech.2006.02.007

[54] Yoshimura T, Okada S, Honda M. Culture of the hydrocarbon producing

microalga *Botryococcus braunii* strain Showa: Optimal CO_2, salinity, temperature, and irradiance conditions. Bioresource Technology. 2013;**133**: 232-239. DOI: 10.1016/j.biortech.2013.01.095

[55] Gouveia JD, Ruiz J, van den Broek LA, Hesselink T, Peters S, Kleinegris DM, et al. *Botryococcus braunii* strains compared for biomass productivity, hydrocarbon and carbohydrate content. Journal of Biotechnology. 2017;**248**:77-86. DOI: 10.1016/j.jbiotec.2017.03.008

[56] Tsutsumi S, Saito Y, Matsushita Y, Aoki H. Investigation of colony disruption for hydrocarbon extraction from *Botryococcus braunii*. Fuel Processing Technology. 2018;**172**:36-48. DOI: 10.1016/j.fuproc.2017.12.004

[57] Jackson BA, Bahri PA, Moheimani NR. Response of *Botryococcus braunii* to repetitive non-destructive extraction of lipids with heptane. In: Chemeca 2018 Conference. 30 September–3 October 2018. Queenstown, NZ: Institution of Chemical Engineers; 2018. pp. 89.1-89.9. Available from: https://search.informit.com.au/documentSummary;dn=047836542036386;res=IELENG

[58] Mehta P, Jackson BA, Nwoba EG, Vadiveloo A, Bahri PA, Mathur AS, et al. Continuous non-destructive hydrocarbon extraction from *Botryococcus braunii* BOT-22. Algal Research. 2019;**41**:101537. DOI: 10.1016/j.algal.2019.101537

[59] Chaudry S, Bahr PA, Moheimani NR. Techno-economic analysis of milking of *Botryococcus braunii* for renewable hydrocarbon production. Algal Research. 2018;**31**: 194-203. DOI: 10.1016/j.algal.2018.02.011

[60] Tasić MB, Pinto LFR, Klein BC, Veljković VB, Filh RM. *Botryococcus braunii* for biodiesel production. Renewable and Sustainable Energy Reviews. 2016;**64**:260-270. DOI: 10.1016/j.rser.2016.06.009

[61] Xu L, Liu R, Wang F, Liu CZ. Development of a draft-tube airlift bioreactor for *Botryococcus braunii* with an optimized inner structure using computational fluid dynamics. Bioresource Technology. 2012;**119**: 300-305. DOI: 10.1016/j.biortech.2012.05.123

[62] Baba M, Kikuta F, Suzuki I, Watanabe MM, Shiraiwa Y. Wavelength specificity of growth, photosynthesis, and hydrocarbon production in the oil-producing green alga *Botryococcus braunii*. Bioresource Technology. 2012; **109**:266-270. DOI: 10.1016/j.biortech.2011.05.059

[63] Casadevall E, Dif D, Largeau C, Gudin C, Chaumont D, Desanti O. Studies on batch and continuous cultures of *Botryococcus braunii*: Hydrocarbon production in relation to physiological state, cell ultrastructure, and phosphate nutrition. Biotechnology and Bioengineering. 1985;**27**:286-295. DOI: 10.1002/bit.260270312

[64] Ge Y, Liu J, Tian G. Growth characteristics of *Botryococcus braunii* 765 under high CO_2 concentration in photobioreactor. Bioresource Technology. 2011;**102**:130-134. DOI: 10.1016/j.biortech.2010.06.051

[65] Liu J, Ge Y, Cheng H, Wu L, Tian G. Aerated swine lagoon wastewater: A promising alternative medium for *Botryococcus braunii* cultivation in open system. Bioresource Technology. 2013; **139**:190-194. DOI: 10.1016/j.biortech.2013.04.036

[66] Talukdar J, Kalita MC, Goswami BC. Characterization of the biofuel potential of a newly isolated strain of the microalga *Botryococcus braunii* Kützing from Assam, India. Bioresource

Technology. 2013;**149**:268-275. DOI: 10.1016/j.biortech.2013.09.057

[67] Ashokkumar V, Rengasamy R. Mass culture of *Botryococcus braunii* Kutz. under open raceway pond for biofuel production. Bioresource Technology. 2012;**104**:394-399. DOI: 10.1016/j.biortech.2011.10.093

[68] Khatri W, Hendrix R, Niehaus T, Chappell J, Curtis WR. Hydrocarbon production in high density *Botryococcus braunii* race B continuous culture. Biotechnology and Bioengineering. 2014;**111**:493-503. DOI: 10.1002/bit.25126

[69] Zhang H, Wang W, Li Y, Yang W, Shen G. Mixotrophic cultivation of *Botryococcus braunii*. Biomass and Bioenergy. 2011;**35**:1710-1715. DOI: 10.1016/j.biombioe.2011.01.002

