Research Spectra 2024; V(1-2): 165-185

Print ISSN: 2394 - 9805
Online ISSN: 2455 - 0256
A Tri-Annual Publication
RESEARCH SPECTRA
An International Multidisciplinary Peer-Reviewed / Refereed Journal

Print ISSN: 2394-9805

Online ISSN: 2455-0256

Volume - V, Issue No. 1-2
(January - August, 2024)

Paper Code
No. - RSV05I1-213

Ekta Shukla, Vibha Srivastava, Yashveer Singh*

Sunbeam College for
Women, Bhagwanpur,
Varanasi

A Hydrocarbon Producing Promising Alga: *Botryococcus:* A review

Ekta Shukla, Vibha Srivastava, Yashveer Singh
Sunbeam College for Women, Bhagwanpur, Varanasi -221005.
Corresponding author email id- yashveersingh.botany@gmail.com

Abstract

Petroleum products have been crucial energy sources for industrial growth, but their increasing demand is rapidly depleting these reserves worldwide. Burning these fossil fuels releases carbon dioxide, contributing to global warming and environmental issues. To address this, using microalgae for photosynthetic carbon dioxide fixation can be a potential solution. Production and use of algal biomass for fuel and valuable chemicals have become essential for energy consumption. The microalga Botryococcus species, particularly B. braunii and B. protuberans, can provide a promising alternative source. These freshwater green microalgae form large colonies and accumulate significant amounts of renewable hydrocarbons in their cell walls through photosynthesis. Botryococcus species exhibit varying growth rates, biomass, and hydrocarbon production, and under optimal conditions, can yield up to 4.5 grams per litre of biomass at the end of the exponential growth phase.

The hydrocarbon content in B. braunii was higher than in B. protuberans at each growth stage. During the resting stage, carotenoids often exceeded chlorophylls, causing the colonies to appear brownish. An oily layer of liquid hydrocarbons was observed on the surface of the culture medium. The highest hydrocarbon content (around 70% of total lipids) was recorded during the resting stage, while productivity was higher in the exponential and early stationary *phases. Radioactive carbon labelling studies showed a decline in polar lipids and an increase in nonpolar lipids, particularly hydrocarbons, during the resting stage. Nitrogen deficiency, in both aerobic and anaerobic conditions, led to a decrease in dry weight, chlorophyll, and protein content, but an increase in carotenoids, carbohydrates, and lipids for both species. Nitrogen starvation resulted in a 1.6-fold increase in lipid content. Anaerobiosis under nitrogen-deficient conditions enhanced lipid production compared to anaerobiosis in nitrogen-supplemented medium. Anaerobiosis induced hydrocarbon biosynthesis more significantly than nitrogen deficiency, but it decreased other nonpolar and polar lipids.*

The microalga Botryococcus species has been extensively studied for its ability to produce biomass that can be used as a promising source of liquid hydrocarbons. Immobilizing the whole cells in alginate beads and growing them in air-lift batch cultures led to a notable increase in carotenoid and lipid contents compared to free-floating cells.

Introduction

The significant dependency of industries and transport on petroleum and its derivatives, coupled with their limited supply, has created serious economic challenges worldwide. These fossil fuels are non-renewable and are being depleted rapidly due to increasing population demands (Aaronson and Dubinsky, 1982). Currently, the unequal distribution of fossil fuel resources is a major cause of conflicts over energy and global warming among nations (Nazloo et al., 2023). In response, the last few decades have seen an intensification of efforts to develop sustainable processes for producing biofuels from microalgae. This has driven scientists to seek new, renewable energy sources derived from plant products to ensure the long-term survival of humanity. Their rapid growth, high lipid production, and adaptability to various environments make microalgae a primary focus in biofuel research.

Given the pressing need for alternative energy resources to meet future demands, biotechnology-driven fuel production is garnering significant interest. Biofuels such as bioethanol, vegetable oils, biogas, biodiesel, and biohydrogen are prime examples of this shift. Recent advancements in the study of microalgae have expanded their potential to produce a diverse array of chemicals, oils, and polysaccharides (Kawamura et al., 2022). Additionally, large-scale cultivation of microalgae has been advocated as a viable source of fats and oils (Borowitzka, 1998; Metzger and Largeau, 2005; Arif et al., 2020), as well as for fuel production (Barclay et al., 1988; Chisti, 2007). This ongoing research highlights the critical role that microalgae may play in addressing global energy challenges.

Algae represent a largely untapped resource for lipids and hydrocarbons, with algal lipids being highly reduced hydrocarbons. The lipid content in algae typically ranges from 20% to 40% of their dry weight. However, under specific environmental and physiological conditions, some algae can produce up to 85% lipids of their dry weight (Dubinsky et al., 1978; Singh and Kumar, 1992). Research in industrial nations such as the USA and Germany has focused on microalgal lipids as a potential source of fuel oils, with initiatives like the Solar Energy Research Institute (SERI) in Colorado spearheading liquid fuel production.

