Molecular Characterization of Squalene Synthase Gene and Fatty Acid Methyl Ester Analysis from a Green Microalga *Botryococcus Braunii* Kutzting

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**Abstract:** The isolation and identification of microalgae were collected from different sampling sites. Among them one strain of *Botryococcus braunii* were selected and the growth kinetic analysis were carried out with BBM medium at room temperature under 12:12 light and dark conditions. The biochemical parameters includes dry biomass, carbohydrate, protein and lipid were analyzed and recorded. All the biochemical constituents of biomass were estimated in *B. braunii*. Extraction and analysis of Fatty Acid Methyl Esters (FAME) of *B. braunii* strains were carried out. Fourier Transform Infra-Red Spectroscopy (FT-IR) was studied in *B. braunii*. The presence of esters, alkanes and alkenes confirms the presence of hydrocarbons from the FAME. For the squalene synthase gene, the phylogenetic analysis were performed and confirmed by using ClustalW and Neighbor joining method in MEGA5 program. The phylogenetic results of the *B. braunii* using the selected genes of squalene synthase confirm the species *Botryococcus braunii*.

**Keywords:** *Botryococcus braunii*, Molecular characterization, Squalene synthase, Biochemical, FT-IR.

1. Introduction

The petroleum fuels are the only available energy source consumed enormously for transportation. There will be a huge price hike and demand for fuel because of its depletion and unsustainability of fossil fuels in the near future. For such conditions, alternate energy sources include exploration of fuel from the underground soil using markers; biodiesel, bioethanol and biomethane production from biomasses. The fossil fuels are the most intrinsic part of our day to day life but in recent decades the emission of greenhouse gases (GHG) has been elevated and led to global warming as an impact of combustion of fossil fuels. Macrocyclic alkanes have been identified in two tertiary crude oils known to contain remains of the fresh water alga *B. braunii*. These alkanes appear to be a novel biomarker for *B. braunii* and add to the catalogue of distinctive hydrocarbons derived from this alga (Audino *et al.*, 2002).

The *B. braunii* are densely-packed radiating cell colonies branching from the center of a roughly spherical unit. They are pyriform shaped usually 13 µm × 7µm in size imbibed in pale yellow to orange red oil like exudations. At the same time, the lipid accumulation vigourously altered led to the increase of unsaponifiable lipids up to 80% among dry mass (Brown *et al.*, 1969; Belcher, 1968; Gelpi *et al.*, 1968; Murray and Thomson, 1977; Knights *et al.*, 1970). The palmitic acid was ultimately present within the hydrocarbon biologically synthesized by *B. braunii* (Largeau *et al.*, 1980). The oleic acid was the potent precursor for the hydrocarbon production much accumulated by *B. braunii* (Templier *et al.*, 1984). The fatty acids are the key intermediates for the biosynthesis of hydrocarbons by carbon fixation from atmospheric air during photosynthesis. Both oleic acid and palmitic acid are the fundamental components of lipid and as a precursor for hydrocarbon accumulation in *B. braunii* (Yamaguchi *et al.*, 1987).

The Squalene synthase otherwise called as Farnesyl diphenylphosphate: Farnesyl diphenylphosphate Farnesyl transferase (EC: 2.5.1.21) which first initiates the catalyzation reaction of isoprenoid pathway towards the production of phytosterols, brassino steroids and triterpenoids (Abe *et al.*, 1993). As a membrane-bound enzyme, the squalene synthase condensates the
two molecules of farnesyl diphtosphosphate to produce a linear 30 carbon compound called squalene. The squalene synthase located with the smooth endoplasmic reticulum, with which the carboxyl terminal fixed to the membrane of endoplasmic reticulum and the amino terminal (catalytic site) found at the cytoplasm (Robinson et al., 1993). The micro alga *Botryococcus braunii* also possess three unique squalene synthase-like (SSL) proteins in addition to squalene synthase; they are SSL-1, SSL-2 and SSL-3 (Niehaus et al., 2011).

2. Materials and Methods

2.1. Sample collection

Fresh water samples were collected by random sampling method from Saidapet and Tirvanmiyur temple tanks, Chennai, Tamil Nadu, India.

Each sample was transported to the laboratory within 24 hours of collection and incubated in the full strength Bold Basal Medium at 22 ± 1˚C and constant light at 50 µmol photons m⁻² s⁻¹ under the photoperiod of 12:12 hour's light/Dark condition.

