Recent Advances in Microalgal Biotechnology

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Industrial Production of Microalgal Cell-Mass and Bioactive Constituents from Green Microalga-*Botryococcus braunii*

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Abstract

The world is facing lack of alternative fuels. The demand for alternate energy is increasing every day and concurrently the depletion of fossil fuels has been so rapid that it could lead to energy crisis in the future. The energy production from photosynthetic micro-organisms such as algae is the solution to this issue, which is providing an eco-friendly alternative to meet energy requirements. Microalgae are potential source of nutrients and health promoting substances, as well as high valuable metabolites that are unique and of high commercial use. In this context, we selected microalgae *Botryococcus braunii* for the biofuel production, which contain 70% hydrocarbons in the biomass on the dry weight basis, and also it accumulates other bioactive compounds such as ether lipids, fatty acids, exo-polysaccharides and carotenoids, which are having high industrial applications. Our recent published results and also current literature on the effect of various culture conditions on biomass, hydrocarbon, lipids and fatty acid production in *B. braunii*, cultivation of *B. braunii* in raceway ponds and photobioreactors, downstream processing of hydrocarbons, bioactive molecules and their use in various applications, biological activities of *B. braunii* extracts with special reference to carotenoids were added to this book chapter. This chapter covers up to date information on the culture conditions, cultivation methods, biomass production, hydrocarbons, chemicals, bioactive constitutes and their biological properties, downstream processing of hydrocarbons from *B. braunii*.

Keywords: Bioactive compounds; Biomass; *Botryococcus braunii*; Hydrocarbons; Lipids; Photobioreactors; Raceway ponds

Introduction

Botryococcus braunii is a unicellular photosynthetic microalgae, member of the *chlorophyceae* (chlorophyta). It is producing large amounts of biomass, lipids, hydrocarbons and other bioactive molecules which can be used in renewable fuel. *B. braunii* is widespread in freshwaters, brackish lakes, reservoirs and ponds [1-3]. It is identified in several countries such as USA, Portugal, France, India, Japan, Philippines, Malaysia, and Thailand etc based on their geographical regions. The r-RNA (16s RNA) sequence of *B. braunii* is compared with other algae species *Characium vacultaum* and *Dunaliella parva* and found to be very close [4]. *B. braunii* is known to accumulate more hydrocarbons under various culture conditions [5-8]. It is divided into three different races-A, B and L based on the characteristics of hydrocarbon they produce. The race-A produces n-alkadienes and trienes (C_{25}-C_{31}), race-B produces botryococcenes (C_{30}-C_{37}) and race L produces tetraterpene (C_{40}) [3,9,10]. *B. braunii* also synthesizes lipids-fatty acids, triacylglycerol, and sterols [11]. Apart from hydrocarbons, *B. braunii* produces other bioactive molecules such as exo-polysacchrides and carotenoids [12,13]. Biomass, lipid and hydrocarbon content varied based on the culture conditions [5-8,12]. Hydrocarbons produced distillate fractionates-gasoline (67%), aviation (15%), diesel fraction (15%) and remaining residual oil by hydrocracking [9]. These fuels are reported to be free from nitrogen and sulfur oxides after combustion. Owing to its lipid and hydrocarbon production, these microalgae recognized for the renewable fuel [14]. Hydrocarbons and lipids are improved by supplying carbon-dioxide [8]. Further, research studies will be required on to improve the optimal culture conditions for the hydrocarbon and lipid production in *B. braunii* spp. The present book chapter is covered on the biomass, hydrocarbon and lipid production from *B. braunii* under various culture conditions, cultivation of *B. braunii* in raceway and photobioreactors, hydrocarbon extraction, bioactive constituents and their use in industrial applications, and also special attention to be paid to biological properties of *B. braunii*.

Morphology and Taxonomy

B. braunii cells were observed a pyramid shaped colloidal cell, green color, and together by a lipid biofilm matrix under microscopic, whereas some of the strains have dark green color with irregular colonies consisting of hundreds of elliptical cells interconnected by strand of tough mucilage. Sometimes *B. braunii* cells are attached to each other by a refringent material that sometimes links two or more distinct clumps of cells, but Metzger and Largeau [3] reported that the morphology of the alga varied in relation to age and culture conditions. *B. braunii* species are very difficult to identify since they have heterogeneity morphology. Many species of *B. braunii* were identified by Inter Simple Sequence Repeats (ISSR) or using standard species or nature of the hydrocarbon synthesis. *B. braunii* genomes are compared by using ISSR fingerprinting tools. ISSR is a potent tool to compare genomes for their identity. ISSR markers were used in the identification of taxonomical studies. Phylogenetic analyses of several strains of *B. braunii* were studied by 18s rRNA sequence [15,16]. 'A' and 'B' race cell sizes are bigger than 'L' race. 'A' race algal cells turns to pale yellow from green stage whereas 'B' and 'L' race algae cells turns to red orange and red brown color. The accumulation of carotenoids in the stationary phase of algae culture was observed. The biopolymers aliphatic and tetra-terpenoid compounds are accumulated in the cell wall of races [17]. These biopolymers are linked with ether bridges and fatty acid esters. Alkenyl phenol is detected in 'A' race. These compounds are increased the solubility of phenolic moiety, prevent biological and chemical degradation in lipid region. Hydrocarbons are stored in outer cell wall of *B. braunii*. Some of the physiological and morphological characteristics of the race 'A', 'B' and 'L' are presented in (Table 1).

Race	Accumulated hydrocarbon	Colony color In stationary	Cell size (length x width)	Biopolymers	References
A	C_{25}-C_{31}	Pale yellow	13 x 7-9 µm	Aliphatic compounds	[9]
A	--	Green	11.0 x 6.4 µm	--	[69]
A	C_{13}-C_{26}	Green to Yellowish green to orange	3-13 µm	--	[70]
B	C_{30}-C_{37}	Red orange	13 x 7-9 µm	Aliphatic compounds	[9]
L	C_{40} H_{78}	Red-brown	8-9 x 5 µm	Tetra-terpenoid	[9]

Table 1: Some of the physiological and morphological characteristics of the 'A', 'B' and 'L' races of *B. braunii*.

Biosynthetic Pathway of *Botryococcenes*

In 'B' race produces botryococccenes (C_{30}) which contain higher levels of triterpenes. Isopenteyl disphosphate and dimethylallyl diphosphate are the fundamental precursors in the isoprepnoid biosynthesis pathway (Figure 1). Isopenteyl disphosphate produces in living organisms by the condensation of acetyl CoA through the mevalonate pathway. Casadevall et al. [18] reported that the mevalonate levels (2-^{14}C) were low in botryococcenes while using labeled experiments. Another experiment proved that botryococcenes and methylated squalenes levels were established by the non-mevalonate pathway after feeding the algae with glucose [19]. In this pathway pyruvate and glyceraldehyde-3-phosphate resulting from glycoclysis are condensed into 1-deoxy-D-xylulose-5-phosphate, in turn transposed into 2-C-methyl-D-erythritol-4-phosphate, the intermediate in IPP biosynthesis via the non-mevalonate pathway [20]. Squalene and botryococcene are triterpenes resulting from farnesyl moieties was reported by Poulter [21]. Actually farnesyl is accumulated into squalene and botryococcenes during a feeding experiment and it could be phosphorylated to its mono and diphophate esters with a cell free extract of *B. braunii* race B [22,23]. The role of farnesyl diphosphate as precursor of botryococcenes did not confirm by incubation of farnesyl diphosphate with cell free extract of *B. braunii*. The radio labeled precursor was incorporated into squalene but not into botryococcenes. This suggested that another derivative of farnesol was intermediate in botryococcenes biosynthesis reported by Inoue et al. [24]. Okada et al. [25] reported that farnesyldiphosphate is a precursor of botryococcenes. Botryococcenes synthase was inhibited by triton X-100 while it stimulates the squalene synthase [22]. Presqualene diphosphate as a common precursor in both squalene and botryococcenes synthesis was proved [26,27]. Either re-arranged or direct cleavage of cylopropane leads to squalene and botryococcenes. Still it is unknown that the single or two enzymes are responsible for squalene and botyrococcene synthesis. Jarstfer et al. [28] reported that recombinant yeast synthesize hydroxyl botryococcenes from presqualene diphosphate and squalene derivatives. The chemical structure of botryococcenes varied in relation to the strain origin, the methylation occurs at various positions depends on genetic factors. Alkylation of squalene in 'B' race, C_{31}-C_{34} higher homologues synthesis by methyl groups of methionine. The cyclo-botryococcenes synthesis is unknown; it may be from methylated botryococcenes or by methylation [29,30].