Among the various microalgae studied for biofuel production, *Botryococcus* stands out due to its unique characteristics. In addition to polysaccharides and carotenoids, *Botryococcus* produces and accumulates hydrocarbons (Kawamura et al., 2020). Although the extraordinary production of large quantities of hydrocarbons is rare in algae, *Botryococcus* is recognized as a promising renewable resource capable of generating high levels of liquid hydrocarbons and lipids. This green colonial microalga can divert a substantial portion of its photosynthate into distinctive long-chain hydrocarbons, including alkadienes lycopadienes, and botryococcene, found in various *Botryococcus* species. Notably, botryococcene accounts for approximately 1% of crude oil from Sumatra (Moldowan and Seifert, 1980).

Other researchers, such as *Dunaliella*, have also demonstrated hydrocarbon production in different forms. The hydrocarbons produced by these microalgae result from the reduction of organic compounds derived from the decarboxylation of fatty acids (Tornabene, 1980). When hydrocracked, hydrocarbons extracted from *Botryococcus* species yield a distillate composed of

67% gasoline, 15% aviation turbine fuel, 15% diesel fuel, and 3% residual oil (Hillen et al., 1982; Matsui et al., 1997).

Algal biofuel is anticipated to become a next-generation carbon neutral bioenergy source because microalgal oils are derived from carbon dioxide fixed through photosynthesis (Wijfells and Barbosa, 2010). Furthermore, the combustion of these fuels is notably free of sulphur and nitrogen oxides. With the shifting landscape of renewable energy sources, *Botryococcus* has been increasingly identified as a valuable algal resource for hydrocarbon production (Nazloo et al., 2024).

Characteristics of *Botryococcus Species*

Botryococcus species are colonial slow growing green microalgae. They are widely found in freshwater and brackish water environments across various continents (Bachofen,1982). Although unicellular, the cells form aggregates or colonies of different sizes. *Botryococcus* is a slow-growing alga, with a doubling time of more than two days (Singh and Kumar,1994). The colonies float on the water surface and form dense blooms, producing a combustible sediment that is a source of boghead coal. The colonies have irregular, touch, hyaline, orange or brown, and yellowish-green mucilaginous envelopes. The oval cells are embedded in a gelatinous matrix containing oils and carotenoids (Singh,1992). The cells are arranged radially at the periphery of young colonies, but become scattered as the colony matures, changing from green to brick red. The cells are closely connected within the colonial envelope by delicate mucilaginous connections. Large compound colonies can reach 800 to 1000 micrometres in diameter, while small simple colonies are up to 70 to 80 micrometres in diameter. The individual cells are approximately 3 micrometres in size.

The cells of *Botryococcus braunii* are fully encased in a slimy envelope, whereas the cells of *Botryococcus* protuberans are only covered at the base. Additionally, *Botryococcus braunii* produces more of this slimy substance. The colonies of *Botryococcus* can regularly multiply through the breakdown or dissolution of the connecting threads. *Botryococcus* is considered unusual in its structure, behaviour, and metabolism, and it appears to have no close relationship with other algal groups.

Growth Pattern and Chemical Behaviour

During growth cultures, both *Botryococcus* species existed in three physiological states, which were characterized by differences in the chlorophyll-carotene ratio and hydrocarbon formation (Singh, 1992).

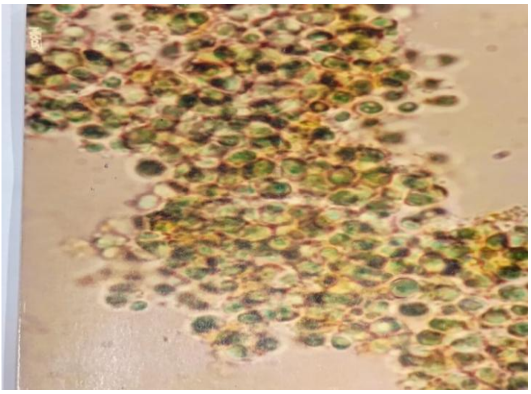

Fig 1. Green exponential growth

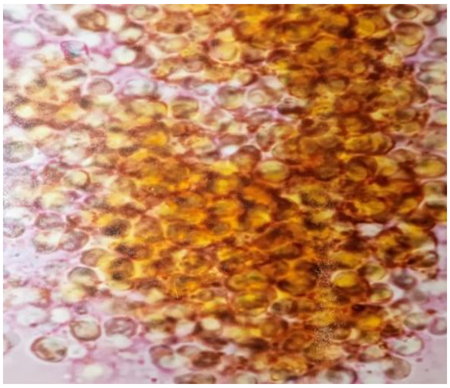

Fig 2. Yellow Stationary growth state

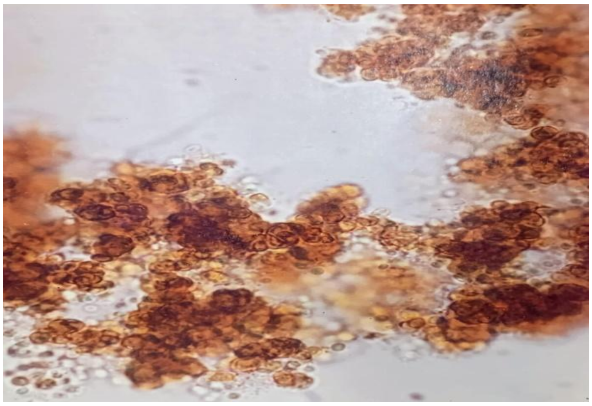

Fig 3. Brown Resting growth State

During the exponential state the colonies are green and heavier than water and the cells are dominated by chlorophyll a and b. The hydrocarbon content was approximately 18 to 20 percent of the total lipids/oils.

