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Original Research Article

Assessing the environmental impact and sustainability of third-generation biofuels from microalgae via HPLC and GC-MS techniques

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Abstract

Background: The issue of fossil fuel depletion has forced us on a quest for modern and eco-friendly alternatives. Microalgal biofuels have managed to move on top for such reasons as, for example, carbon neutrality and lipid production without causing any harm to crop, which has attracted extensive interest to them.

Aim and Objective: This study is done to estimate the lipid production potential of a few microalgal strains at different environmental conditions that are suitable for biofuel applications. It also aims to find out which fatty acids are the significant ones to be used as biofuel and eventually to select the microalgal strain that is the best candidate for biofuel production.

Materials and Methods: The lipid production of four types of microalgae (*Chlorella* sp., *Scenedesmus* sp., *Chlorococcum* sp., and *Botryococcus* sp.) was augmented by cultivation under several environmental conditions.

Results: The highest lipid content of 25°C was identified by *Botryococcus* sp., while *Chlorella* sp. reached 22.8% at 35°C. GC-MS analysis showed that the highest fat content was in the *Chlorococcum* sp. and *Botryococcus* sp. species, with palmitic, linolenic, and stearic acids being the main fatty acids, which pointed out the fact that *Botryococcus* sp. should be the first one enchaining the biofuel production.

Conclusion: Large-scale lipid production in micro- and algae could be tackled by optimization of environmental conditions. This could potentially lead to a more sustainable biofuel industry, the most promising seed of which is *Botryococcus* sp.

Keywords: Biofuel production, Environmental conditions, Lipid optimization, Microalgae.

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1. Introduction

At the present period, global energy demand is significantly increasing, and this has been making a big problem. This crisis is almost wholly caused by the release of what is commonly referred to as "greenhouse gases (GHGs)" from the burning of "fossil fuels".[1] The use of energy carriers contributes to the excessive releasing of CO2 and other pollutants, hence polluting the environment.[2] Annual reports of the WEO of 2019 reveal that while CO2 emission has increased by 0.5%, overall world primary power consumption has figured out to rise by 1.3%.[3] However, most of the global power demands are pertained with finite resources like non-renewable sources, which are depleted in the near future at the present rate of use.[4] The exhaustion of fossil resources, commercial evolution, worldwide reliance on energy, and increase in fuel costs has resulted in energy crisis.[5] Moreover, enhanced fuel ingestion and ineffective use have aggravated the situation.[6] Crisis has now evolved into an environmental crisis around the globe.[6]

As a result, the use of fossil fuels has brought a shortage inenergy crisis, pollution and environmental deterioration.[7] Therefore, people are looking for other types of energy, for instance bioenergy that is basically a type of renewable energy. Different kinds of biofuels in the industries include 1 biomolecular biofuel and 2 advanced biofuels. The biofuels can be of first second third and fourth generation based on the raw materials that are used in the production of the biofuels.

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The first-generation biofuel is derived from biomass obtained from food products the second-generation biofuel is gotten from lignocellulosic biomass.[8] The 3rd generation of renewable resources includes algal biomass, and its capacity of renewal is within the possibilities. Instead, the fourth generation uses genetically modified (GM) algal biomass. The use of biofuel production at the global level was at 127.7 billion liters in 2014 and 201 billion liters in 2020. That is why, in bolstering competitiveness special emphasis should be placed on such factors as, for instance, the output rate and quality of the product, supply chain issues, cost at the onset of production as well as stock keeping and market acceptability. Among the available biofuels Algal based biofuels are accounted as the option for cleaner fuels, with market share projection upto 2050 of 5000 billion dollars. Algae has the potential to be environmentally friendly and can help decrease GHG emissions.[9,10] If it is developed as an integrated bio refinery, it might also have a beneficial economic effect. To ensure sustainability and economic viability, it is necessary to address significant challenges in both the upstream and downstream processes. The aim of the study is to optimize the production of lipids in four selected microalgal strains "*Chlorella* sp., *Scenedesmus* sp., *Chlorococcum* sp., and *Botryococcus*" under varying environmental conditions (temp, light intensity, and pH) to assess their potential for sustainable biofuel production**.** The research is focused on investigating the impact of deviating temperature from 25°C to 35°C with a gap of 5°C on lipid synthesis in certain microbial strains. This study aims to examine the effect of different light intensities (3, 4, and 5 lux) on lipid accumulation in "*Chlorella* sp., *Scenedesmus* sp., *Chlorococcum* sp., and *Botryococcus*sp".[11,12]

2. Materials and Methods

2.1. Microbial strain isolation

Four algal strains were obtained from the algal culture collection of the Department of Microbiology, Faculty of Science, Chaudhary Charan Singh University, Meerut City, Uttar Pradesh, Pin – 250 001. Provides a systematic arrangement of data, allowing for easy reference to each strain's details, including its unique strain number, taxonomic description, and the source from which it originated.