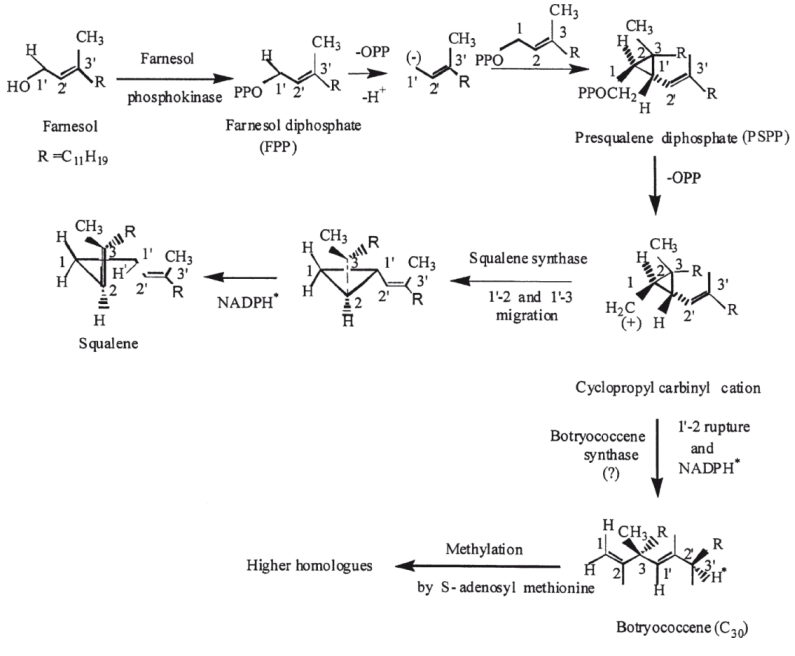

Figure 1: Biosynthetic pathway of *Botryococcenes* (C_{30}) (adapted from Banerjee et al. [9]).

Hydrocarbons in Races of *B. braunii*

B. braunii is divided into three different races-'A', 'B' and 'L' based on the characteristics of hydrocarbon they produces (Table 2 and Figure 2). The race-A produces n-alkadienes and trienes (C_{25}-C_{31}), race-B produces botryococcenes (C_{30}-C_{37}) and race L produces tetraterpene (C_{40}) [3,9,10] Race 'A' and 'B' were identified in continental, temperate and tropical lakes, whereas 'L' race was found in tropics. Hydrocarbon content varied strain to strain depending on the climatic zones. 'A' race exhibited around 20-60% of hydrocarbons on the dry weight basis which collected from the Bolivain strain from Lake Overjuyo and indigenous strains [8,11]. The hydrocarbon content in 'B' race was found to 40% on dry weight basis [31,32], whereas in race 'L' showed hydrocarbon content 8% in Thailand strains [33]. Relative percentage of hydrocarbon content and lipids in 'A', 'B' and 'L' races of *B. braunii* is presented in (Table 3).

Race A	Race B	Race L
$C_{25}H_{48}$	$C_{30}H_{50}$	$C_{40}H_{78}$
$C_{27}H_{48}$	$C_{31}H_{52}$	--
$C_{27}H_{51}$	$C_{32}H_{54}$	--
$C_{27}H_{52}$	$C_{33}H_{56}$	--
$C_{29}H_{54}$	$C_{34}H_{58}$	--
$C_{29}H_{56}$	$C_{35}H_{60}$	--
$C_{31}H_{58}$	$C_{36}H_{62}$	--
$C_{31}H_{60}$	$C_{37}H_{64}$	--

Table 2: Hydrocarbons produced by 'A', 'B' and 'L' races in *B. braunii*.

Strain name	Race	Hydrocarbon (%)	Lipid (%)	References
MCRC-Bb	A	20	26	[8]
N-836	A	22	28	[8]
CFTRI-Bb-1	A	34	25	[8]
SAG-30.81	A	46	--	[12]
LB-572	A	33	--	[12]
N-836	B	30	--	[13]
Yamanaka	A	16	--	[32]
Berkeley	B	37	--	[32]
Yayoi	B	33	--	[32]
Dawin	B	35	--	[32]
TRG	--	--	26	[32]
KMITL-2	--	--	55	[35]
Showa	B	39	--	[42]
765	--	24	13	[47]
IPPAS H-252	--	--	20	[58]
--	B	--	50	[90]
Kutz No LB 807/1Droop 1950 H-252	--	13	17	[152]
--	A	--	18	[153]
Jillamatong	A	20	--	[154]
Overjuyo-3	B	25	--	[154]
Paquemar	B	25	--	[154]
GUBITJTBB1	--	52	57	[155]
--	B	22	--	[156]
FACHB-357	B	19.4	42	[157]

Table 3: Hydrocarbon and lipid content in 'A', 'B' and 'L' races of *B. braunii* strains.

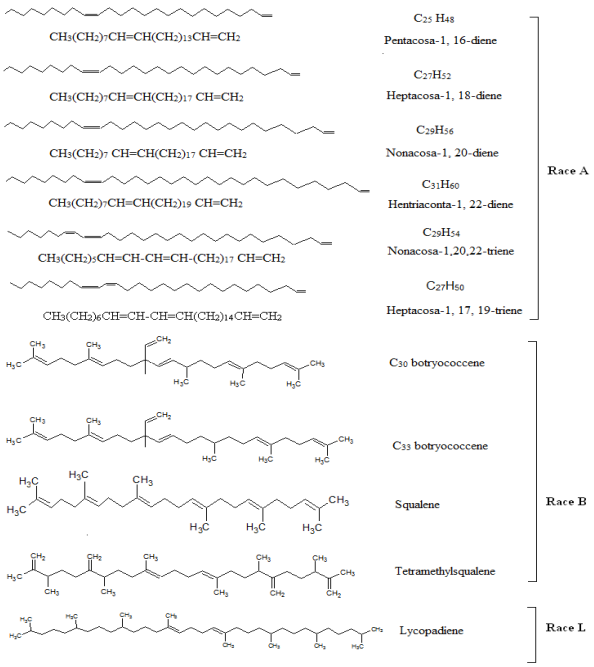

Figure 2: Types of hydrocarbons produced by 'A', 'B' and 'L' races of *B. braunii* (adapted from Metzger and Largeau, [3], Banerjee et al. [9]).

Effect of Culture Conditions on *B. braunii*

The culture conditions such as pH, temperature, irradiance, nitrogen, phosphorus, carbon dioxide and salinity effects on the growth, biomass, hydrocarbon and lipid production of *B. braunii* were reported by various researchers [6-8,12,13,34-37].

Effect of pH, temperature, irradiance

The pH was important parameter which had great influence on the growth, biochemical composition and also the form of enzymes [38]. Culture pH is adjusted to 7.4 before inoculation, while increased in pH was detected during the growth of algae [7,8,14]. Usually, increase in pH was observed in the cultivation due to the consumption of dissolved carbon-dioxide for photosynthesis [7,8,14]. A change in pH was observed in carbon-dioxide and salinity cultures during the growth of algae [7, 8]. Effect of various pH (6.0-8.5) on the growth and hydrocarbon production in B. brauni strains (SAG-30.81 and LB 572) were reported by Dayananda et al. [34]. The results showed that *B. braunii* strains can be able to grow in the all tested pH, increased in biomass yield was observed at pH-6.0. The culture pH was increased; might be due to the utilization of biocarbonate by algae [7]. However culture pH did not effect on biomass and hydrocarbon production. Recently, Ren et al. [39] reported that the algal growth and lipid production were affected by the culture pH (6.0-11.0) in algae.