The colonies gradually turned yellow-orange due to the accumulation of carotenoids, and the cells lined up on the surface of the culture medium. During the steady state, a yellowish-green layer is formed. The buoyancy is due to the accumulation of oil in the colonial matrix.

The orange-brown resting state is characterized by the production of orange-coloured oil, often in such quantity and abundance that the contents of the cells are completely masked and the entire colony appears orange or brick red. There was also an oily layer on the surface of the medium that is very viscous due to soluble carbohydrates (extracellular carbohydrates) that were dissolved in the culture medium (Yashveer Singh, 2000).

Under optimal culture conditions, *Botryococcus* species (*B. braunii* and *B. protuberans*) grew exponentially up to 22-24 days after a lag phase (green rest) of 3-5 days. The steady state was found between day 25 and 35. From day 35 to 50, an orange-brown resting state occurs (Singh and Kumar, 1994). The maximum biomass was recorded during the early steady state and in the resting stage the cells lost their dry weight. This may be due to excretion and lysis of cells. Botryococcus also exists in three hydrocarbon carbon distribution ranges (C-17, C-27 and C-40) depending on the growth phase of the algae. About 85 percent of the dry weight is hydrocarbons in the orange-brown resting phase (Tornabene, 1982; Casdevall et al., 1985).

In *Botryococcus*, hydrocarbons are produced internally and are often secreted outside the cells, where they can be observed as an oily layer between and around the cells (Largeau et al., 1980). In *B. braunii*, hydrocarbons accumulate in the intra colony matrix outside the cell wall, which may allow hydrocarbon milking without killing the algae (Jackson et al., 2017).

On a dry weight basis, carotenoids exceed chlorophylls after steady state. Carotenoids are mainly yellow isoprenoid polyene pigments derived from lycopene. Carotenoids typically make up 0.1 percent of the dry weight of microalgae, however, some algae (*Dunaliella* salina) produce greater amounts of carotenoids in the form of beta-carotene under certain environmental conditions. In green algae, during the senescence and rest phase, accumulation of carotenoids occurs (Borowitzka et al., 1984).

During the exponential phase, most of the metabolic energy is used in the synthesis of chlorophylls, proteins, and intracellular carbohydrates. However, when cells transition from the exponential state to the steady state and resting state, the energy is predominantly used for lipid synthesis. The excretion of hydrocarbons in *Botryococcus* species may be a way to dispose of excess energy and thus avoid damage to cells due to photooxidation or other adverse conditions.

The biomass production and chemical profile of *Botryococcus* cells grown in batch cultures showed decreasing patterns in dry weight, protein, chlorophyll, and carbohydrate content; On the contrary, its carotenoid and lipid content gradually increased from the steady state to the resting state. Total lipids in both species (*B. braunii* and *B. protuberans*) gradually increases and biomass (dry weight) decreases as culture is aged. During the resting state, cells lose their dry weight may be due to secretory behaviour and cell lysis. The reduction in cellular carbohydrate content during steady state may be due to the conversion of carbohydrates to lipids. It has also been reported that degradation of chlorophylls and an increase in carotenoids often accompany lipid synthesis (Lien and Spencer, 1983).

Hydrocarbon Production

Under optimum culture conditions, a 60 percent decline in the total lipids (non-polar and polar) has been observed in *Botryococcus* species from the exponential to the resting phase. At the same time, hydrocarbons increase up to 70 percent of the total lipids. The decrease in total lipids and increase in hydrocarbons with increasing culture age in both species might be due to the conversion of major nonpolar lipid components such as fatty acids, triglycerides, aldehydes, and sterols into hydrocarbons (Table -1).

Table 1 Hydrocarbon production by *Botryococcus* species under normal batch culture conditions

± -Means Standard Deviation of three independent Replicates (Source: Kumar and Singh 1996)

Species	**Lipid Composition (% of Total lipids)**	**Physiological States**		
		Exponential state	**Stationary state**	**Resting state**
B. braunii	Hydrocarbons	18.2±0.6	48.7±1.1	70.2±2.2
	Nonpolar lipids	52.2±1.2	32.4±1.6	18.3±1.4
	Polar lipids	29.3±1.8	18.9±0.5	11.5±0.8
B. protuberans	Hydrocarbons	20.7±1.1	47.5±1.4	59.4±2.3
	Nonpolar lipids	49.3±2.0	31.3±1.2	23.1±1.2
	Polar lipids	30.1±1.8	21.2±1.1	17.5±0.7

In Algae the accumulated lipids are chiefly esterified fatty acids and are frequently associated with

substantial increases in carotenoids. Fats accumulate in the cells as other cell constituents decrease (Shifrin and Chisholm,1981). In sharp contrast, *Botryococcus* species accumulate hydrocarbons mainly during the stationary and resting states, and the productivity of hydrocarbons was found to be higher during the exponential and early stationary states (Kumar and Singh,1996).