Strain No.	Taxonomic Description	Origin/ Source
AC1	*Chlorella* sp.	
AC2	*Botryococcus* sp.	Department of Microbiology, CCSU
AC3	*Chlorococcum* sp.	
AC4	*Scenedesmus* sp.	

2.2. Growth and maintenance of microalgae

The algal strains were cultured in BG-11 Medium, which was chemically described and adjusted following Stanier's specifications (1971). The light intensity ranged from 52-55 micromole photon meter square per second, and the at 28 ± 2 degrees Celsius temperature was maintained. **Table 1** that the pH of the medium was maintained between 7.5 and 7.5 to optimize culture growth. For culture maintenance, a "solid agar-based medium was prepared by dissolving" twelve to fifteen grams of agar in one liter of media with a concentration of 1.2 to 1.5 percent, followed by autoclaving. Cultures were streaked on Agar based BG – 11 Medium after fourteen days of incubation period for independent colonies under standard condition for cultural. These colonies were then transferred to flasks with 50mililiter of BG-11 Medium for a period of 14 days. The strains under investigation exhibited a range of morphological traits, and their identities were confirmed using identification keys provided by Desikachary.[13]

2.3. Morphology and morphometry

Morphological studies were observed on the strains collected from the exponential growth phase. A minimum of thirty measurements were conducted on each morphological characteristic using a light microscope on fresh material. The micrographs were captured with the digital compact camera. By using Imagepro + program (version 4.5). The growth pattern and colour of the thallus for all strains were consistently seen in both solid and liquid medium until the exponential phase, which occurred on the 14th day of incubation.

2.4. Materials required

A homogenized microalgal suspension involves for several reagents, such as 0.2N of $HClO4$ in a 2:1 v/v ratio, nitrogen gas for evaporation, water, chloroform, methanol, potassium dichromate solution, standard palmitic acid, BG-11 medium for microalgae cultivation, and bligh and dyer lipid extraction reagent. Proportions of $H2SO4$ and $C2H5Cl3O$.

2.5. Lipid production in different cultural conditions

For the purpose of studying how to optimize culture conditions for increased lipid synthesis, four genera of microalgae—*Chlorella*, *Botryococcus*, *Chlorococcum*, and *Scenedesmus*—were chosen. Over the course of 28 days, these cultures were exposed to variable climatic and cultural conditions in culture room while being cultivated on BG-11 culture medium.[14] The variables studied included variations in "temperature (25-, 30- and 35-degree Celsisus), light intensity (3, 4 and 5 lux), and pH (6.0, 6.5 and 7.3)".

2.6. Lipids sampling and their processing

The dry weight concentration of total lipids was measured using a homogenized microalgal solution, with palmitic acid serving as the reference. The determination of dry weight was conducted by utilizing a specified volume suspension of microalgal according to an established approach.[15] Each experiment consisted of three replications, and the data collected were subjected to statistical analysis.[16] Lipids obtained from three specific taxa were analysed for composition of their fatty acid, and methyl esters were synthesized.[17]

2.7. Lipid purification

After 14 days of incubation, a "homogenized microalgal suspension was centrifuged at 3000 RPM for 15 minutes." The pellets were mixed with cold perchloric acid, incubated at 4ºC for 15 minutes, and then centrifuged at 7000 RPM. A 25 mL chloroform-methanol (2:1) solution was added, mixed, and left for 5 minutes at room temperature, then centrifuged at 7000 RPM. The supernatant was collected, water added and centrifuged at 4000 RPM. The lower organic phase evaporated to 2 mL under nitrogen. After evaporating 0.5 mL of the sample, adding potassium dichromate, and heating the mixture in a water bath set to boil for 45 minutes, the process was continued. Absorbance was recorded at 350 nm, and "lipid content was quantified" as mg/g of dry weight.[18]

2.8. Lipid characterization

2.8.1. High performance liquid chromatography (HPLC)

The lipids in selected microalgal strains were analyzed and characterized using HPLC after extraction via Bligh and Dyer method and conversion to Fatty Acid Methyl Esters (FAME) through trans-esterification. HPLC analysis was conducted with a C18 reverse-phase column for effective lipid separation. FAMEs were separated under isocratic conditions using a methanol: water solvent system, and lipid components were identified via a UV detector at a specific wavelength. Retention times were compared with lipid standards, and lipid purity was assessed by the area under the chromatographic peaks. This method enabled accurate quantification of major lipids relevant for biofuel production.