Some of the algae species can grew under higher temperature and irradiance [40-42]. Similarly, *B. braunii* grew the temperature at 30 ± 2°C under 1.2 ± 0.2 klux irradiance and 16:8 h light dark cycles [7,8] and it varies depends on the algal species. Temperature may effect on the fatty acid composition in the algae. Irradiance plays a major role in photosynthesis i.e. they convert CO_2 into organic compounds such as sugars, using the energy from light. *B. braunii* can be able to grow under high and low light intensities. High irradiance alters fatty acid synthesis to produce more saturated and mono-unsaturated fatty acids that mainly make up neutral lipids. Fatty acid composition changes especially in saturated one while increasing temperature in the culture [43]. Recently, Yoshimura et al. [42] reported that effect of various combinations of temperature (5-45°C) and irradiance (0-2000 µmol m^{-2} s^{-1}) on growth and hydrocarbon production in *B. braunii* strain (Showa), the maximum specific growth of 0.50/day was observed at 30°C with irradiance 850 µmol/m^2/s and hydrocarbon content was increased with specific growth rate.

Irradiance is the major factor which influence on growth and other high value products of algae. Low irradiance causes a reduction in dry weight while high irradiance causes biochemical damage to the photosynthetic machinery. Effect of light intensity on biomass and lipid content in *Botryococcus braunii* (KMITL-2) was investigated by Ruangsomboon [35]. The effect of light and dark cycles on growth and lipid contents in *Botryococcus braunii* (KMITL-2) were studied by establishing light and dark cycles (L:D, h:h) of 12:12, 14:10, 16:8, 24:0 under illumination of 87.5, 200, and 538 µE/m^2/s, at 25°C with constant bubbling of air. Results showed that the biomass yield was observed same at 24:0, 16:8 and 14:1 light and dark cycles, whereas the highest lipid content was obtained under 16:8 light and dark conditions. The biomass yield was found to be higher in 87.5 µE/m^2/s compared to 200 and 538µE/m^2/s. Major carotenoid lutein in *Botryococcus braunii* (LB-572) was enhanced at various light intensities reported by Rao et al. [44]. Similar results were observed when *Botryococcus braunii* exposed to various irradiance at light and dark cycles [36,45]. Effect of various conditions on the growth and hydrocarbon production of *B. braunii* strains are presented in (Table 4).

Strain name	Race	Temp(oC)	Irradiance (µmol/m^2/s)	Light (h)	CO_2 (%)	Growth rate	Doubling time	Hydrocarbon (%)	References
LB-572	A	26	20	16	2	0.07	10.6	28	[7]
Gottingen (807/1)	A	25	131	14	1	0.3	2.3	44	[14]
Showa	B	25	240	12	2	--	--	38	[32]
KMITL-2	--	25	200	24	0	0.1	7	55	[35]
Showa	B	30	850	14	1	0.5	1.4	--	[42]
Showa	B	25,30	839	14	10	0.44	--	39	[42]
Showa	B	23-25	250	24	0	0.12	--	39	[46]
Showa	B	23-25	250	24	0.3	0.42	--	29	[46]
765	--	25	150	24	20	0.13	5.5	24	[47]
IPE-001	B	25	35	16	1	0.15	4.5	64	[80]
Yayoi	B	25	240	12	2	0.2	3.5	40	[122]
UC-58	--	25	250	24	1	0.42	1.7	--	[133]
CHN-357	--	25	303	12	0	0.2	3.5	10	[158]
NIES-836	--	25	303	12	0	0.09	7.7	35	[158]
UK-807-2	--	25	303	12	0	0.18	3.8	65	[158]

Table 4: Effect of culture conditions on the growth and hydrocarbon production of *B. braunii* strains (Adapted from Yoshimura et al. [42]).

Effect of Carbon dioxide (CO_2)

Carbon dioxide (CO_2) is necessary for microalgae to maintain the culture pH in medium. Recently, many studies were conducted on the carbon-dioxide effects on biomass, lipid, fatty acid composition and hydrocarbon production in *B. braunii* strains [8,42]. CO_2 favors the accumulation of lower chain botryococcenes (C_{30}-C_{32}) in *B. braunii* while sparged with ambient air contain higher botryococcenes (C_{33}-C_{34}) [46]. Methylation steps leading from C_{30} to C_{31} and C_{32} are faster in CO_2 enriched cultures than steps leading to C_{33}, C_{34} and higher homologues. In autotrophic media, growth and hydrocarbon content in *B. braunii* were improved by utilizing exogenous carbon source. We also reported that, the carbon dioxide effects on biomass, hydrocarbon and fatty acid profile in various indigenous species of *B. braunii* (LB-572, SAG 30.81, MCRC-Bb, N-836, CFTRI-Bb-1, and CFTRI-Bb-2) at 0.5, 1.0, and 2.0% (v/v) levels [8]. In the all tested levels, *B. braunii* was grown without any change in culture pH. Palmitic acid and oleic acid levels were increased in the strain *B. braunii* (LB-572) with CO_2 treatment at 1% and 2%. Hydrocarbon content was found to be 20% in the *B. braunii* LB-572, CFTRI-Bb-2, CFTRI-Bb-1, and N-836 strains, whereas it was less than 20% in the SAG 30.81. Another study evaluated the effect of carbon dioxide (2-20%) in *B. braunii*-765 [47]. The results showed that the strain can able to grow in all the CO_2 tested levels with aeration rate at 0.2 vvm, without any changes in culture pH. The maximum biomass yield was found to be 2.31 (g/L) at 20% CO_2 concentration on 25th day and also enhanced in hydrocarbon content and algal cell size with the increase of CO_2 levels.

Effect of Nitrogen

Nitrogen plays a major role in microalgae for the growth and lipid accumulation. It is supplied to *B. braunii* in the form of nitrate

(NO_3-), nitrite (NO_2-) and ammonia (NH_4+), which regulates nitrogen metabolism in algae. Nitrogen deficiency lead to lipid accumulation in *B. braunii* was reported [48,49]. Zhila et al. [49] reported that *B. braunii* accumulates Triacylglycerols (TAGs) with higher amount of oleic acid under nitrogen limitation conditions, whereas changes in both fatty acid composition and polar lipids under nitrogen deficiency culture. Many algal species accumulates TAGs under nitrogen starvation, which contain more monounsaturated fatty acids [39]. Potassium nitrate, calcium nitrate, sodium nitrate and ammonium nitrate effects on hydrocarbon and fatty production in *B. braunii* (LB-572 and SAG 30.81) were evaluated by Dayananda et al. [34]. In the results, hydrocarbon content was enhanced in both *B. braunii* (LB-572 and SAG-30.81) strains by potassium nitrate treated culture. Oleic acid is the major fatty acid was found in all the nitrate treated groups. Ammonia (NH_3) used as nitrogen source which causes the culture pH decline to 4, where the cells were damage due to nitrate reductase enzyme inactivation. Hydrocarbon content decreased when *B. braunii* exposed to NH_3, this indicates a diversion of acetyl Co-A from hydrocarbon synthesis pathway to amino acid synthesis pathway [50]. The effects of nitrogen (0-3.5 mg/L) on growth of *B. braunii* (764 and 765) at two different light intensities (60 and 110μmol/m^2) were evaluated by Sun et al. [51]. In the results observed that growth of *B. braunii*-764 with all nitrogen treatments were significantly higher at 110 μmol/m^2 compared to other treatments. Lack of nitrogen limits protein synthesis and thus increased lipid [52] or sometimes carbohydrates accumulation [53]. Biomass and lipid content in *Botryococcus* (KMITL-2) was enhanced by initial nitrogen concentration at 86 mg/L [35].