At this stage 25 mL culture in Erlenmeyer Flasks was shaken manually in a full strength medium where sub cultured for further analysis. Most samples were enriched by this way for dominant strains until the samples taken for serial dilution or streaking on to solid media used for strain isolation.

2.2. Growth kinetics of the microalga *Botryococcus braunii*

The green microalga *Botryococcus braunii* utilized in this research study was already collected, isolated and pure culture was maintained in the Algal culture collection laboratory, Department of Plant Biology and Plant Biotechnology, Presidency College (Autonomous), Chennai-600005.

The microalga *Botryococcus braunii* was allowed to grow in a Bold Basal Medium (BBM) liquid broth (400 ml in 1L Erlenmeyer flask with 4 % inoculum) and incubated for 16 days under 12:12 hrs of dark and light (Crompton 40 W, Cool day light 6500 K at an intensity of 2000 lux.) and dark respectively at room temperature (28 oC) for 16 days.

The optical absorbance values were analyzed for 16 days at 680nm in an UV-Vis. Spectrophotometer (Hitachi U-2900).

The Growth kinetics (K) and generation times (G) of all the microalga Botryococcus braunii was calculated by the following equations 1 and 2 (Qin, 2005).

\[
K = \frac{(\log OD_f - \log OD_i)}{T \times 3.322}
\]

Where, ODf : final optical density, ODi : initial optical density, T : time in days

Equation 2: Following equation was used for Calculation of generation time (G) in days:

\[
G = \frac{0.301}{K}
\]

2.3. In vitro mass cultivation

The microalga *Botryococcus braunii* were then subjected to *in vitro* mass cultivation in a 5L Erlenmeyer flask with 2L of BBM broth and incubated under 12:12 hrs of light illumination (Crompton 40 W, Cool day light 6500 K at an intensity of 2000 lux.) and dark respectively at room temperature (28°C) for 16 days.

2.4. Harvest and estimation of dry biomass

The biomass after *in vitro* mass cultivation was harvested by centrifugation at 8000 rpm for 5 min. at 20°C. The concentrated biomass in the pellet was then incubated for 24 hours at 35°C for overnight. The dry biomass was wiped using a sharp sterilized surgical blade and weighed gravimetrically using an electronic weighing balance.

2.5. Biochemical constituents

2.5.1. Estimation of total Chlorophyll a and Chlorophyll b

The green photosynthetic pigment Chlorophyll a and Chlorophyll b content of *B. braunii* were estimated by Jeffrey and Humphery (1975) method and its equation given below.

\[
\text{Chlorophyll a} = 11.93\times 10^{-6}E_{664} - 1.93\times 10^{-6}E_{647} \mu gml^{-1}
\]

\[
\text{Chlorophyll b} = 20.36\times 10^{-6}E_{647} - 5.50\times 10^{-6}E_{664} \mu gml^{-1}
\]

The absorbance values at 664 and 647 nm were recorded daily for about 16 days using a UV-Vis. Spectrophotometer.

2.5.2. Estimation of total carotene

The total carotene content of the green microalga *Botryococcus braunii* was determined everyday up to 16 days based on the formula is given below.

\[
\text{Carotene (µg/ml)} = A_{450} \times 25.2
\]

2.5.3. Extraction and estimation of carbohydrate

The *Botryococcus braunii* culture was centrifuged at 8000 rpm for 5 min. around 20°C and the obtained pellet with biomass was conserved.
Then the biomass was allowed to sonicate (Equitron Ultrasonic Cleaner) at 53 KHz for 20 min. at room temperature (28°C) to lyse the cells. The condensate sample was then centrifuged at 8000 rpm for 10 min. at 20°C; the obtained supernatant was used as a crude extract for the estimation of carbohydrate.

The total carbohydrate was determined based on the method well defined by Dubois et al. 1959 by Phenol Sulfuric acid method. The crude sample was treated with 5% phenol and Conc. Sulfuric acid. Then the whole content was incubated for 10-15 min. at room temperature. The optical absorbant values were measured at 490 nm using a UV-Vis. Spectrophotometer. The total carbohydrate content was determined by comparing the absorbance values with the standard graph.