[70] Rao AR, Ravishankar GA, Sarada R. Cultivation of green alga *Botryococcus braunii* in raceway, circular ponds under outdoor conditions and its growth, hydrocarbon production. Bioresource Technology. 2012;**123**:528-533. DOI: 10.1016/j.biortech.2012.07.009

[71] Bazaes J, Sepulveda C, Acién FG, Morales J, Gonzales L, Rivas M, et al. Outdoor pilot-scale production of *Botryococcus braunii* in panel reactors. Journal of Applied Phycology. 2012;**24**:1353-1360. DOI: 10.1007/s10811-012-9787-3

[72] Ozkan A, Kinney K, Katz L, Berberoglu H. Reduction of water and energy requirement of algae cultivation using an algae biofilm photobioreactor. Bioresource Technology. 2012;**114**:542-548. DOI: 10.1016/j.biortech.2012.03.055

[73] Cheng P, Ji B, Gao L, Zhang W, Wang J, Liu T. The growth, lipid and hydrocarbon production of *Botryococcus braunii* with attached cultivation. Bioresource Technology. 2013;**138**:95-100. DOI: 10.1016/j.biortech.2013.03.150

[74] Ashokkumar V, Agila E, Sivakumar P, Salam Z, Rengasamy R, Ani FN. Optimization and characterization of biodiesel production from microalgae *Botryococcus* grown at semi-continuous system. Energy Conversion and Management. 2014;**88**:936-946. DOI: 10.1016/j.enconman.2014.09.019

[75] Nascimento IA, Marques SSI, Cabanelas ITD, Pereira SA, Druzian J, De Souza CO, et al. Screening microalgae strains for biodiesel production: Lipid productivity and estimation of fuel quality based on fatty acids profiles as selective criteria. Bioenergy Research. 2013;**6**:1-13. DOI: 10.1007/s12155-012-9222-2

[76] Fan JY, Chiu HC, Wu JT, Chiang YR, Hsu SH. Fatty acids in *Botryococcus braunii* accelerate topical delivery of flurbiprofen into and across skin. International Journal of Pharmaceutics. 2004;**276**:163-173. DOI: 10.1016/j.ijpharm.2004.02.026

[77] Choi GG, Kim BH, Ahn CY, Oh HM. Effect of nitrogen limitation on oleic acid biosynthesis in *Botryococcus braunii*. Journal of Applied Phycology. 2011;**23**:1031-1037. DOI: 10.1007/s10811-010-9636-1

[78] Ruangsomboon S. Effect of light, nutrient, cultivation time and salinity on lipid production of newly isolated strain of the green microalga, *Botryococcus braunii* KMITL 2. Bioresource Technology. 2012;**9**:261-265. DOI: 10.1016/j.biortech.2011.07.025

[79] Ruangsomboon S. Effects of different media and nitrogen sources and levels on growth and lipid of green microalga *Botryococcus braunii* KMITL and its biodiesel properties based on fatty acid composition. Bioresource Technology. 2015;**191**:

377-384. DOI: 10.1016/j.biortech.2015.01.091

[80] Tran HL, Kwon JS, Kim ZH, Oh Y, Lee CG. Statistical optimization of culture media for growth and lipid production of *Botryococcus braunii* LB572. Biotechnology and Bioprocess Engineering. 2010;**15**:277-284. DOI: 10.1007/s12257-009-0127-7

[81] Yeesang C, Cheirsilp B. Low-cost production of green microalga *Botryococcus braunii* biomass with high lipid content through mixotrophic and photoautotrophic cultivation. Applied Biochemistry and Biotechnology. 2014; **174**:116-129. DOI: 10.1007/s12010-014-1041-9

[82] Cabanelas ITD, Marques SSI, de Souza CO, Druzian J, Nascimento IA. *Botryococcus*, what to do with it? Effect of nutrient concentration on biorefinery potential. Algal Research. 2015;**11**:43-49. DOI: 10.1016/j.algal.2015.05.009

[83] Pérez-Mora LS, Matsudo MC, Cezare-Gomes EA, Carvalho J. An investigation into producing *Botryococcus braunii* in a tubular photobioreactor. Journal of Chemical Technology and Biotechnology. 2016;**91**: 3053-3060. DOI: 10.1002/jctb.4934

[84] Wijihastuti RS, Moheimani NR, Bahri PA, Cosgrove JJ, Watanabe MM. Growth and photosynthetic activity of *Botryococcus braunii* biofilms. Journal of Applied Phycology. 2017;**29**:1123-1134. DOI: 10.1007/s10811-016-1032-z

[85] Pangestuti R, Kim SK. Biological activities and health benefit effects of natural pigments derived from marine algae. Journal of Functional Foods. 2011; **3**:255-266. DOI: 10.1016/j.jff.2011.07.001