Effect of Phosphorus

Phosphorus is supplied to algae growth in the form of KH_2PO_4 and K_2HPO_4. Microalgae can absorb phosphate in the requirement of the cells. The phosphate can store in intracellular granules. The cells utilize phosphate when the extracellular phosphate supplies run out. Growth was enhanced in algae with the phosphate deficiency culture conditions [14]. Metabolites production in algae was increased by supplying phosphate. Phosphate released in the medium as the cell lyses in the declined stage of culture, while increased in the initial concentration of phosphate in the medium. Growth and hydrocarbon content in *B. braunii* was increased in the excess amount of phosphate, this may be changes in the nitrogen: phosphate (N: P) ratio in the culture medium, which influence on lipid accumulation in algae [14]. Effect of various phosphorus levels (0.15-0.77 mg/L) on growth of *B. braunii* (764 and 765) at 60 and 110 μmol/m^2 light intensities were studied by Sun et al. [51]. In the results, growth rate of *B. braunii* (764 and 765) at 60 μmol/m^2 showed no significant difference. The average growth rates of *B. braunii* (764) at 110 μmol/m^2 were significantly lower than other phosphorus treatments. Initial phosphorus concentration on growth and lipid content in *B. braunii* (KMITL-2) was observed, the results showed the maximum biomass was found to be 1.91 g/L at 444 mg/L phosphorous concentration, whereas the highest lipid content was found to be 54% at a phosphorus concentration of 222 mg/L [35].

Effect of Salinity

Microalgae are incredibly tolerant to changes in salinity. It is effected on microalgae in different ways osmosis stress, ion stress and changes of the cellular ion ratios due to the selective ion permeability of the membrane while changes in salinity. The better algae growing conditions for most species is at a salinity level that is slightly lower than that of their native habitat, which is obtained by diluting sea water with tap water [54]. Effect of various salinity concentrations on growth, biomass, hydrocarbons, lipids, fatty acids and secondary metabolites in race 'A' of *B. braunii* was reported by Ranga Rao et al. [7]. In the results, the biomass yield was enhanced and bioactive constituent changes were observed at all the tested levels of salinity. Fatty acids-palmitic acid (1.7 fold) and oleic acid (2 fold) were increased in both 34 mM and 85 mM salinity treated culture, whereas 2 fold increased in carotenoid content at 85mM salinity concentration. Maximum hydrocarbon content was observed in 51 mM and 68 mM of salinity. The lipid content of *B. braunii* in salt concentration was higher than control group (without salt) [55]. Similarly Vazquez-Duhalt and Arredondo-Vega [56] reported that the lipid content in cells of B. brauni (Austin & Gottingen) was enhanced in the presence and absence of NaCl. Earlier reported that, increase in salinity may results in a slightly increase in total lipid content of algae [47,53]. Secondary metabolites-carotenoids in *B. braunii* were enhanced by providing salt stress [6,7]. The effect of salinity on growth, biomass, lipid, fatty acid and hydrocarbon production in various *B. braunii* species were reported [36,57,58] Growth and lipid production of four *Botryococcus braunii* (TRG, KB, SK, and PSU) strains were tested, all the strains were survived higher salinity concentration, whereas growth of SK, TRG and KB strains was decreased. The lipid content in SK, TRG and PSU strains were decreased when the salinity was increased [36]. Seawater containing medium was used for the hydrocarbon production in *B. braunii* (Showa) where the 90% hydrocarbon obtained without any pretreatment [57]. In another study conducted on the effect of 0.3 and 0.7 M NaCl on biomass yield, lipid content, and fatty acid profile of *Botryococcus braunii* (IPPAS H-252) in different phases of the culture, the culture growth was inhibited for first three days, due to considerable changes in the lipid and fatty acid profile. In the later phases of the culture, algae biomass increased, and the degree of unsaturation was increased, mainly due to rise in the content of polyenoic acid [58].

Cultivation of *B. braunii*

Raceway ponds

Micro algal cultivation is promising method for the production of high value metabolites- biomass, lipids and hydrocarbons which are very used for the biofuel feed stock [59]. Algae are grown in raceway ponds and photo-bioreactors for the scale up studies (Figure 3). A raceway pond contains oval shape, closed loop channels, maintained depth in between 20 cm to 40 cm, and mixing is provided by paddle wheels. Raceway ponds are constructed by cement and plastics [60]. Continuous mixing gives equal light to the culture and also prevents sedimentation in pond. The raceway pond cultivation system is very cheap compared to closed system. This system needed investment in terms of light and other operations [61-63].

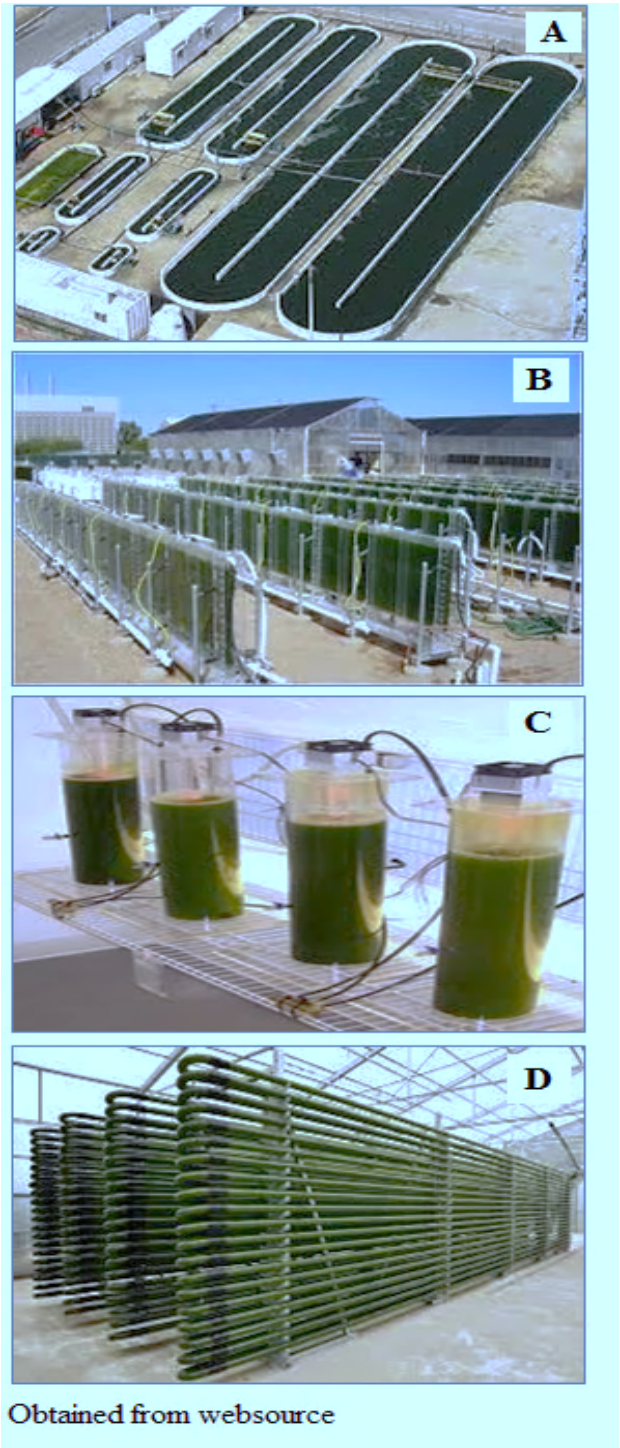

Figure 3: Common microalgae cultivation in raceway ponds (A), flat panel photobioreactors (B), bubble column photobioreactors (C) and tubular photobioreactors (D).