2.8.2. Gas liquid chromatography (GLC)

GLC was used to analyse the fatty acid distribution in lipids isolated from four microalgal strains. Lipids Dyer and Bligh method was used for lipid extraction, converted to FAMEs via transesterification, and injected into a gas chromatograph with a capillary column. The column separated the fatty acids based on volatility and molecular weight, with a flame ionization detector identifying the compounds. With reference standards to determine the fatty acid composition of each strain the retention times of the peaks were compared, providing valuable information for biofuel production.

3. Results

3.1. Lipid production in different conditions

Present findings suggest the comparative scarcity of lipids in the kingdoms of algae subject to differing temperatures. For example, *Botryococcus* sp. had the most profound accumulation of lipids by achieving a 36.1% reading at 25°C. Still, this was gradually reduced to 26.2% at 30°C and 23.8% at 35°C. As the matter of lipid accumulation was the second in the same topic of *Chlorella* sp., the writer noted the decrease of 19.8%, 17.4%, and 22.8% at 25°C, 30°C, and 35°C, respectively. Firstly, in the case of *Chlorococcum* sp., it was the different way when it came to the fluctuation of lipid content, as it was a rise from 21.9% at 25°C up to 27.9% at 30°C first, and then it went down to 20.4% at 35°C. Meanwhile, *Scenedesmus* sp. has shown the most stable lipid content; the numbers to represent them were 19.6%, 21.9%, and 22.3%; consequently, the temperature changes were negligible.

From the efforts of this experiment, the role of the temperature factor in lipid biosynthesis of the algae was clearly shown to be the most influential in the case of *Botryococcus* sp. with maximum lipid occurrence of 25°C yet at 30°C. There was a sharp decrease in the amount of lipids. This implies that an increase in temperatures might lead to an enhancement of the cellular energy requirement, which in turn affects the biosynthesis efficiency of lipids. According to the results of the experiment, lipid content and light were the factors that rated the growth of algae on a scale where the *Botryococcus* sp. was on top of that. The next in place was *Chlorococcum* sp. *Scenedesmus* sp. and *Chlorella* sp. instead were found to have a lower lipid accumulation.

The greatest amount of lipid content regarding dry weight was detected at a 4-lux lighting condition, while it was comparatively lower at 3 and 5 lux. To be more specific, the lipid amounts at the three light intensities (3, 4, and 5 lux) were as follows: *Botryococcus* species had a content of 14.5%, 22.8%, and 21.4% at the three different intensities; *Chlorococcum* species recorded a content value of 15.3%, 24.2%, and 21.4% at the light intensities of 3, 4, and 5 lux; *Chlorella* species, on the other hand, indicated a content of 21.0%, 21.4%, and 19.8% at the lighting intensities of 3, 4, and 5 lux; lastly, *Scenedesmus* species were found to have a content of 14.7%.(**Table 2**)

Within the incubation medium, three pH levels (6.0, 6.5, and 7.3) were evaluated. The highest lipid content was observed at a 7.3 (24.8%) pH compared to the 6 and 6.5 (21% and 22.4%) pH. Different genera employed in the current work exhibited various patterns of lipid behaviour.(**Table 3**)

Table 1: Different incubation temperatures (oC) impact on the overall lipid content in certain microalgal species

Strains		***Chlorella* sp.**	***Botryococcus* sp.**	***Chlorococcum* sp.**	***Scenedesmus* sp.**
25	**mg/g**	198	361	219	196
	% lipid	19.8	36.1	21.9	19.6
30	**mg/g**	174	262	279	219
	% lipid	17.4	26.2	27.9	21.9
35	**mg/g**	228	238	204	223
	% lipid	22.8	23.8	20.4	22.3