B. braunii is classified into three different races (A, B, and L) depending on the type of hydrocarbons it synthesizes (Dayananda *et al.,* 2006). Race- A produces C23 to C33 odd-numbered alkadienes. These linear olefins can constitute up to 61 percent of the dry weight. The L- race produces a single hydrocarbon C40 to C78, a tetraterpene known as lycopadiene and constitutes up to 2-8 percent of the dry biomass (Banerjee *et al.,* 2002, Metzger *et al.,* 2005, Dayananda *et al.,2006*). Race-B produces triterpenoid hydrocarbons, C30 to C37 terpenoid hydrocarbons referred to as botryococcene, and C31 to C34 methylated squalenes and it can accumulate high levels of hydrocarbons ranging from 27 to 86 percent on a dry weight basis.

It appears that *Botryococcus* behaviour differs somewhat from the one commonly reported for many microalgae. Both species accumulate mostly hydrocarbons instead of triglycerides and this accumulation does not require blockage of cell division because it can proceed during the exponential growth state. Hydrocarbon production and accumulation are affected by the changes in the physiological status of the cells. The conspicuous formation of lipids by algae is the result of the accumulation of storage triglycerols. Many genera of class Chlorophyceae, Xanthophyceae, and Bacillariophyceae have oil storage as a diagnostic feature instead of or in addition to starch. Except B*otryococcus* species all the algae primarily produce triglycerols, not hydrocarbons. The fundamental difference between triglyceride formation in higher plants and algae is that in the case of algae, the oil-accumulating cells are themselves fully photosynthetic. This means that the complete pathway from carbon dioxide fixation to triglycerol synthesis can be modulated by the same cells.

Hydrocarbons Production under Nitrogen Limitation and Anaerobiosis

The maximum lipid concentration was found in green algae, *Monollantus salina*, followed by *Nannochloropsis salina* cultivated for nine days in nitrogen-depleted conditions. Besides lipids, changes in protein, carbohydrate, and fatty acids are also measured in phytoplankton (Harrison et al., 1990) and growth becomes limited by nitrogen deficiency. Variations in the nitrogen content of the culture medium also cause changes in the composition of carotenoids. Increasing nitrogen levels in the medium leads to an increase in the biomass, protein, and chlorophyll contents in freshwater green and blue-green algae (Piorreck and Pohl,1984). In *Dunaliella* species, nitrogen starvation results in the accumulation of C40 isoprenoid beta-carotene in the chloroplast (Ben-Amotz and Avron,1983), and in *Isochrysis* species, the accumulation of C37 alkenone (Ben - Amotz et al., 1985). In these algae, it appears that the cyclic and isoprenoid hydrocarbons are non-degradable end products that accumulate under slow growth and stress conditions. In *Chlorella vulgaris* under nitrogen-deprived conditions, polysaccharides (starch) were found to be 55 percent of the dry weight whereas, under nitrogen-replete conditions, it is only 20 percent (Behrens et al., 1989).

Anaerobic conditions facilitate the biosynthesis of lipids and hydrocarbons in both species of *Botryococcus*. The process of anaerobiosis triggers lipid biosynthesis via the activation of an oxygen-sensitive pyruvate dehydrogenase enzyme (Inui et al., 1987). Inui et al. (1982) documented the processes of lipid degradation and carbohydrate synthesis in the green algal species, *Euglena gracilis*. In the year 1988, Coleman et al. noted that under anaerobic environments, nitrogen-deficient cells undergo carbohydrate degradation and simultaneously synthesize lipids via wax ester fermentation, which subsequently enhances lipid accumulation. A study also showed a reduced hydrocarbon production in certain green algae, including *Botryococcus braunii*, under optimal growth conditions whereas, increase in the hydrocarbon production was observed under conditions that limit growth (Ben-Amotz et al. 1985). Additionally, it has been established that anaerobiosis influences the composition of fatty acids and lipids in algal species. The accumulation of hydrocarbons and various neutral lipids is augmented under anaerobic circumstances, attributed to incomplete oxidation processes. Research has demonstrated that the biosynthesis of both hydrocarbons and oxygenated compounds occurs via fatty acid precursors. Existing literature indicates that investigations concerning *Botryococcus* species predominantly concentrates on growth, lipid, and hydrocarbon production (related to physiological states) and composition under optimal growth conditions (Wolf et al., 1985; Casdevall et al., 1985; Kumar and Singh, 1996; Singh, 2000). To date, scant information exists regarding lipid production in *Botryococcus* species under conditions of nitrogen deficiency and anaerobiosis (Singh and Kumar, 1992).

From time to time the investigations on *Botryococcus* species amid nitrogen starvation and anaerobiosis have focused on lipid and hydrocarbon production, simultaneously it was also observed that the growth rate and chlorophyll content showed a marked reduction under nitrogen-deficient conditions.