[86] Ambati RR, Gogisetty D, Gokare RA, Ravi S, Bikkina PN, Su Y, et al. *Botryococcus* as an alternative source of carotenoids and its possible applications—An overview. Critical Reviews in Biotechnology. 2018;**38**: 541-558. DOI: 10.1080/07388551.2017.1378997

[87] Mulders KJ, Lamers PP, Martens DE, Wijffels RH. Phototrophic pigment production with microalgae: Biological constraints and opportunities. Journal of Phycology. 2014;**50**:229-242. DOI: 10.1111/jpy.12173

[88] Tonegawa I, Okada S, Murakami M, Yamaguchi K. Pigment composition of the green microalga *Botryococcus braunii* Kawaguchi-1. Fisheries Science. 1998; **64**:305-308. DOI: 10.2331/fishsci.64.305

[89] Matsuura H, Watanabe MM, Kaya K. Echinenone production of a dark red-coloured strain of *Botryococcus braunii*. Journal of Applied Phycology. 2012;**24**:973-977. DOI: 10.1007/s10811-011-9719-7

[90] Grung M, Metzger P. Algal carotenoids 53; secondary carotenoids of algae 4; secondary carotenoids in the green alga *Botryococcus braunii*, race L, new strain. Biochemical Systematics and Ecology. 1994;**22**:25-29. DOI: 10.1016/0305-1978(94)90111-2

[91] Okada S, Tonegawa I, Matsuda H, Murakami M, Yamaguchi K. Botryoxanthin B and α-botryoxanthin A from the green microalga *Botryococcus braunii* Kawaguchi-1. Phytochemistry. 1998;**47**:1111-1115. DOI: 10.1016/S0031-9422(98)80082-4

[92] Nakamura H, Matsunaga S, Kawagishi H, Okada S. Effects of 2-azahypoxanthine on extracellular terpene accumulations by the green microalga *Botryococcus braunii*, race B. Algal Research. 2016;**20**:267-275. DOI: 10.1016/j.algal.2016.10.023

[93] Ambati RR, Ravi S, Aswathanarayana RG. Enhancement of carotenoids in green alga-*Botryococcus braunii* in various autotrophic media

under stress conditions. International Journal of Biomedical and Pharmaceutical Sciences. 2010;**4**:87-92. Available from: https://www.researchgate.net/profile/Dr_Ranga_Rao_Ambati/publication/235352513

[94] Rao AR, Baskaran V, Sarada R, Ravishanka GA. *In vivo* bioavailability and antioxidant activity of carotenoids from microalgal biomass—A repeated dose study. Food Research International. 2013;**54**:711-717. DOI: 10.1016/j.foodres.2013.07.067

[95] Rao AR, Sarada R, Baskaran V, Ravishankar GA. Antioxidant activity of *Botryococcus braunii* extract elucidated *in vitro* models. Journal of Agricultural and Food Chemistry. 2006;**54**: 4593-4599. DOI: 10.1021/jf060799j

[96] Buono S, Langellotti AL, Martello A, Bimonte M, Tito A, Carola A, et al. Biological activities of dermatological interest by the water extract of the microalga *Botryococcus braunii*. Archives of Dermatological Research. 2012;**304**: 755-764. DOI: 10.1007/s00403-012-1250-4

[97] Liu L, Pohnert G, Wei D. Extracellular metabolites from industrial microalgae and their biotechnological potential. Marine Drugs. 2016;**14**:191. DOI: 10.3390/md14100191

[98] Öner ET. Microbial production of extracellular polysaccharides from biomass. In: Fang Z, editor. Pretreatment Techniques for Biofuels and Biorefineries. Berlin Heidelberg: Springer; 2013. pp. 35-56. DOI: 10.1007/978-3-642-32735-3_2

[99] Brockhausen I. The role of galactosyltransferases in cell surface functions and in the immune system. Drug News & Perspectives. 2006;**19**: 401-409. DOI: 10.1358/dnp.2006.19.7.1021491

[100] Matuso N, Kaneko S, Atsushi K, Kobayashi H, Kusakabes I. Purification and characterization and gene cloning of two α-L-arabinofuranosides from *Streptomyces chartreusis* GS 901. Biochemical Journal. 2000;**346**:9-15. DOI: 10.1042/bj3460009

[101] Shin HY, Park SY, Sung JH, Kim DH. Purification and characterization of α-L-arabinopyranosidase and α-L-arabinofuranosidase from *Bifidobacterium breve* K-110, a human intestinal anaerobic bacterium metabolizing ginsenoside Rb2 and Rc. Applied and Environmental Microbiology. 2003;**69**:7116-7123. DOI: 10.1128/AEM.69.12.7116-7123.2003