Table 2: Investigation of the impact of different light intensities (lux) on the overall lipid content in certain microalgal species

Strains		***Chlorella* sp.**	***Botryococcus* sp.**	***Chlorococcum* sp.**	***Scenedesmus* sp.**
3	**mg/g**	153	228	145	147
	% lipid	15.3	22.8	14.5	14.7
4	**mg/g**	210	242	214	201
	% lipid	21	24.2	21.4	20.1
5	**mg/g**	198	214	210	187
	% lipid	19.8	21.4	21	18.7

Table 3: Investigation of the impact of different pH levels on the overall lipid content in certain microalgal species

Strains		***Chlorella* sp.**	***Botryococcus* sp.**	***Chlorococcum* sp.**	***Scenedesmus* sp.**
6	**mg/g**	178	224	189	188
	% lipid	17.8	22.4	18.9	18.8
6.5	**mg/g**	182	210	206	201
	% lipid	18.2	21	20.6	20.1
7.3	**mg/g**	208	248	211	212
	% lipid	20.8	24.8	21.1	21.2

Table 4: *Botrycoccus species* fatty acid profile

S. No.	**Rentiontime (min.)**	**Chemical name**	**Area (%)**
1.	3.472	n-Decane	2.24
2.	4.301	7 – Methyl- heptadecane	1.67
3	4.793	4-Methyl- undecane	7.83
4.	6.312	n-Dodecane	5.72
5.	6.122	2-Methyl decane	2.43
6.	7.248	Oxalic acid	4.26
7.	7.632	n-Tridecane	6.47
8.	8.513	3-Methyl dodecane	1.63
9.	8.696	2,6,10- trimethyl dodecane (Farnesane)	1.79
10.	9.011	n-Tetradecane	4.94
11.	15.270	Methyl hexadecanote (Palmitate)	35.42
12.	23.830	2,6,10,14,18,22-Tetracosahexaene (Squalene)	2.64