Contamination is the major problem in open and closed system due to the environmental fluctuations [64,65], and is required to monitor to keep culture in good condition. Contamination occurs in the ponds by other algae, bacteria or predator's-ciliates, rotifers, amoeba and cysts, and can be partially reduced or controlled by initial cell concentration, culture pH, chemical and physical treatments [65,66]. Agitation is essential as accumulation of biomass in one placed. Generally, cultures run in batch or continuous or semi-continuous. The batch cultivation was the best to maintain fresh algal inoculum as starting material for every batch. To obtain higher biomass yield and productivity depends the initial cell, biomass and nutrient concentrations, which plays a key role in maintaining healthy culture [67,68]. Our aim is to produce more biomass with minimum energy inputs, it is recommended to use marine forms to control or minimize contamination from other algal or zooplankton species. Recently a few research studies were conducted on the cultivation of *B. braunii* in raceway ponds [13,69,70]. *B. braunii* species-LB-572 and N-836 were grew in raceway and circular ponds reported by Ranga Rao et al., [13] in the results, the hydrocarbons of C_{20}–C_{30} carbon chain length were higher in raceway and circular ponds on 18th day cultivation was observed, whereas the major fatty acids were found to be palmitic and oleic acids in the both raceway and circular ponds. Ashok kumar and Regasamy [69] reported that *B. braunii* strains-Kutz, AP-103, AP-104 and AP-105 were isolated from the Indian freshwater bodies and cultivated them in raceway ponds for the biomass and lipid production, in the results observed that the maximum biomass, lipid, hydrocarbon content was found to be 1.8 g/L, 19% and 11% in AP-103 strain. In addition 33% carbohydrate and 18% protein contents were observed under raceway ponds cultivation. The major fatty acids- heptadecane, hexadecane, oleic, linolenic and palmitic acids were identified. Similarly, the maximum biomass yield in *B. braunii* (Mahabali) was found to be 2 g/L (w/w) on 14th day cultivation in raceway ponds reported by Dayananda et al. [70].

Photobioreactors

Photobioreactors (PBRs) are designed to grow microorganisms in closed system [71,72]. Recently they have received a great use in microalgae cultivation for the biomass production. PBRs can be optimized according to the biological and physiological characteristics of the algal species being cultivated. A uni-algal culture can be maintained in photobioreactors whereas in raceway ponds it is not possible. Environment sensitive algal species can easily grow in photobioreactors which gives more productivity. Based on their design, PBRs are considered to have several advantages compared to raceway ponds. In PBRs can be control various parameters such as pH, temperature, mixing, light, CO_2, O_2, prevent evaporation, reduce CO_2 losses, allow to higher cell densities and volumetric productivities. PBRs offer safe, protect environment and prevent contamination by microorganisms. PBRs have several disadvantages such as bio-fouling, overheating, oxygen accumulation, difficult in large scale, to install high operating cost, cell damage by shear stress and degradation of material used for the PBRs [73]. Biomass and lipid productivity in PBRs was higher than in raceway ponds [74,75]. Generally three parameters such as volumetric, areal and illuminated surface productivity used to calculate the biomass productivity in algae production systems [76].

PBRs-flat panel, tubular, bubble column, annular (cylindrical), air lifting is used for mass culture of algae. Tubular PBRs are made with plastic or glass tubes with U bends to capture more sunlight. The tubes diameter are 5-10 cm. Algal culture are circulated through mechanical pumps [60]. These PBRs have high surface volume; hence light capture is higher and gives higher biomass yield. Microalgae are successfully cultivated in tubular PBRs [77-79]. Tubular PBRs require a large land area for a given volume of reactor and this is a significant disadvantage. Bubble columns and airlift reactors are more compact than tubular devices can offer more advantages for large scale culture [77]. Airlift systems have been used for *Botryococcus, Porphyridum cruentum, Haematococcus pluvialis, Phaeodactylum tricornutum* [80-82]. Annular (cylindrical) PBRs vertically designed with aeration provided from the bottom and illuminated through transparent walls. They offer efficient light, temperature and agitation to algal cells. Mixing is very important that increases the cell concentration when exposed to light and minimize the dark volume of the reactor. It also enhances mass transfer between nutrients, facilitates dissipation of heat and prevents oxygen [60]. Efficient illumination can be achieved by internal light which distributes light into cultures [83]. Light and dark cycles can influence on photosynthesis. Enhancing productivity in cultivation of algae by supplying flashing light causes additional cost [84]. Now a day's LED photodiodes are used for illumination instead of fluorescent lights. Continuous illumination can be achieved by solar radiation during the day time whereas night time can use solar power driven LED lights to enhance productivity [85]. Light transmission can be increased by increasing the reactor that is exposed to light. Improving light penetration in PBRs was evaluated by Carvalho et al. [86]. Plastic bags are used for microalgae outdoor cultivation system. Flat panel consist of joined transparent plates to store the culture on which the culture is illuminated from one or both sides and stirred by aeration. Flat panel PBR offers biomass production of photoautotrophic microorganisms. Flat vessels are made for outdoor mass cultivation of algae under photoautotrophs [87,88] which is very easy to build desired light path. The factors to be considered in the design of flat panels are the collection of solar radiation, to remove oxygen, to avoid nutrient gradients and to control temperature. In order to get irradiance 100 $\mu E/m^2/s$ in panel reactors, to maintain cell density 1 g/L and flat panel depth should be below 0.07 m. The optimum light path was 0.1 m for *Nannochloropsis sp*. in flat panel was reported by Zou and Richmond [89] where the biomass productivity reached 0.5 g/l/d. The flat panel reactors can achieve high biomass yield due to their light illumination surface area to volume. Mixing is provided by supplying CO_2 enriched air which used as carbon sources to increases cell density and biomass productivity.

Today, raceway ponds and PBRs used for *Botryococcus* culture for the biomass production [13,47,69,70,79]. Batch and continuous mode was tested in open and closed systems. Continuous mode is feasible for higher biomass production. Continuous culture mode in air lift PBRs used for *B. braunii* at small scale was reported by various authors [14,72,77,78]. Maximum hydrocarbon content was observed in bubble column photobioreactor by supplying 1% CO_2-enriched air with 10 klux irradiance [77,78]. In bubble column photobioreactors, the biomass yield was reached 7 kg/m^3 and hydrocarbon content was found to 50% in *B. braunii* culture was reported by Kojima and Zhang [90]. Compared with closed aseptic culture vessels, open pond reactors can provide a moderate surface to volume ratio at a much lower cost per unit volume [91]. However the culture conditions in open systems are less controlled than in closed reactors, consequently the biomass productivity was low compared with closed PBRs. In open culture systems contain mixed populations and not desired algae. Open culture system is the most reliable for producing large amounts of biomass, for extraction of lipids and hydrocarbons. Recently reported that the biomass productivity of *B. braunii* strains in raceway ponds [13,47,69,70] was comparatively same with the photobioreactors [47], this might be due to the strain difference, culture conditions (CO_2 supply, initial cell concentration and salinity) and also specific design of raceway ponds and photobioreactors. Based on the current literature, biomass and areal biomass productivity of *B. braunii* species in the cultivation of raceway and photobioreactors comparison with other algal species are presented in (Table 5).