2.5.4 Extraction and estimation of protein

About 400 ml of the *Botryococcus braunii* culture was centrifuged at 8000 rpm for 5 min. around 20°C and the obtained pellet with biomass was conserved. Then the biomass was allowed to sonicate (Equitron Ultrasonic Cleaner) at 53 KHz for 20 min. at room temperature (28°C) to lyse the cells. The condensate sample was then centrifuged at 8000 rpm for 10 min. at 20°C; the obtained supernatant was used as a crude extract for the estimation of protein.

The estimation of protein in this present study was done based on the method described by Bradford (1976). The protein content of the unknown sample was determined in comparison with the standard graph of the known sample. The standard graph for protein was constructed using Bovine serum albumin as a standard known protein. About 1 ml of the crude sample used to determine the total protein content with 5 ml of the Bradford reagent. After incubation for 15 min. the content was subjected to determine the absorbance values at 595 nm in a UV-Vis. Spectrophotometer.

2.5.5. Extraction and estimation of lipid

The microalga biomass was harvested by centrifuging at 8000 rpm for 5 min. The resulted pellet was well homogenized and sonicated at 53 KHz for 10 min. at room temperature. The total lipid constituent was determined based on the method described by Folch et al. in 1957. A 6 ml of chloroform and methanol (2:1 ratio) was mixed with the extract and vortexed. The extract was then centrifuged at 8000 rpm for 5 min. The resultant liquid phase was washed with 0.9% of NaCl and vortexed for 5 min. The entire content was then centrifuged at 2000 rpm for 5 min. which resulted to form two distinct phases. The upper phase conserved due to its richness in lipid and the lower aqueous phase was discarded. The total lipid content was determined gravimetrically after the complete evaporation of the solvent (Upper phase).

2.6. Extraction and analysis of Fatty Acid Methyl Esters (FAME) by Gas Chromatography and Mass Spectroscopy

The aqueous microalga sample was subjected to direct transesterification. Reagent 1 was poured to the fresh algal samples and vortexed. The mixture was incubated in a water bath at 100°C for 5 min. Reagent 2 was added and vortexed for 5-10 min. followed by thermal incubation at 80°C and rapidly cooled down to 4°C. Reagent 3 was added the mixture and mixed gently up to 10 min. Two phases were seen blatantly from which, the lower phase was conserved. To the content 3 ml of reagent 4 was added followed by mixing for 5 min. and obtained upper phase was removed. The lower phase rich in fatty acid methyl esters were stored in a vial at 4°C in a refrigerator.

The tranesterified Fatty Acid Methyl Esters were analyzed with the help of a gas chromatography equipped with flame ionization detector (FID) (Perkin Elmer, USA). A SP-2560 column (100 m × 0.25 mm I. D., 0.20 µm) (Sigma, Germany) along with standard fatty acid Supelco 37 Component FAME mix from Supelco (Bellefonte, PA, USA) was employed. About 5 µl of the sample was injected and the GC conditions were injector temperature: 260°C; Column temperature: 140°C and detector temperature: 260°C. Helium was used as a carrier gas with the flow rate of 1 ml/min. The unknown FAME’s were determined in comparison with the retention times of the standard FAME’s (Supelco) using a mass spectra from NIST library.

2.7. Fourier transforms infra-red spectrometric (FT-IR) analysis of FAME

The FAME sample was analyzed in infra-red spectrometer (Perkin Elmer model spectrum – 1 PC). The FT-IR spectra with the resolution of 4 cm⁻¹, Scan Number: 3 were performed after the evaporation of the lipid fraction on Thalium bromide tablets. The FT-IR spectrums of all the FAME samples were obtained as a percentage of transmission ranged from 450 cm⁻¹ to 4000 cm⁻¹.
2.8. Molecular identification of *Botryococcus braunii*

2.8.1. Isolation of RNA

About 300 µl of the culture was ground into fine powder in liquid nitrogen using a mortar and pestle. A 2 ml of solution I was added while grinding to make homogenous mixture and allowed to thaw completely with intermittent grinding. Then 800 µl of nuclease free water was added by mixing and grinding. The whole content was transferred to two 2 ml microcentrifuge tubes and kept undisturbed for 5 min. at room temperature. Chloroform (200 µl) was added to each tube and vortexed briefly for less than 10 seconds and kept undisturbed for 10 min. at room temperature. Then the whole mixture was allowed to centrifuge for 10 min. at 4°C and the upper aqueous phase was transferred into to fresh microcentrifuge tubes. Then about 0.6 volumes of isopropanol were added and vortexed briefly less than 10 seconds and left undisturbed for 10 min. at room temperature. Then after centrifugation at 13,000 rpm for 10 min. at 4°C the supernatant was discarded and the obtained pellet with RNA was washed with 70 % ethanol, air dried and dissolved in 50 µl of DEPC-treated water. The extracted RNA was separated and analyzed using 1.5 % Agarose in submarine gel electrophoresis.