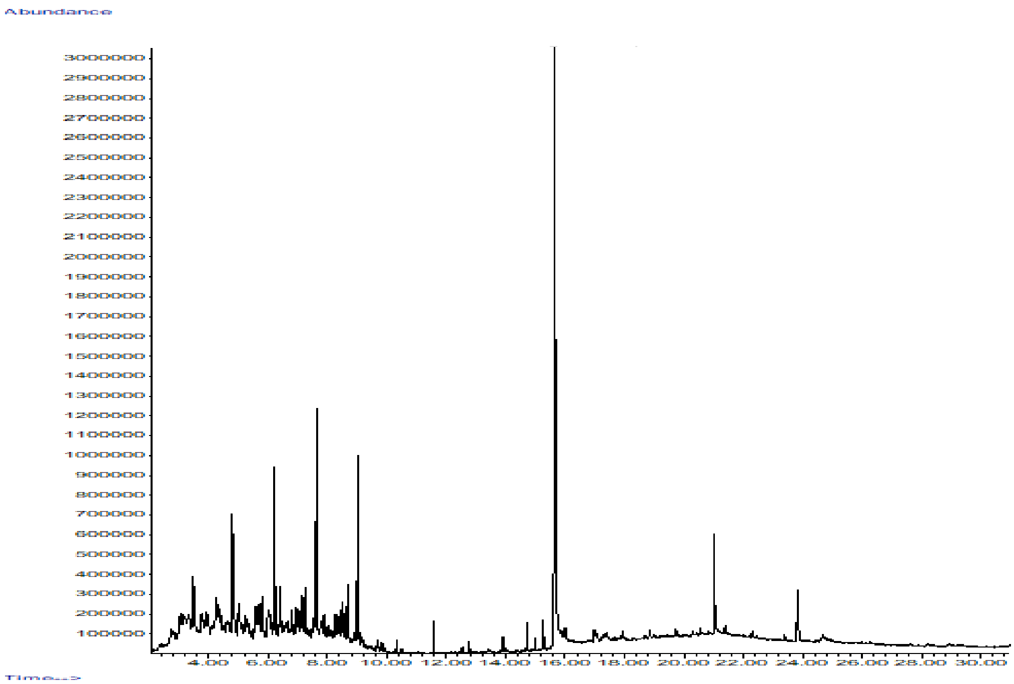

Figure 1: *Botrycoccus* species fatty acid profile

Table 5: *Chlorococcum species* fatty acid profile

Chemical Name	Retention Time (min.)	Area (%)
Methyl 9,12,15-octadecatrienoate (Linolenate)	17.09	11.57
Methyl 5,9,12-octadecatrienoate	16.907	3.53
Methyl 9,12-octadecatrienoate (Linolenate)	17.015	2.19
Methyl 9,12,15-octadecatrienoate (Linolenate)	17.374	2.42
Methyl 9,12,15-octadecatrienoate	17.905	1.28
Methyl 4,7,10-hexadecatrienoate (Gamolenate)	14.801	1.2
Methyl 4,7,10,13-hexadecatetraenoate	12.864	1.22
Methyl 7,10-hexadecadienoate	14.95	0.58
Methyl hexadecanoic (Palmitate)	15.299	8.24
Methyl-heptadecanoate (Margareate)	16.306	0.6
Methyl methacrylate octadecyl ester	19.11	2.28
n-Undecane	4.394	0.42
n-Dodecane	5.312	0.23
n-Decane	3.477	0.25
Methyl tetra decanoate	11.027	0.08
Methyl dodecanoic (Laurate)	11.794	0.08
n-Tridecane	7.631	0.31
n-Tetradecane	9.01	0.21
9,11-Dimethyl tetra cyclo tetradecane	12.455	0.16
Methyl pentadecanoate	13.2	0.13
Methyl cis-8,11,14-Eicosatrienoate	17.374	2.42
Sesquiasbinene hydrate	16.383	0.98
Methyl octadecanoate (Stearate)	17.299	1.28
Methyl 9-hexadecenoic (Palmitoleate)	15.013	0.58
Methyl 9,12,15-octadecatrienoate (Linolenate)	16.987	1.17
Methyl tetracosanoate (Lignocerate)	22.646	0.33

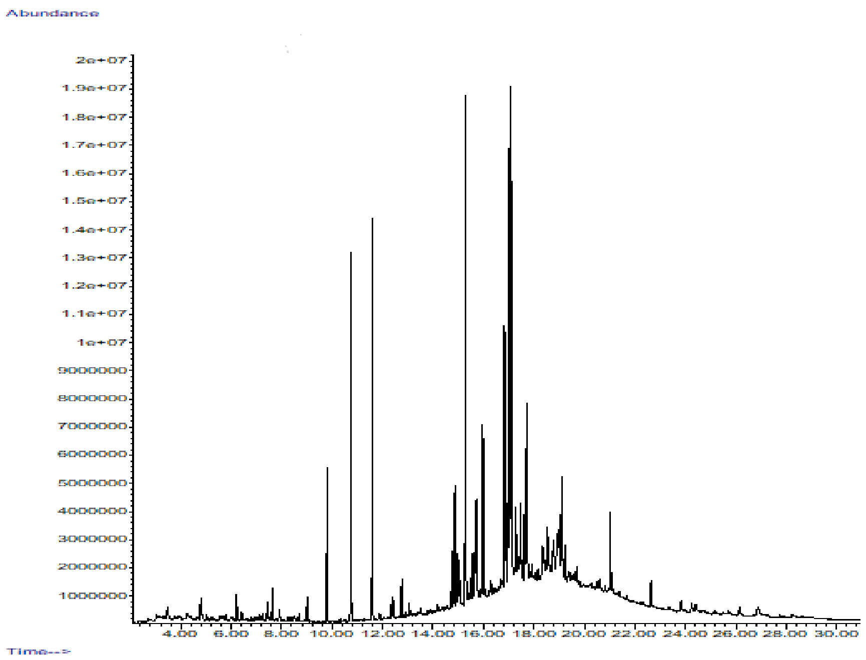

Figure 2: *Chlorococcum* species fatty acid profile

Table 6: *Chlorella species* fatty acid profile

Chemical Name	Retention Time (min.)	Area (%)
Methyl 9-Octadecenoate (Elaidate)	17.095	8.74
Methyl 9,12,15-octadecatrienoate (Linolenate)	17.05	11.03
n-Undecane	4.793	6.16
n-Dodecane	6.212	5.5
n-Decane	3.471	3.71
Methyl 5,9,12-octadecatrienoate (Gamma-linolenate)	16.832	2.07
n-Tridecane	7.637	5.51
Methyl octadecanoate (Stearate)	17.284	1.89
Methyl hexadecanoic (Palmitate)	15.276	30.2
n-Tetradecane	9.01	3.4
Methyl 9,12-octadecatrienoate (Linolenate)	16.987	7.51