Nitrogen deficiency adversely affects the biosynthesis rate of alpha-Aminolevulinic acid, which serves as the primary committed precursor in chlorophyll synthesis (Ohmori et al., 1984). The lipid content of both species is augmented under nitrogen-deficient growth conditions (Singh and Kumar, 1992). Cells under anaerobic conditions exhibited a higher lipid production compared to aerobic cells, with peak lipid values recorded at 72 percent in *B. braunii* and 62 percent in *B. protuberans* under nitrogen-deficient anaerobic environments. To elucidate the influence of nitrogen availability on the regulation of carbon utilization for lipid biosynthesis, efforts were made to modify lipid contents by incubating cells under anaerobic conditions for a duration of 12-15 days in nitrogen-sufficient or nitrogen-deficient growth media. The enhancement of lipid content in both *Botryococcus* species is more pronounced under aerobic cultures in nitrogen-free media than in those subjected to anaerobic conditions in nitrogen-supplemented media (Table -2).

Table 2 Content and Composition of Lipids in by *Botryococcus* species grown under optimum nitrogen sufficient, nitrogen deficient and anaerobic conditions.

Lipid	**Optimum**		**Without Nitrogen**		**Anaerobiosis**	
	B. braunii	***B. protuberans***	***B. braunii***	***B. protuberans***	***B. braunii***	***B. protuberans***
Content*	38.6±1.2	35.2±1.2	61.41±2.1	52.2±1.6	47.8±0.3	44.6±3.3
Composition**						
Hydrocarbon	56.4±1.8	47.2±1.6	64.6±1.2	56.0±0.5	72.0±1.2	62.0±1.8
Nonpolar lipids***	18.0± 0.8	32.5±1.8	19.1±1.3	33.4±1.0	10.5±0.7	25.5±1.1
Polar lipids	25.6±1.6	20.3±1.1	16.3±1.1	10.6±1.5	15.5±1.2	11.3±0.6

*** Percentage of dry weight, ** Percentage of total lipids *** Except Hydrocarbons,**

± -Means Standard Deviation of three independent Replicates (Source: Kumar and Singh 1992)

The relative concentration of hydrocarbons within the total lipid composition of both species is significantly elevated in anaerobic cultures (approximately 18%) in comparison to those cultivated under optimal conditions. In nitrogen-deficient aerobic cultures, there exists merely an 8% enhancement in the ratio of hydrocarbons to total lipids (Singh and Kumar, 1992). Anaerobic conditions result in a reduction of nonpolar lipids (excluding hydrocarbons) and the polar lipid fractions, while nitrogen deficiency induces a slight increase in the ratio of nonpolar lipids to polar lipids relative to total lipids. Although the concentration of predominant polar lipids is altered when cells are subjected to nutritional stress, the relative proportions of individual components remain fundamentally invariant (Ben-Amotz et al., 1985). Tornabene et al. (1983) observed that under conditions of low nitrogen concentration, the green alga *Neochloris oleoabundans* exhibits a high lipid content, with over 70 percent of these lipids being non-polar, such as triglycerides, alongside a minimal presence of hydrocarbons.

In a study on *Botryococcus* species, Singh and Kumar (1992) performed anaerobiosis in nitrogen-free and nitrogen-containing media by placing the cultures within an anaerobic incubator (Don Whitley Scientific Ltd, England). A mixture of nitrogen and carbon dioxide gas (9:1 v/v) was introduced into the incubator at a pressure of 2.15 kg per square centimetre. Hydrogen was supplied at a pressure of 0.86 kg per square centimetre. They observed substantial quantities of hydrocarbons under experimental conditions. The findings suggest that hydrocarbons, being chemically reduced derivatives, may have been synthesized more efficiently during anaerobiosis compared to other lipid fractions. This study may corroborate previous research indicating that *Botryococcus* species are indeed distinct within the microalgal domain for their capacity to produce and accumulate considerable amounts of lipids, primarily consisting of hydrocarbons, under specific stress conditions (Yoshimura et al., 2013; Arif et al., 2020; Areco et al., 2022).

Ultraviolet -B Radiation and Hydrocarbons Production

Ultraviolet rays (<400 nm) constitute a fundamental component of our solar system. Based on wavelength, ultraviolet radiation is systematically categorized into three distinct types: UV-A (320-400 nm), UV-B (280-320 nm), and UV-C (100-280 nm) (Newton et al. 1979). The implications of greenhouse gases, especially global warming, can be largely linked to the increased levels of UV-B radiation affecting the Earth's surface. The shorter the wavelength of UV-B radiation, the more pronounced the deleterious effects (Raghuvanshi and Singh,1991). Under controlled greenhouse conditions, flora subjected to UV-B radiation exhibited an approximate 50% decrease in growth. In higher plant species, reductions in leaf area, as well as both fresh and dry biomass, and photosynthetic efficacy have been documented across numerous ultraviolet-B-sensitive species (Worrest,1986). Microalgae are integral to the food web and the biotic framework within any aquatic ecosystem. These phytoplankton experience the adverse effects of heightened ultraviolet-B radiation, a consequential alteration of the entire ecosystem may ensue. In marine vegetative organisms, UV-B radiation has profound impact on various metabolic pathways, pigmentation, and community structure (Worrest,1986). Investigations into the effects of ultraviolet-B radiation conducted by Hader et al. (1986) and Dohler et al. (1986) on diatoms and blue-green algae have revealed a decline in primary productivity alongside an elevation in certain secondary metabolites. Singh (2000) investigated the effects of ultraviolet-B radiation on the development and metabolism of *Botryococcus* species positing it as an environmental stressor. The ultraviolet-B radiation source was provided by Fotodyne, Inc. USA, utilizing a lamp with a primary output at 312.67 nm. The requisite radiation dosage can be modulated by varying the distance between the UV-B lamp and the algal specimen. Approximately 50 percent cell mortality was observed at a dosage of 6 Watts per square meter of ultraviolet-B radiation following a treatment duration of 40-45 minutes in both species. Complete cellular loss was documented after exposure duration of 110 minutes.