Microalgae	Mode of cultivation	Biomass productivity or areal biomass productivity (g/L/dorg/m^2/d)	References
B. braunii (LB-572)	Raceway	0.11	[13]
B. braunii (N-836)	Raceway	0.05	[13]
B. braunii (765)	Photobioreactor	0.09	[47]
B. bruanii (AP-103)	Raceway	0.11	[69]
B. braunii (Mahabal)	Raceway	0.10	[70]
Phaeodactylum	Tubular	1.19	[71]
Sprulina	tubular	0.62	[84]
B. braunii (FACHB-357)	Single layer		
photobioreactors	5.50	[157]	
B. braunii (LB-572)	Panel reactors	0.02	[155]
B. braunii (LB-572)	Biofilm photobioreactor	0.71	[160]
Chaetoceros	Bubble column	3.31	[161]
Chaetoceros	Air lift reactor	4.09	[161]
Chlorella kessleri	Tubular	0.12	[162]
Scenedesmus obliquus	Tubular	0.20	[162]
Spirulina	Tubular	0.39	[162]
Dunaliella	Flat plate	1.50	[163]
Haematococcus	Air lift reactor	0.80	[164]
Monodus	Bubble column	0.03	[165]
Strain name			

Nannochloropsis	Flat panel	0.22	[166]
Nannochloropsis	Flat plate	0.22	[166]
Nannochloropsis	Raceway	11.0	[167]
Nannochloropsis	Flat panel	27.0	[167]
Nannochloropsis	Tubular	25.0	[167]
Nannochloropsis salina	Raceway	0.20	[168]
Phaedactylum	Tubular	1.38	[169]
Phaeodactylum	Flat plate	1.38	[170]
Phaeodactylum	Tubular	1.19	[72]
Scenedesmus rubescens	Raceway	0.02	[171]
Scenedesmus sp.	Raceway	0.19	[172]
Spirulina platensis	Raceway	0.18	[173]
Synechocystis aquatilis	Flat panel	1.00	[174]

Table 5: Biomass and areal biomass productivity of *B. braunii* comparison with other algal species in raceway and photobioreactors cultivation.

Hydrocarbon Extraction

B. braunii produces variety of hydrocarbons which contain higher and lower carbon chain. Biomass can be harvested by filtration or centrifugation. Hydrocarbons are accumulated in outer cell wall. *B. braunii* cell wall is very thick and it is very difficult to extract or break [3]. Hydrocarbons can be recovered by solvent extraction process, high pressure, sonication or supercritical fluid extraction process. High pressure extraction for hydrocarbons used at bench scale. Hydrocarbons can easily extracted by non-toxic solvents which are inexpensive, easily available, and immiscible with water, low boiling point and reusable. For obtaining higher hydrocarbon recovery, dry algal cells can be used for the hydrocarbon extraction instead wet cells [3]. However, dewatering and drying of the biomass is not a cost-effective method for industrial applications. Wet biomass cells contain higher percentage of water; the algal cells reduce contact of the non-polar solvents with the outer cell wall [92]. Wet cells are aggregated and form clumps during the extraction process. Hydrocarbon recovery yield was influenced by physiological status of culture. Frenz et al. [93] reported that the hydrocarbon recovery was high in exponential growth phase of algae which was observed in photobioreactor cultivation. However, the strain, solvent selection, extraction time and temperature are the key parameters to get the efficient hydrocarbon recovery.

The biocompatible solvent selection is very critical because the presence of organic solvent can retard the cell growth. Based on the current literature, various solvents-hexane, n-octane, dodecane, acetone and ethyl acetate extraction methods were used for hydrocarbon recovery [93-97]. Terpenoids in *B. braunii* biomass was extracted by using solvent hexane [98]. Another solvent method, supercritical CO_2 extraction method used for hydrocarbons recovery, optimal time was found to be 30 min at pressure 30 MPa [99,100]. This extraction method was expensive, non-toxic, reusable, contains low viscosity and CO_2 allow quick extraction of solids. Algal hydrocarbons solubility in CO_2 extract was increased with increasing pressure [100]. Recently, Cho et al. [95] reported that n-octane was best for the hydrocarbon recovery when compared to dodecane, tetradecane and hexadecane solvents which gives 48% hydrocarbon recovery at 6h time. Three non-polar solvents-n-hexane, ethyl acetate and acetone at 50°C temperature with 10.3 MPa pressure used for hydrocarbon extraction [101], the results showed n-hexane was the efficient solvent extraction method for higher hydrocarbon recovery, further he confirmed accelerated solvent extraction (ASE) was 6% higher than Sox-let method at 6 h and 60 ml solvent which saved time and solvent. Major hydrocarbons (C_{32}, C_{33}, C_{34}) recovered in dimethyl ether used as extraction solvent whereas in n-hexane extract was found to be C_{30} and C_{31} hydrocarbons reported by Kanda et al.[102] Dayananda et al. [12] reported that the hydrocarbon extraction was 33% in *B. braunii* (LB-572) and 46% in *B. braunii* (SAG-30.81) obtained using hexane extraction method. In our laboratory studies, hydrocarbon extracted for *B. braunii* strains using n-hexane showed 34%, 20% and 22% hydrocarbon recovered in CFTRI-Bb1, MCRC-Bb and N-836 strains of *B. braunii* reported by Rao et al. [7,8]. Based on these findings, n-hexane was the efficient solvent for the hydrocarbon extraction. Hydrocarbon recovery from various *B. braunii* species using solvent extraction methods is presented in (Table 6).

Strain name	Solvents/temp/pressure/extraction time	Hydrocarbon recovery (%)	Reference
CFTRI-Bb-1	n-hexane	34	[7]
MCRC-Bb	n-hexane	20	[7]
N-836	n-hexane	22	[7]
LB-572	n-hexane	33	[12]
SAG-30.81	n-hexane	46	[12]
Showa	n-hexane	39	[42]
Showa	n-hexane	90	[57]
UTEX-572	n-octane, 6h time	48	[94]
Showa	Hexane, at 50°C, 10.3 MPa pressure, 5 min	40	[101]
SAG 807-1	1,8-diazabicyclo-[5.4.0]-undec-7-ene/octanol (1:1)	16	[140]
SAG 807-1	1,8-diazabicyclo-[5.4.0]-undec-7-ene	15	[140]
SAG 807-1	1,8-diazabicyclo-[5.4.0]-undec-7-ene/ethanol (1:1)	12	[140]
Showa	n-hexane at 100°C	97	[175]

Table 6: Hydrocarbon recovery from biomass of *B. braunii* strains by using solvent extraction methods.

Bioactive Constitutes and their Use in Industrial Applications

B. braunii produces variety of bioactive molecules such as hydrocarbons, alkanes, ether lipids, fatty acids, polysaccharides and carotenoids which have high demand in industrial applications such as fuel, food and feed applications (Table 7). In addition, *B. braunii* is used in plant growth promoter studies in plant tissue culture systems. Carotenoids and exopolysaccharides from *B. braunii* showed various biological properties in *in vitro* and *in vivo* studies. These bioactive molecules are described briefly in the below.

Bioactive molecules	Industrial Application
Hydrocarbons	Fuels, electricity, natural gas, liquid fuel, petroleum gas,petrol, diesel, power plants, homes
Alkanes	Fuel oils, natural gas gasoline, polymers, paints, plastics, drugs, cosmetics, detergents,
Either lipids	Cytotoxic effects on tumor cells, chemical indicators in neoplasms, chemical mediators, Fat absorption, human food, animal feed

Fatty acids	Human nutrition, nutraceutical and pharmaceutical applications, Gene interactions, Acylation or proteins, Membrane fluidity, substrate specificity
Exopolysaccharides	Food, paint, laundry, textile, adhesive, binding agent, coating, emulsifying agent, stabilizer and thickening agent
Carotenoids	Antioxidant properties, anticancer activities , bioavailability , food, ingredients, feed, nutraceutical and pharmaceutical applications,

Table 7: Bioactive molecules from *B. braunii* and their industrial applications.