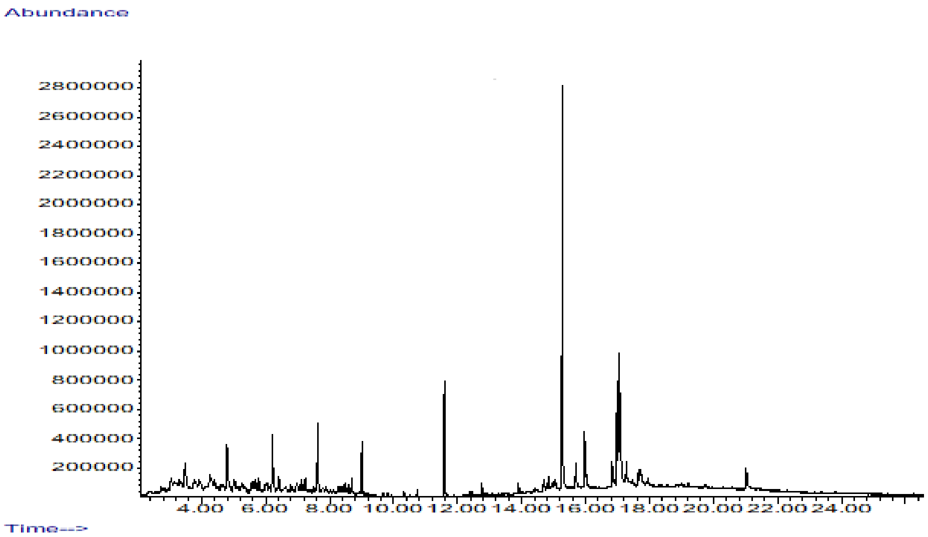

Figure 3: *Chlorella* species fatty acid profile

Table 7: *Scenedesmus species* fatty acid profile

Chemical Name	Retention Time (min.)	Area (%)
Methyl 9-Octadecenoate (Elaidate)	14.095	7.14
Methyl 9,12,15-octadecatrienoate (Linolenate)	16.05	11.31
n-Undecane	5.793	6.16
n-Dodecane	3.212	5.5
n-Decane	2.471	4.01
Methyl 5,9,12-octadecatrienoate (Gamma-linolenate)	15.832	2.07
n-Tridecane	6.637	4.99
Methyl octadecanoate (Stearate)	13.284	1.91
Methyl hexadecanoic (Palmitate)	15.276	29.1
n-Tetradecane	9.01	3.4
Methyl 9,12-octadecadienoate (Linolenate)	15.987	7.51

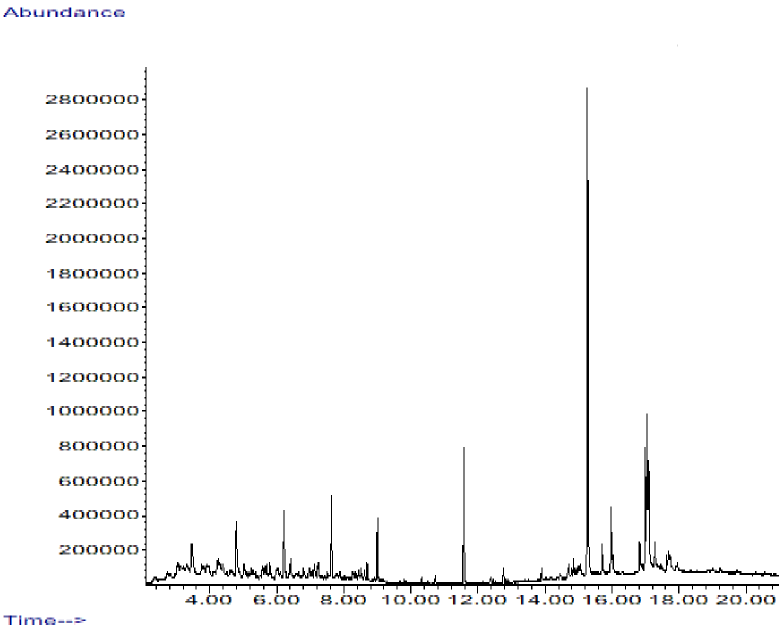

Figure 4: *Scenedesmus* species fatty acid profile

Table 8: HPLC RT and parent ion mass values obtained from a mass spectroscopy analysis of lipids isolated from *Chlorella, Scenedesmus, Botryococcus*, and *Chlorococcum*