Hydrocarbon production in both species was enhanced following 40-45 minutes of ultraviolet-B radiation exposure, yet diminished after 60 minutes.

In aquatic vegetation, ultraviolet radiation detrimentally affects physiological processes such as photosynthesis and enzymatic functions involved in protein and carbohydrate synthesis. The USEPA (1987) has reported that an increase in the dosage of ultraviolet-B radiation correlates with a reduction in biomass within phytoplankton. It is also well-established that certain algal species synthesize extracellular secondary metabolites as a coping mechanism against unfavourable conditions. The observed stimulation of lipids and hydrocarbons in *Botryococcus* may be indicative of this adaptive response.

Immobilization of Cells

Immobilization of algal cells may create anaerobic conditions and enhance enzymatic activity. It has been proposed to digest the algal biomass to produce organic compounds by fermentation with cell immobilization by entrapment in alginate gel (Friedl *et al.,* 1991). In comparison to batch cultures where free cells are used, immobilized cells offer certain specific advantages such as - physical stabilization of cells, no washout of cells, biomass retention and prevention of overgrowth, and easy separation of cells and excreted products. Immobilized algal cells have many potential applications including biocatalysts for biotransformation and biosynthesis and wastewater treatment through bioaccumulation of heavy metals. Biotransformation by immobilized algae include the production of sulphated polysaccharides by a red alga Porphyridium (Guddin *et al,*1984), glycolate and glycerol from green microalga *Chlorella* and *Dunaliella,* (Kerby and Stewart,1988) and Hydrocarbons from *Botryococcus braunii* (Bailliez *et al,*.1985, Bailliez *et al,*1988) respectively.

A study on the effects of whole-cell immobilization in sodium alginate beads was carried out by Singh (2003) on varied parameters such as growth, photosynthetic activity, lipids, and hydrocarbon production in *Botryococcus* species with growth states. He observed that after 30 days of incubation, rapid bleaching takes place in free cultures of *Botryococcus* species. While immobilized algae were still deep green even at the end of the stationary state. Immobilization stimulates and stabilizes photosynthetic oxygen evolution, chlorophyll, and carotenoid contents, even during the resting state. This might be due to the immobilized cells offering biomass retention in microbes. The carotenoid content in alginate gel entrapped cells increases significantly in both the species after the exponential growth state and this increase was maintained up to the resting state. Maximum lipids and Hydrocarbons were recorded in immobilized cells of resting state followed by stationary state (Table -3).

Table 3 Effects of immobilization of *Botryococcus* cells on lipids and hydrocarbon production at the end of physiological states.

Species	**Contents (% of Dry weight)**	**Exponential state (24th day)**		**Stationary state (35th Day)**		**Resting state (50th Day)**	
		Free	Immobilized	Free	Immobilized	Free	Immobilized
B. braunii	Lipids	36.62±1.8	37.8±2.0	39.5±2.2	42.48±1.8	43.92±2.0	45.80±2.4
	Hydrocarbons	20.15±1.6	19.82±1.2	47.65±2.4	52.45±2.6	68.15±2.8	72.50±2.8
B. protuberans	Lipids	38.80±1.3	36.12±1.2	37.84±1.3	40.72±2.0	41.25±2.2	44.60±2.0
	Hydrocarbons	21.30±1.7	20.64±1.6	45.86±1.4	60.73±2.5	63.40±2.6	70.34±2.9

Results are the means of ± SD of three independent replicates.

Cultures were harvested on indicated days

The of value of hydrocarbon in percent of total lipids (Source: Singh 2003)

Degradation of chlorophyll and an increase in certain carotenoid pigments often accompany lipid synthesis during stationary and resting states in *Botryococcus* species (Singh,1992).

The observed increase in lipid and hydrocarbon accumulation by Singh (2003) is in agreement with the findings of Bailliez *et al. (*1988). Algae immobilized in alginate beads can transform oleic acid (a fatty acid) into hydrocarbons. Immobilization of cells creates anaerobic conditions and anaerobiosis appears to trigger lipid synthesis in *Botryococcus* species. In both species, under any culture conditions, lipids and hydrocarbon accumulation occur mainly during the resting growth state. The studies carried out by Cheng *et. al., (2014)* on effects of nitrogen source and nitrogen supply model on the growth and accumulation of immobilized biofilm cultivation of *Botryococcus braunii* confirm the previous studies.

There are some issues with the cultivation process of *Botryococcus* species as compared to other microalgae. *Botryococcus* species grow very slowly, with a doubling time of 3-5 days, making it time-consuming and expensive to produce its biomass.