Source of Hydrocarbons

B. braunii contain three different races such as 'A', 'B' and 'L'. These are divided based on the characteristic of hydrocarbons they produce. Race 'A' produces odd numbered n-alkadienes and alkatrienes. Race A produces 60% of the olefins in the dry cell mass of the green state colonies [103]. Two unusual hydrocabrons-C_{27} H_{51} and C_{27} H_{48} tetrane reported in race A strain which was isolated from Lake Overjuyo, Bolivia. Chemical structures (30) and hydrocarbons (50) were reported in 'A' race of *B. braunii* [11] and most of the compounds were identified monoenes, tetraenes and odd carbon numbered. The hydrocarbon distribution depends on the genetic factors which varied strain to strain during the cultivation under identical conditions [104]. Oleic acid is the main precursor of dienes and trienes reported by Templier et al. [105,106]. The 'B' race produces poly methylated unsaturated triterpenes, called botryococcenes and they can exist as isomers with the same carbons but different structures. Squalene and C_{31}- C_{34} methylated squalenes were synthesized by 'B' race in *B. braunii* [11,30]. These molecules were isolated from Bolivian strain of *B. braunii* [107]. The botyrococcene (C_{30}) was the precursor for higher homologous compounds reported by Metzger et al. [29]. Botrycoccene can accumulate around 20-80% in the dry cell biomass [108]. A 50 botryococcenes were purified in 'B' race reported by Metzger and Largeau, [11]. The 'L' race produces tetraterpene ($C_{40}H_{78}$), known as lycopadiene [109] and they can accumulates 2-8% of the dry cell biomass [33]. Lycopadiene was the sole hydrocarbon detected in *B. braunii* strains from Thailand and Ivory Coast [110].

Source of Alkanes

Alkanes are nothing but saturated hydrocarbons found in *B. braunii* [111]. Cell accumulates methylated aldehydes, is generated from fatty acids via methylation process by S-adenosyl methionine, and then converted them into aldehydes by fatty acyl reductase which requires ATP, CoA, and NADH for its activity. Fatty acyl reductase was solubilized in 0.1% octyl beta-glucoside, purified by blue A and pamitoyl agarose column chromatography and its molecular weight was found to be 35 kDa by SDS-PAGE [112,113]. Alkanes are obtained by the conversion of aldehydes which are generated from the fatty acids by methylation process; alkanes are losing one molecule of carbon in this process. Alkane's conversion was occurred via decarboxylation which requires decarbonylase enzyme activity in anoxic conditions. It is located in microsome, it may convert carbon monoxide in *B. braunii* into carbon dioxide which is necessary for organisms to accumulate higher amount of hydrocarbons and the enzyme can be inactivated by metal chelators. Saturated hydrocarbons such as docosane, hexacosane and heptacosane were reported in *B. braunii* (N-836) [114].

Source of Ether Lipids

'A', 'B' and 'L' races of *B. braunii* are producing variety of either lipids. Ether lipids made up with various triacylglycerols, glycerophosphatides and isopranoid dialkyldiglycerol tetraethers. 'A' race produces ether lipids like alkadienyl-O-alkatrienyl ether, resorcinolic either and alkenyl-O-botryalyl ether reported by various authors [115-117]. It is producing high amount of alkadienyl-O-alkatrienyl ether where the strain isolated from Lake Overjuyo, Bolivian and another from Lake Coat Herno from France [115]. These species contain 40% of ether lipids in the exponential phase of algae whereas in stationary phase their levels were low [118]. These ether lipids are close to hydrocarbons. However hydrocarbons levels were very low in these strains during the growth phase of algae. One more 'A' race strain was isolated from Lake Overjuyo, Bolivian which contains 35% of phenoxy ether lipids on the dry weight basis [117]. Another strain isolated from Maddingley brick Pits, UK which showed 5% of ether lipids on the dry biomass. Either lipids linked with phenoxy bonds or hydrocarbons which derived from alkadienes, thus give rise to high molecular weight lipid compounds. These lipids structurally derive from the coupling of alkadienes, alkenylresorcinols, alkenylhydroquinols or branched aldehydes by aldol condensation called botryals. Alkenyl-O-botryalyl ether is ether lipid which attached to hydroxyl group is esterified by oleic acid [117]. In 'B' race, ether lipids such diepoxy-tetramethylsqualene, botryolin A and botryoxanthin-A, B was reported [110,119-121). Minor components of botryolins were isolated from Ivory Coast. These triterpenoid triethers contain a tetramethylsqualene carbon skeleton. Braunixanthin-1,2 were isolated from the Japanese strain reported by Okada et al. [122]. They contain an alkylhdroquinol moiety mid chain bound by ether bridges to enhinenone and a tetramethysqualene derivative. This contains a tetrahydrofuran ring, which derives from the cyclisation a diepoxy-tetramethylsqualene [110]. 'L' race produces various ether lipids, recently reported that diepoxy-lycopane, lycopanerol 'A', lycopanerol 'F' and lycopanerol 'H' isolated from *B. braunii* which linked with tetrahydrophyran ring containing lycopanes [110,119,123,124]. Lycopanerol 'F' found in the India and Ivory Coast strains which associated three tetrahydrofuran containing lycopanes with ether bridges. Lycopanerol 'H' found in the Ivory Coast strain which includes tetrahydrofuran and tetrahydropyran containing lycopene, alkylphenol, phenoxy bonds. Accumulation of lycopanerol in the 'L' race was found to be 10% on the dry weight basis. These compounds are formed as terpenoid, alkylphenol, resorcinol and non-terpenod with ether/phenoxy bonds. Each compound derived from the diepoxy-lycopane which containing one or three tetraterpenoid with tetrahydrofuran or tetrahydropyran [106].

Source of Fatty Acids

Microalgae species - *Botryococcus, Chlorella, Scenedesmus, Dunaliella, Nannochloropsis* and *Chlorococcum* etc accumulates high amount of lipids [61] Among the algal species, *Botryococcus braunii* is one considered to be a potential source for biofuel feedstock which produces high amount of fatty acids [6,7,13]. *Botryococcus* accumulates lipids in the range of 2-86% on the dry weight basis; this content was varied by culture conditions and specificity of alga [11]. Lipids contain various fatty acids such as palmitic acid ($C_{16:0}$), oleic acid ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic acid ($C_{18:3}$). Accumulation of fatty acids in the exponential phase was higher compared to stationary phase of algae. *B. braunii* contain methylated branched fatty acids and these are known to inhibit endothelial cell and leukocyte proliferation [113,125,126]. Fatty acids profile - $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{22:0}$ in *B. braunii* was reported in raceway and circular pond cultivation [13]. Enhanced oleic and palmitoleic acid content in *B. braunii* (LB-572) under salinity culture conditions [6].

Source of Exopolysaccharides

B. braunii produces Exopolysaccharides (EPS) but a few species are used in commercially importance. These are used in industrial applications such as food, paper, paint, laundry, textile and adhesive. Use of the polysaccharides depends on their rheological characteristics. Microbial polysaccharides have a great impact since their use in various applications. Polysaccharide production associated with pathogenicity of microorganisms. EPS produced by *Porphyridium curentum* used in commercial applications, whereas EPS produced by *Chlamydomonas Mexicana* considered to be soil conditioner [127,128]. Similarly, *B. braunii* is producing exoploysaccharides which

accumulates in the ranges from 250 g/m³ in 'A' and 'B' races and 1 kg/m³ in 'L' race [14,129]. Cells in *B. braunii* are made up with mucilaginous polysaccharides; these are dissolved in culture medium, increases in viscosity of culture medium. Polymers-galactose, fucose, arabinose, rhamonse, uronic acids, and non-sugars are identified in *B. braunii*. Among the polymers, galactose is the major compound in the heterogeneouse polysaccharide. Some of the unusual sugars namely 3-O-methyl fucose, 3-O-methyl rhamnose and 6-O-methyl hexose were reported in *B. braunii*. EPS content 4-5.5 Kg/m³ in *B. braunii* UC-58 was reported by Fernandes et al. [130]. The polysaccharide content was higher in decline phase of algae compared to exponential and stationary phase. Enhanced polysaccharide content in nitrogen deficiency conditions, where the degrading nitrogen contains macromolecules and accumulating polysaccharides and lipids. EPS content was declined, when urea and ammonia used as nitrogen source. The consumption of urea and ammonia causes decrease in culture pH, this gives low polysaccharide production. Optimum nitrate concentration was 8mM and temperature ranges from 25-30°C for polysaccharide production in *B. braunii*. EPS synthesis under continuous illumination was exhibit higher than cells grown under cyclic illumination [131]. The degree of biopolymer was decreased when the culture was exposed out of the optimal temperature. *Auriobacterium barkeri* associated with *B. braunii* culture, however they did not effect on the exoploysaccharide synthesis [130]. Sulfated polysaccharides isolated from microalgae species, used in anti-adhesive property, inhibited HeLaS3 human cell line by algal polysaccharides [132]. EPS is a potential source of uronic and fucose, which can be used in reduce metal ions toxicity and substrate in chemical synthesis [133].