Solution I: phenol saturated with tris (hydroxymethyl) aminomethane buffer to a pH of 6.7 ± 0.2. To this sodium dodecyl sulphate [SDS; 0.1% (w/v)], sodium acetate [NaOAc; 0.32 M (w/v)] and ethylenediaminetetra acetic acid (EDTA; 0.01 M final concentration from a stock solution of 0.5 M, pH 8.0) were added.

2.8.2. cDNA synthesis

The first strand of cDNA was synthesized using 3 µl of RNA sample with SMART IV oligonucleotide and CDS/3’ PCR primer 1 µl each. After thorough mixing the content was incubated at 72°C for 2 min. and cooled on ice for 2 min. After spinning briefly, 5X first strand buffer (2 µl), DTT (20mM) (1µl), dNTP mix (10mM) (1µl) and Power script RT (1µl) were added and mixed. After incubation for 1 hr at 42°C, the content was stored at -80°C in an ice.

2.8.3. PCR amplification

A PCR was performed in a total volume of 30 µl containing 15 µl master mixture, 1 µl of µM each of primer and 1 µl of cDNA template. PCR conditions were as follows: denaturation at 94°C for 5 min; 35 cycles of 94°C for 1 min, primer -specific annealing temperature at 45 sec and extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The PCR products were resolved by electrophoresis in a 1% agarose gel in 1 × TAE buffer. The gels were pre-stained with 10 mg/ml ethidium bromide.

### TABLE 1 : Primer details used for amplification

<table>
<thead>
<tr>
<th>Gene (Accession no)</th>
<th>Primer sequence (5'-3')</th>
<th>Annealing temperature°C</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squalene synthase-like 3</td>
<td>F:TCGGGAAGTCTTGCAGCACC</td>
<td>54.9</td>
<td>250</td>
</tr>
<tr>
<td>(HQ585060)</td>
<td>R:AAGCACCCTTAGCTGAAACCTTT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.8.4. Gene sequence and submission to NCBI GenBank

Amplified PCR product purified using Qiaquick PCR purification kit (QIAGEN, USA). Sequencing reactions were carried out in both directions using same forward and reverse primers used for amplification with BigDye Version 3.1 kit (Applied Bio-systems) on an ABI-PRISM 3730 DNA Sequencer (Applied Bio-systems). Ambiguous sequences from the base called sequences were corrected with Chromas (Version 2.01) and the sequences were assembled with Bio-Edit (Version 7.0.9.0). The search for sequence homolog of potential gene product was made using the BLASTn and BLASTx program (NCBI).

2.8.5. Phylogenetic Analysis

Deduced amino acid sequence of respective protein from other sources was retrieved from NCBI database and alignment was performed using ClustalW. Phylogenetic tree analysis of Squalene synthase, SSL1, SSL2 and SSL3 protein sequence with other related sequences was performed using neighbour-joining method in MEGA 5 program (Tamura et al., 2007).

3. Results

3.1. Sampling sites

The abiotic factors of fresh water samples were collected by random sampling method from Saidapet and Tirvannmiyur temple tanks Chennai. Each samples were transported to the laboratory...
within 24 hours and incubated in the Bold Basal Medium at 22 ± 1°C and constant light at 50 µmol photons m⁻² s⁻¹ under the photoperiod of 12:12 hour’s light/Dark condition. The major algae present in collection site was identified as *Botryococcus braunii* (Fig.1).

![Fig. 1. Botryococcus braunii strain](image)

### 3.2. Growth kinetics and generation time

The log phase was started from the third day of *in vitro* cultivation and there is an increase in the stationary phase up to sixteenth day (Fig. 2). The death stage was not seen but the deposition of biomass was obvious. The growth kinetic (K) was 0.099 and the generation time (G) was 3.02.