However, algal biofuel is one possible solution to the problems of sustainable energy supply and global warming. The history of cultivating and breeding microalgae is much shorter than for crop plants and it is at a primitive stage but it is open for improvements in the cultivation and biomass production using current genetic engineering and microalgal biotechnological tools and techniques.

Conclusion

It can be concluded that the green microalga Botryococcus species, in addition to producing hydrocarbons, are reducing the buildup of atmospheric carbon dioxide, which is a major driving force in global warming projections. This can be accomplished by reducing the use of fossil fuels, removing carbon dioxide from the atmosphere, and capturing or ut ilizing the carbon dioxide emitted by the combustion of fossil fuels before it enters the atmosphere.

Acknowledgments

Authors are thankful to the Management of Sunbeam College For Women, an autonomous NAAC accredited 'A' grade college, Bhagwanpur Varanasi, UP for providing reference books and e-library facilities.

References

- Aaronson S and Dubinsky Z (1982) Mass production of microalgae. Experientia **38** :36-40 Arif M, Bai Y, Usman M, Jalaloh M, Harraz FA, and Al -Assiri MS (2020) Highest accumulated microalgal lipids (polar and nonpolar) for biodiesel production with advanced wastewater treatment: role of lipidomics. Bioresour Technol **298** Article -122299.
- Areco M, Rojas M, Noseda D, Passucci V, Rotella N, and Curutchet G (2022) Effect of nitrogen source and nickel concentration on green microalga *Botryococcus braunii* growth and its remediation potential. J Appl Phycol pp1-14.
- Bachofen R (1982) The production of hydrocarbons by *Botryococcus braunii*. Experientia **38**: 47-49.
- Bailliez C, Largeau C, and Casadevall E (1985) Growth and hydrocarbon production of *Botryococcus braunii* immobilized in calcium alginate gel. Appl Microbiol Biotechnol **23**: 99-105
- Bailliez C, Largeau C, Casadevall E, Yang LW, and Berkaloff C (1988) Photosynthesis, growth and Hydrocarbon production of *Botryococcus braunii* immobilized by entrapment and adsorption in polyurethane foams. Appl Microbiol Biotechnol **29**: 141-147.
- Banerjee A, Sharma R, Chisti Y, and Banerjee UC (2002) *Botryococcus braunii*: A renewable source of hydrocarbon and other chemicals. Crit Rev Biotechnol **22**(3): 245-279.
- Barclay W, Wyman C, Lewin RA, and Cheng L (1988) Development of microalgal systems for the production of liquid fuels. In: Stadler T, Verdus MC, Karamanon Y, Morvan H and Christaen D (eds), Algal Biotechnology. Elsevier, Amsterdam, 55-64.

- Behrens PW, Bingham SC, Hoeksema SD, Cohoon DL, and Cox JC (1989) Studies on the incorporation of CO2 into starch by *Chlorella vulgaris*. J Appl Phycol **1**: 123-130.
- Ben- Amotz A and Avron M (1983) Accumulation of metabolites by halotolerant algae and its industrial potential. Annu Rev Microbiol **37**: 95-119.
- Ben- Amotz A, Tornabene TG, and Thomas WH (1985) Chemical profile of selected species of microalgae with emphasis on lipids. J Phycol **21**: 72-81.
- Borowitzka LJ, Borowitzka MA, and Moulton TP (1984) The mass culture of *Dunaliella salina* for fine chemicals: from lab to pilot plant. Hydrobiologia **116**: 115-121.
- Borowitzka MA′ (1988) Fats, Oils and Hydrocarbons: In Borowitzka MA and Borowitzka LJ (eds), Microalgal Biotechnology. Cambridge University Press, Cambridge. 257-387.
- Casadevall E, Dif D, Largeau C, Guddin D, Chaumont D and Desanti O ((1985) Studies on batch and continuous cultures of *Botryococcus braunii*: A hydrocarbon production in relation to physiological states, cell structure and phosphate nutrition. Biotechnol Bioeng. **27**: 286- 295.
- Cheng P, Wang J and Liu T (2014) Effects of nitrogen source and nitrogen supply model on the growth and accumulation of immobilized biofilm cultivation of *Botryococcus braunii*. Bioresour. Technol **166**: 527-533.
- Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv **25**: 294-306.
- Coleman LW, Rosen BH and Schwartzbach SD (1988) Environmental control of carbohydrate and lipid synthesis in Euglena. Plant Cell Physiol **29**: 423-432.
- Dohler G, Biermann I Zink J (1986) Impact of UV-B radiation on photosynthetic assimilation of 14C- bicarbonate and inorganic 15N- compounds by cyanobacteria. Z Naturforsch C, Biosci, **41**(4)**:** 426-432.
- Dubinsky Z, Berner T and Aaronson S (1978) Potential of large-scale algal culture for biomass and lipid production in arid lands. Biotech Bioeng Symp **8**: 51-68.
- Gelpi E, Oro Z, Schneider HJ and Bennett ED (1968) Olefins of high molecular weight in two microscopic algae. Science **161**: 700-702.

- Hader DP, Watanabe M and Furuya M (1986) Inhibition of the mortality in the cyanobacterium *Phormidium uncinatum* by solar and monochromatic ultraviolet irradiation. Plant Cell Physiol **27**: 887-894.