Source of Carotenoids

Microalgae are producing high value added products, in particularly carotenoids which have broad industrial applications still studies are required to improve their culture conditions or cultivation methods in order to be economically competitive in the market [134]. Some algal species accumulate high concentration of carotenoids under stress conditions [6,7,44]. Similarly, *B. braunii* is known to produce hydrocarbons, lipids, exopolysaccharides and carotenoids [135,136]. The accumulation of carotenoids in 'A', 'B' and 'L' race of *B. braunii* was reported by various authors [44,120-122,136-139]. *B. braunii* changes from green to brown, red orange and pale yellow due to the accumulation of secondary carotenoids. Carotenoids-β-carotene, echinenone, canthaxanthin, lutein, violaxanthin, loroxanthin, and neoxanthin are produced in 'B' and 'L' races in linear phase [138]. However, lutein is the major carotenoid (22-29%) reported in the linear phase of these races. Canthaxanthin and echinenone are the dominating carotenoids in the stationary phase [138]. Grung et al. [139] reported the presence of adonixanthin in 'L' race in stationary phase. Botryoxanthin-A [120], botryoxanthin-B, and α-botryoxanthin [121], braunixanthin 1 and 2 [122] are new carotenoids isolated from the 'B' race. Total carotenoid content in 'A' race of *B. braunii* was found to be 0.28% under sodium chloride stress conditions was reported by Ranga Rao et al. [6]. Carotenoids-violaxanthin, lutein, astaxanthin, zeaxanthin and β-carotene were characterized and quantified in 'A' race of *B. braunii*-LB-572 [44,140]. Biomarkers of 12 carotenoids in *B. braunii* were reported by Muntean et al. [136], however, the major carotenoid lutein was identified in 'A' race [140]. Further these carotenoids are characterized and identified by mass spectra [137]. Hydroxylation of hydrocarbon carotenoids is known to be responsible for the formation of 3-hydroxy cyclic carotenoids and epoxy carotenoids. The presence of traces of β-carotene in *B. braunii* may therefore be related to the conversion of these molecules to lutein. This may be a reason for the higher content of lutein in this alga. This alga represents a potential source of lutein, a commercially interesting carotenoid of application in aquaculture and poultry farming, as well as in the prevention of diseases related to age related macular degeneration.

Source of Elicitor

Enhancement of secondary metabolites by elicitation is one of the new strategies which are gaining commercial application. Elicitors are chemical compounds, either biotic or abiotic, which on contact with higher plants trigger the production of secondary metabolites [141]. Secondary metabolite production in plant tissue culture system was enhanced by various microalgal extracts such as *Spirulina, Haematococcus, Scenedesmus, Synechococcus, Nostoc* and *Botryococcus* [142-146]. Similarly, *B. braunii* extracts had some bioactive principles responsible for increasing secondary metabolite production in plant culture system [142,143]. Recently, the effect of *B. braunii* extracts on the growth and secondary metabolite production in *C. frutescens* callus culture was evaluated by Sharma et al. [142]. In the results, increase in seed germination, root, shoot and leaf length in both light and dark conditions in *C. frutescens* over the control group. The total chlorophyll and carotenoid content increased 2 fold after 15 days in *C. frutescens* culture at 8 mg/L *B. braunii* extract was when compared with control group. Major metabolites-vanillin and vanillylamine are intermediates in the capsaicin biosynthesis pathway, increased during the experimental period and this was reflected in the enhancement of capsaicin in the extract-treated callus cultures. Both capsaicin and vanillin content were increased in the *Capsicum* suspension cultures by using *B. braunii* extracts as elicitor. Vanillylamine in 1.5-fold, capsaicin in 2.5-fold and vanillin in 2-fold were higher than the control group after incubation with *B. braunii*. Earlier *B. braunii* extracts showed growth-promoting effects on roots of *Tagetes erecta* at the 8ppm level reported by Murakami [143].

Biological Activity of *B. braunii*

Microalgae biomass is generally used in various applications such as food and feed applications. Since the biomass contains high value metabolites-carbohydrates, proteins, total carotenoids, phenolic, nucleic acids and fibre [147]. After extracting hydrocarbons and lipids from the biomass has high value co-products which have rich in nutritional benefits. It is used in various industrial applications such as aquaculture, poultry and nutraceutical in animal consumption. Microalgae products are available in the market with mixture of carotenoids; fatty acids which are utilized in the food industry. Algal biomass approved in commercial applications in several countries. Many research studies were conducted on use of algal biomass and carotenoids in food, feed and nutraceutical applications [134,148,149]. Microalgae *B. braunii* is known to produce high value compounds-carotenoids, fatty acids, lipids, polysaccharides and hydrocarbons from 'A', 'B' and 'L' races. Carotenoids-violaxanthin, astaxanthin, lutein, zeaxanthin, chlorophylls a and b, and α, β-carotene were identified in the *B. braunii*, among the carotenoids, lutein represents more than 75% of the total carotenoids. *B. braunii* extracts showed 70% antioxidant activity in DPPH and hydroxyl radical scavenging model systems and rat tissues at 10 ppm level of carotenoids [140]. Carotenoids in 'A' race of *B. braunii* showed biological activity in *in vitro* and *in vivo* models [137,140,150,151]. The antioxidant activity was enhanced in rats, due to the bioactive molecules in the biomass. Lutein in *B. braunii* biomass was enhanced bioavailability and antioxidant enzymes levels in rat tissues after feeding biomass as source of lutein [137,150]. Exoplolysaccharides (EPS) rich in *Botryococcus braunii* have various biological activities such as anti-adhesive against bacterial infections, cytotoxic effects and anti-tumor properties (Guzman Murillo and Ascencio, 2000). Antibacterial activity of *B. braunii* extracts were tested against important clinical bacterial isolates such as *Bacillus subtilus, Bacillus cereus, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes, Micrococcus luteus, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus,Streptococcus fecalis* and *Yersinia enterocolitica* using agar-well diffusion assay method reported by Ranga Rao et al. [147]. Results proved that the ethyl acetate extract was showed highest inhibition against *E. aerogenes*. Based on the published data, *B. braunii* extracts can be used as bacteriostatic agents for suitable applications.

Conclusion

Based on the current studies, *B. braunii* produces higher amount of biomass, hydrocarbons, lipids and other bioactive constituents such as ether based lipids, fatty acids, polysaccharides and carotenoids which can be used for commercial applications. Various culture conditions were enhanced the growth, hydrocarbon content and other bioactive molecules in *B. braunii*. Raceway ponds and photobioreactors were developed for the scale-up studies. Contamination levels were minimized in culture by using photobioreactors. Hydrocarbon recovered by using various solvent extraction methods. Polysaccharides and carotenoids of *B. braunii* were showed biological properties in *in vitro* and *in vivo* models. *B. braunii* extracts used as elicitor in plant tissue culture system which enhanced secondary metabolite production in *Capsicum frutescens*. Algal hydrocarbons have high octane rate to use as motor fuel. *B. braunii* can removed nitrates and phosphates from the wastewater. The current chapter could provide the production of hydrocarbons and other bioactive constitutes from *B. braunii* and their use in industrial applications.

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