![Fig. 2: Growth curve of the green microalga Botryococcus braunii](image)

### 3.3. Estimation of pigments

The green photosynthetic pigments Chlorophyll a and b were comparatively low with carotene. Chlorophyll a and b were 6.62 µg ml⁻¹ and 9.79 µg ml⁻¹ respectively. The carotene content was about 16.95 µg ml⁻¹. The pigments were produced high during the proliferation of the microalga *Botryococcus braunii* and increase in the production of the pigments were occurred at the last three days of culture (Fig. 3).

![Fig. 3: The production of the three important pigments by Botryococcus braunii](image)
3.4. Biochemical constituents

The harvested and dried biomass of the microalga *Botryococcus braunii* was about 243 mg ml\(^{-1}\). The total protein content was very low with 12.68 mg ml\(^{-1}\) followed by total carbohydrate and total lipid content with 63.05 mg ml\(^{-1}\) and 69.29 mg ml\(^{-1}\) respectively. The protein content was found very low with only 8.74 %. The lipid content was produced high with 47.77 % of the total biochemical constituents. The total carbohydrate content was ranked next to the lipid content with 43.47 % (Fig. 4). The lipid content was found high and the carbohydrate content was found approximately equal to the lipid content but the protein content was ultimately very low.

![Fig. 4: Biochemical constituents of Botryococcus braunii](image)

3.5. Analysis of fatty acid methyl esters (FAME) by gas chromatography and mass spectrometry (GC-MS)

The total fatty acid methyl esters extracted from the microalga *Botryococcus braunii* was about 78.17 % (Fig. 5) which constitutes Thioacetic acid C27:0 (12.34 %), 9-Hexadecenoic acid C34:0 (9.05 %), n-Hexadecanoic acid C16:0 (17.69 %), 16-Octadecenoic acid C19:0 (22.63 %), 9- Octadecenoic acid C21:0 (16.46 %). The occurrence of high cetane number compounds in the FAME of *Botryococcus braunii* confirms the presence of long chain fatty acids and hydrocarbons.

![Fig. 5: GC chromatogram of Botryococcus braunii](image)
3.6. Fourier transforms infra-red spectrometric (FT-IR) analysis of FAME

More number of alkane groups (C-H and =C-H) were reported from the FAME of *Botryococcus braunii* where 2972, 2932, 2884, 2658, 1466, 1380 and 649 cm\(^{-1}\). The alkene groups (=C-H and C-H) were obviously clear by the peak at 952, 817 and 777 cm\(^{-1}\) (Table 2) (Fig. 6). The esters (C-O) were obtained based on the peaks at 1129 and 1001 cm\(^{-1}\). The presence of alkanes and alkenes virtually confirms the occurrence of long chain hydrocarbons produced by the microalga *Botryococcus braunii*.

### Table 2: FT-IR of Functional Groups of Constituents from *Botryococcus braunii*

<table>
<thead>
<tr>
<th>Peaks</th>
<th>Bond</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3360 cm(^{-1})</td>
<td>N-H</td>
<td>1°, 2° amines, amides</td>
</tr>
<tr>
<td>2972, 2932, 2884,2658 cm(^{-1})</td>
<td>C-H</td>
<td>Alkanes</td>
</tr>
<tr>
<td>1648 cm(^{-1})</td>
<td>N-H</td>
<td>1 amines</td>
</tr>
<tr>
<td>1466, 1380 cm(^{-1})</td>
<td>C-H</td>
<td>Alkanes</td>
</tr>
<tr>
<td>1341 cm(^{-1})</td>
<td>N-O</td>
<td>Nitro compounds</td>
</tr>
<tr>
<td>1306 cm(^{-1})</td>
<td>C-N</td>
<td>Aromatic amines</td>
</tr>
<tr>
<td>1161 cm(^{-1})</td>
<td>C-H -CH(_2)X</td>
<td>Alkyl halides</td>
</tr>
<tr>
<td>1129, 1001 cm(^{-1})</td>
<td>C-O</td>
<td>Alcohols carboxylic acids, esters, ethers</td>
</tr>
<tr>
<td>952 cm(^{-1})</td>
<td>=C-H</td>
<td>Alkenes</td>
</tr>
<tr>
<td>817, 777 cm(^{-1})</td>
<td>C-H</td>
<td>Alkenes</td>
</tr>
<tr>
<td>649 cm(^{-1})</td>
<td>=C-H</td>
<td>Alkenes</td>
</tr>
<tr>
<td>491 cm(^{-1})</td>
<td>C-Br</td>
<td>Alkyl halides</td>
</tr>
</tbody>
</table>