HPLC peak	1	2	3	4
Microalgae	*Chlorella*	*Scenedesmus*	*Botryococcus*	*Chlorococcum*
Lipid	Sphingolipids	Sphingolipids	Sphingolipids	Sphingolipids
HPLC Retention Time (min)	10.76	21.5	33.9	7.767
% Total lipid	19.1	20.3	22.9	19.8
Parent ion mass	124.38	156.6	193.8	115.4

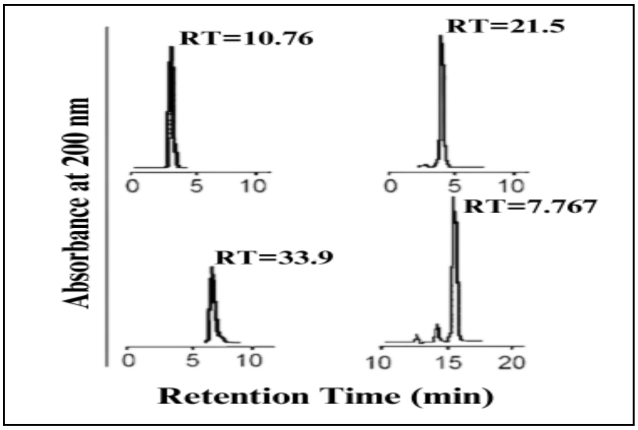

Figure 5: HPLC of standard lipids (1) *Chlorella*, (2) *Scenedesmus*, (3) *Botryococcus*, and (4) *Chlorococcum*

3.2. Lipid purification and characterization

3.2.1. Profile of fatty acid

Botryococcus sp. fatty acid compounds' methyl esters were isolated by GC-MS technology. The lipids were transesterified first, and then after transition, the sterols of these were analysed into various research components to include saturated hydrocarbons like n-dodecane, 7-methyl heptadecane, 2-methyl decane, 4-methyl undecane, n-decane, 2, 6, 10-trimethyl dodecane, and n-tetradecane, and the unsaturated hydrocarbon, tetracosahexaene. The primary regained fatty acid (palmitic acid) took nominal space on the chromatogram, accounting for only 35.42%, while the accompanying acid (oxalic acid) reached 4.26%. All the compounds were available, in which a total hydrocarbon percentage was found to be 37.55% and could be attributed 26.95 to acid components.

3.2.2. Chemical constituents profiling

The *Chlorococcum* sp. GC-MS investigation resulted in the identification of 27 main constituents, which happened to include as well as consist of components of hydrocarbons (HCs), fatty acids, and other compounds. The hydrocarbons were less than 2%, while fatty acids constituted 30.63%. The FAME acids were found in the greatest quantity and were mostly composed of methyl 9, 12, 15-octadecatrienoate (11.57%), methyl 8, 11, 14-eicosatrienoate (2.19%), methy l 9, 12-octadecadienoate (6.37%), and methyl octadecatrienoate (3.53%). A total of eleven compounds, including hydrocarbons that amounted to 35.28% and fatty acid methyl esters that supplied 29.34%, were identified by the GC-MS analysis of *Chlorella* sp. These longer fatty acids provided were the least fatty acid methyl ester to be measured after hexadecanoate, with the most prominent being methyl octadecatrienoate (11.03%), methyl octadecenoate (elaidate, 8.74%), and methyl 9, 12-octadecadienoate (7.5%). The GC-MS analysis of *Scenedesmus* sp. showed that eleven compounds were present and that hydrocarbons (28.18%) and fatty acid methyl esters (24.14%) were the most predominant elements. Both *Scenedesmus* and *Chlorella* species have oil profiles where hydrocarbons (37.55%) and fatty acids (28.95%) are the most abundant.