- Harrison PJ, Thomas PA, and Caderwood GS (1990) Effects of nutrients and light limitation on the biochemical composition of phytoplanktons. J Appl Phycol **2**: 45-56.
- Inui H, Miyatake K, Nakano Y, and Kitaoka S (1982) Wax ester fermentation in *Euglena gracilis* FEBS letters **150**: 89-93.
- Inui H, Ono K, Miyatake K, Nakano Y, and Kitaoka S (1987) Purification and characterization of pyruvate: NADP oxidoreductase in *Euglena gracilis*. J Biol Chem **262** :9130-9135.
- Kerby NW and Stewart EDP (1988) The biotechnology of microalgae and Cyanobacteria. In Rogers LJ and Gallon JR (eds), Biochemistry of Algae and Cyanobacteria. Proceed Phytochem Soc Europe, Clarendon Press, Oxford **28** :329-334.
- Kumar HD and Singh Y (1996) Lipid profile and Hydrocarbon production by *Botryococcus* species. Current Res Plant Sc **2**: 71-80.
- Lien S and Spencer KG (1983) Microalgal production of oils and lipids. In Class DL and Elliot HH (eds), Energy from Biomass and Wastes. Institute of Gas Technology Chicago,1107-1122.
- Metzger P and Casadevall E (1988) Chemical variability in the oil rich alga *Botryococcus braunii*. In Rogers LJ and Gallon JR (eds) Biochemistry of the Algae and Cyanobacteria. Proceed Phytochem Soc Europe, Clarendon Press, Oxford, 99-100.
- Metzger P, Allard B, Casadevall E, Berkaloff C and Coute A (1990) Structure and chemistry of a new chemical race of *Botryococcus braunii* that produces lycopadiene. J Appl Phycol **26**:258-266.
- Metzger P and Largeau C (2005) *Botryococcus braunii*: a rich source of hydrocarbons and related ether lipids. Appl Microbiol Biotechnol **66**: 486-496.
- Moldowan JM and Seifert WK (1980) First discovery of botryococcene in petroleum. J Chem Soc Chemical Communication **19**: 912-914.

- Nazloo EK, Moheimani NR and Ennaceri H (2023) Graphene based catalyst for biodiesel production: Characterization and performance. Science Total Environ 859: Article -160000.
- Nazloo EK, Danesh M, Sarrafzadeh MH, Moheimani NR and Ennaceri H (2024) Biomass and Hydrocarbon production from *Botryococcus braunii*: A review focusing on cultivation methods. Science Total Environ **926**: Article -171734.
- Newton JW,Tyler DD and Slodki ME (1979) Effect of UV- B radiation on blue-green algae. Appl Environ Microbiol **37**: 1133- 1141.
- Ohmori M, Wolf FR and Bassham JA (1984) *Botryococcus braunii* C/N metabolism as affected by ammonia addition. Archive Microbiol **140**: 101-106.
- Piorreck M and Pohl P (1984) Formation of biomass, total protein, chlorophylls, lipids and fatty acids in green and blue -green algae during one growth phase. Phytochemistry **23**: 217-223.
- Raghubanshi AS and Singh JS (1991) Increasing atmospheric abundance of radioactively active trace gases.: Causes and Consequences. Tropical Ecol. **32(1)**: 1-23.
- Shifrin NS and Chisholm SW (1981) Phytoplankton lipids: Environmental influence on production and possible commercial applications. In: Shelef G and Soeder CJ (eds), Algal Biomass. Elsevier/ North Holland Biochemical Press, Amsterdam, 627-645.
- Singh Y (2000) Studies on a hydrocarbon rich microalga, *Botryococcus* at different physiological states. J Indian Bot Soc **79**: 115-128.
- Singh Y (2001) Effects of increased solar ultraviolet -B radiation on growth and metabolism of green alga *Botryococcus*. Nature and Biosphere.6(1&2) :14-20.
- Singh Y (2003) Photosynthetic activity and lipid and hydrocarbon production by alginate immobilized cells of *Botryococcus* in relation to growth phase. J Microbiol Biotechnol **13(5)**: 687-691.
- Singh Y and Kumar HD (1992) Lipid and hydrocarbon production by *Botryococcus* species under nitrogen limitation and anaerobiosis. World J Microbiol Biotechnol **8**: 121-124.

- Singh Y and Kumar HD (1994) Growth of *Botryococcus* species in improved medium. Phykos **33(1,2**): 77-87.
- Tornabene TG (1982) Microorganisms as hydrocarbon producers. Experientia **38**:43-46.
- Tornabene TG, Holzer G, Lien S and Burris N (1883) Lipid composition of nitrogen starved green alga, *Neochloris oleoabundans*. Enzyme Microbiol Technol **5**: 435-440.
- USEPA (1987) An assessment of the effects of ultraviolet -B radiation on aquatic organisms. In: Assessing the Risks of Trace Gases that can Modify the Stratosphere. EPA400/1-87/00lC .**12**: 1-33.

- Wolf FR, Nemethy RK, Blanding JH and Bassham JA (1985) Studies on the biosynthesis of unusual acyclic isoprenoids in the alga, *Botryococcus braunii*. Phytochemistry **24**: 730-737.

◆