3.7. Molecular identification

#### 3.7.1. Isolation of RNA and cDNA synthesis

The agarose gel image showing the separated total RNA as two different bands where 28S and 18 S rRNA was found along with all the RNAs (Fig. 7). The complimentary cDNA was synthesized for the gene Squalene synthase (SQS) and were qualitatively determined by 1.5 % agarose gel electrophoresis. The single band of the gel image resulted the cDNA which was then subjected to sequence based on Sanger’s dideoxy sequencing (Fig. 8). The sequenced SQS gene was submitted to NCBI through BankIt, GenBank and the temporary BankIt
submission number was retrieved (BankIt submission no. 1897235) and the accession number yet to be received.

**Fig. 7**: The bands in the agarose gel image showing the extracted RNA from *B. braunii*.

**Fig. 8**: The single band in lane 1 shows the cDNA of the gene squalene synthase (SQS) of *B. braunii*.

### 3.7.2. Phylogenetic analysis

The phylogenetic tree represents the evolutionary relationship of the gene squalene synthase (SQS) of *Botryococcus braunii* with other organism. The *Botryococcus braunii* strain SP-1 was found in a separate clade but near to *Botryococcus braunii* Q9SDW9 and the last three clades illustrates the green microalgae (Fig. 9). One more dendrogram was constructed by NCBI BLAST Pair wise alignment of Neighbour joining method with maximum sequence difference of 0.005. In which, the clade (1Cl|Query_82295) was found in a separate clade which was found nearer to the other *Botryococcus braunii* and confirms molecularly that the gene is squalene synthase (SQS) and the strain belongs to *Botryococcus braunii* (Fig. 10).

![Phylogenetic tree](image)

**Fig. 9**: Evolutionary relationships of Squalene synthase (SQS) obtained from *Botryococcus braunii*
4. Discussion

*Botryococcus braunii* is a green colonial microalga widespread in freshwater and brackish lakes, reservoirs, ponds, or even ephemeral lakes situated in continental, temperate, alpine, or tropical zones (Wake and Hillen 1980, 1981; Aaronson *et al.*, 1983; Huszar and Reynolds 1997; Huang *et al.*, 1999; Metzger and Largeau 1999; Volova *et al.*, 2003). This alga is characterized by a conspicuous ability to synthesize and accumulate a variety of lipids. These lipid substances include numerous hydrocarbons, i.e. highly reduced compounds comprising only carbon and hydrogen as elements (Brown and Knights 1969; Knights *et al.*, 1970), and a number of specific ether lipids (Metzger *et al.*, 1993; Metzger and Largeau 1999). An important feature of this alga is the production of numerous ether lipids of a new type which are not glycerol derivatives like those occurring in all other living organisms.

In each race, ether lipids are closely related to hydrocarbons and in some strains their production can be largely dominant. Lastly, non-polysaccharide biopolymers of very high molecular weight (104 Da to 4×106 Da), polyaldehydes and polyacetals have been isolated from lipid extracts of *B. braunii*. Their occurrence and possible functions as precursors of the insoluble polymeric material building up the outer walls of the alga were recently reviewed by Metzger and Largeau (2002). The recognition of biological markers in ancient and recent sediments provides valuable information regarding the origin of deposited organic matter (Seifert and Moldowan, 1981). In this study growth kinetics of *B. braunii* was showed good result at the log phase was started from the third day of in vitro cultivation and there is an increase in the stationary phase up to sixteenth day. The death stage was not seen but the deposition of biomass was blatantly obvious. The growth kinetic (K) was 0.099 and the generation time (G) was 3.02.

In recent years, interest in the use of green alga as a source of biofuels has increased due to the need to reduce green house gas emissions and because of depletion of world petroleum reserves (Hu *et al.*, 2008). For algae to produce enough oil to meet fuel demands, large scale culturing of algae and monitoring of oil production will be required (Chisti, 2007, 2008). Current analysis methods for monitoring algal oil production are complicated, time consuming and destructive (Huang., 2010). In this investigation showed the biochemical constituents such as the total protein content was very low with 12.68 mg m⁻¹ followed by total carbohydrate and total lipid content with 63.05 mg ml⁻¹ and 69.29 mg
ml¹ respectively. The protein content was found very low with only 8.74 %. The lipid content was produced high with 47.77 % of the total biochemical constituents. The total carbohydrate content was ranked next to the lipid content with 43.47 %. The lipid content was found high and the carbohydrate content was found approximately equal to the lipid content but the protein content was ultimately very low.