[102] Cepák V, Přibyl P. Light intensity and nitrogen effectively control exopolysaccharide production by the green microalga *Botryococcus braunii* Trebouxiophyceae. Genetics and Plant Physiology. 2018;**8**:24-37. Available from: http://www.bio21.bas.bg/ippg/bg/wp-content/uploads/2018/08/GPP_8_1-2_2018_24-37.pdf

[103] Dayananda C, Sarada R, Rani MU, Shamala TR, Ravishankar GA. Autotrophic cultivation of *Botryococcus braunii* for the production of hydrocarbons and exopolysaccharides in various media. Biomass and Bioenergy. 2007;**31**:87-93. DOI: 10.1016/j.biombioe.2006.05.001

[104] Bayona KCD, Garcés LA. Effect of different media on exopolysaccharide and biomass production by the green microalga *Botryococcus braunii*. Journal of Applied Phycology. 2014;**26**: 2087-2095. DOI: 10.1007/s10811-014-0242-5

[105] García-Cubero R, Cabanelas ITD, Sijtsma L, Kleinegris DM, Barbosa MJ. Production of exopolysaccharide by *Botryococcus braunii* CCALA 778 under laboratory simulated Mediterranean climate conditions. Algal Research.

2018;**29**:330-336. DOI: 10.1016/j.algal.2017.12.003

[106] García-Cubero R, Wang W, Martín J, Bermejo E, Sijtsma L, Togtema A, et al. Milking exopolysaccharides from *Botryococcus braunii* CCALA778 by membrane filtration. Algal Research. 2018;**34**: 175-181. DOI: 10.1016/j.algal.2018.07.018

[107] Kumar D, Kastanek P, Adhikary SP. Exopolysaccharides from cyanobacteria and microalgae and their commercial application. Current Science. 2018;**115**:234. Available from: https://www.currentscience.ac.in/Volumes/115/02/0234.pdf

[108] Volkman JK. Acyclic isoprenoid biomarkers and evolution of biosynthetic pathways in green microalgae of the genus *Botryococcus*. Organic Geochemistry. 2014;**75**:36-47. DOI: 10.1016/j.orggeochem.2014.06.005

[109] Kavitha G, Kurinjimalar C, Sivakumar K, Kaarthik M, Aravind R, Palani P, et al. Optimization of polyhydroxybutyrate production utilizing waste water as nutrient source by *Botryococcus braunii* Kütz using response surface methodology. International Journal of Biological Macromolecules. 2016;**93**:534-542. DOI: 10.1016/j.ijbiomac.2016.09.019

[110] Markou G, Nerantzis E. Microalgae for high-value compounds and biofuels production: A review with focus on cultivation under stress conditions. Biotechnology Advances. 2013;**31**: 1532-1542. DOI: 10.1016/j.biotechadv.2013.07.011

[111] Kavitha G, Kurinjimalar C, Sivakumar K, Palani P, Rengasamy R. Biosynthesis, purification and characterization of polyhydroxybutyrate from *Botryococcus braunii* K. International Journal of Biological Macromolecules. 2016;**89**:700-706. DOI: 10.1016/j.ijbiomac.2016.04.086

[112] Arévalo-Gallegos A, Garcia-Perez JS, Carrillo-Nieves D, Ramirez-Mendoza RA, Iqbal HM, Parra-Saldívar R. *Botryococcus braunii* as a bioreactor for the production of nanoparticles with antimicrobial potentialities. International Journal of Nanomedicine. 2018;**13**:5591. DOI: 10.2147/IJN.S174205

[113] Sawayama S, Minowa T, Yokoyama SY. Possibility of renewable energy production and CO_2 mitigation by thermochemical liquefaction of microalgae. Biomass and Bioenergy. 1999;**17**:33-39. DOI: 10.1016/S0961-9534(99)00019-7

[114] Ruangsomboon S, Sornchai P, Prachom N. Enhanced hydrocarbon production and improved biodiesel qualities of *Botryococcus braunii* KMITL 5 by vitamins thiamine, biotin and cobalamin supplementation. Algal Research. 2018;**29**:159-169. DOI: 10.1016/j.algal.2017.11.028

[115] Calderón NDG, Bayona KCD, Garcés LA. Immobilization of the green microalga *Botryococcus braunii* in polyester wadding: Effect on biomass, fatty acids, and exopolysaccharide production. Biocatalysis and Agricultural Biotechnology. 2018;**14**: 80-87. DOI: 10.1016/j.bcab.2018.02.006

[116] Al-Hothaly KA. An optimized method for the bio-harvesting of microalgae, *Botryococcus braunii*, using *Aspergillus* sp. in large-scale studies. MethodsX. 2018;**5**:788-794. DOI: 10.1016/j.mex.2018.07.010