A common feature of the fatty acid distributions in sediments is the presence of C₂₀-C₃₀ saturated straight-chain fatty acids that show a strong predominance of even chain-lengths. In many types of sediment, particularly those from lacustrine environments, these are probably derived from the surface waxes of higher plants (Eglinton and Hamilton, 1967). However an increasing body of analytical data suggests that microalgae (Rezanka and Podojil, 1986; Dunstan et al., 1992) and perhaps bacteria (Volkman, 1988) can also produce these fatty acids albeit in small amounts (typically <2%) relative to C₁₄-C₂₀ fatty acids. These long-chain fatty acids are minor constituents in the gas chromatogram of fatty acids from Vischeria punctuate. Recent data for Scenedesmus communis suggest that the C₂₈ fatty acid in this green alga may play a role in the formation of the aliphatic biopolymer in its cell wall (Schouten et al., 1998), although this does not preclude other roles within cell.

Three distinct races of B. braunii have been described based on the types of hydrocarbons that each accumulates (Metzger and Largeau, 2005). Race A accumulates fatty acid-derived alkadienes and alkatrienes (Gelpi et al., 1968), race L accumulates the tetraterpene lycypadiene (Metzger et al., 1990), and race B accumulates triterpenes, predominately botryococcene, squalene, and their methylated derivatives (Okada et al., 1995). The oils accumulate both in intracellular oil bodies and in association with an extracellular matrix (Weiss et al., 2010), which in race B consists largely of long-chain, cross-linked biopolymers formed in part from acetalization of polymethyl squalene diols (Metzger et al., 2007). Di- and tetra-methylated botryococcenes are generally the most abundant triterpenes accumulating in race B with smaller amounts of tetramethylated squalene (Huang and Poulter, 1989) and other structural derivatives of squalene and botryococcene that range from C₃₁ to C₃₇ accumulating to various levels in different strains and in response to variable culture conditions (Metzger et al., 1985).

Squalene biosynthesis has been extensively investigated because it is positioned at a putative branch point in the isoprenoid biosynthetic pathway directing carbon flux to sterol metabolism, and thus represents a potential control point for cholesterol biosynthesis in man (Bergstrom et al., 1993). Evidence for a two-step reaction mechanism catalyzed by squalene synthase has been described (Poulter, 1990).

The Botryococcus braunii strain SP-1 was found in a separate clade but near to Botryococcus braunii Q9SDW9 and the last three clades illustrates the green microalgae. One more dendrogram was constructed by NCBI BLAST Pair wise alignment of Neighbour joining method with maximum sequence difference of 0.005. In which, the clade (1Cl|Query_82295) was found in a separate clade which was found nearer to the other Botryococcus braunii and confirms molecularly that the gene is squalene synthase (SQS) and the strain belongs to Botryococcus braunii. The amino acids were retrieved from the squalene synthase (SQS) gene of Botryococcus braunii and its three dimensional molecular structure was predicted. Among the basic two different secondary structures of the protein, helical structures were seen and the β-pleated sheet was absent. The superimposed study on the squalene synthase protein of Botryococcus braunii with human squalene synthase recorded that the two molecular structures resemble similar with slight conformational differences.

Extensive investigations of squalene synthase including site direct mutagenesis (Gu et al., 1998) and structural elucidation of 3-dimensional structure (Pandit and Yousuf, 2002) have focused on five highly conserved domains (domains I–V) thought associated with catalysis (Robinson et al., 1993). Okada et al. (2000) described the functional characterization of a squalene synthase gene from B. braunii race B. In that work, degenerate oligonucleotide primers complementary to several of the conserved domains were used to amplify a small region of a putative squalene synthase gene, and that gene fragment was then used to isolate a full length cDNA from a cDNA library.

5. Conclusion

The biochemicals derived from biological materials as depositions, could be used as indicator (Biological and Biochemical marker) for new and essential compounds and oil. The final conclusion is
oil exploration could be carried out by using Botryococcus braunii microalgae as a biological as well as biochemical marker. This may be indigenous, cost effective, ecofriendly, efficient and intensive method for the oil production.

References