3.2.3. Gas liquid chromatography

GLC chromatogram reveals the lipid profiles of four microalgal strains: *Chlorella* sp refers to a species of micro algae that belongs to the *Chlorella* genus. *Botryococcus* sp., *Chlorococcum* sp., and *Scenedesmus* sp. Every single strain also shows different peaks in the chromatographic analysis depending on their Lipid components at different retention time values. *Botryococcus* sp. has the highest peak of 33minutes, and according to the figure it has the highest lipid content than the other strains which make it favourable for biofuel production. The presence of long-chain fatty acids at this peak is more than appropriate for biofuel conversion purposes. *Chlorella* sp. has moderate peak at about 10 minutes that can be an indication that it contains fewer lipids as compared to *Botryococcus* sp., but it still remains in the level of the reasonable amount of lipids to be used in a variety of biofuel related applications. *Chlorococcum* sp. has a smaller peak at 8 minutes signifying the presence of lipids although in smaller quantity as compared to protein suggesting low lipid production. Lastly, *Scenedesmus* sp. has a clearly visible peak at 21 min. Signal is quite intense indicating lipids, but the concentration was still lower than in *Botryococcus* sp. Overall, the chromatogram focused *Botryococcus* sp. as the second richest among lipids among the strains examined herein. While *Chlorella* sp. and *Chlorococcum* sp. have comparatively lesser yet significant lipid content interestingly.

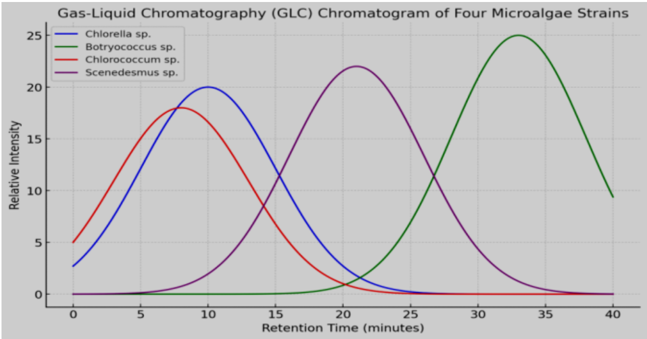

Figure 6: GLC of microalgae

4. Discussion

The study confirms a significant influence of temperature itself on lipid biosynthesis in algae and *Botryococcus* sp., exhibiting the highest lipid content at a temperature of 25°C, with the following lower lipid content at others. Hashem *et al.*[19] described a similar situation because when the temperature was high, the cells required more energy, and this led to the oil-making reactions to slow down. This is the idea that you need to get the right temperature to make the most of biofuel that PACE Ltd. fit into their work.

Lost deeds, indeed, appeared in the absence of temperature; conversion was the highest at 30°C in

Chlorococcum sp., whereas *Botryococcus* sp. dropped beyond 25°C, which was the same as what Gao *et al.* found.[20] In any case *Scenedesmus* sp. kept its lipid content at almost constant values, which indicated its toughness, not to mention the sentence by Patel *et al.*[21] The findings of different algae species' diverse behavior toward temperature enhanced the urgency of developing specific strategies for each species to boost lipid yield.

Another environmental parameter that contributed proportionally to the lipid content is the light intensity. *Botryococcus* sp. reached the maximum lipid content under all light levels, which were also CO2 concentrations, followed by *Chlorococcum* sp., *Scenedesmus* sp., and *Chlorella* sp.[22,23] Hence, the study indicates that light and temperature control are of equal importance when it comes to the utilization of solar radiation to the best of the biofuel production capacity, and this point was also made previously by Robles-Iglesias *et al.*[22]

All in all, the research paper correctly summarized that the integration of the best growing conditions in respect to temperature, light, and nutrients to produce plant lipids will be achieved, and that is why the paper is presented by Patel *et al.*[21] More research is essential for the large-scale development and the experimental design that will confirm the findings.[22]

The findings of moderate light brightness (about 4 lux) imply that the micro-alginate lipid production is stimulated, especially in *Botryococcus* and *Chlorella* species that exhibit the highest fat level with this protocol. The same results are also found in the study of Singh *et al.*, where it is shown that the production of lipid in microalgae is optimized with moderate light intensities (~4-5 lux). The application of high light intensities, which are above 5 lux, in the specific case will lead to light-induced photo-inhibition due to excessive formation of reactive oxygen species (ROS) and impaired lipid synthesis. This result is in line with the findings of Singh *et al.*, where the authors observed that at high light intensities the inhibition of the photosynthesis process could govern the limitation of lipid in the microalgae.[24] Furthermore, Li, *et al.* indicated that microalgae prefer live in a slightly alkaline medium with more significant growth, and lipid accumulation, which in turn is supportive of the most favourable conditions for microalgal cultivation.[25,26]

The obtained results bring to light the fact that *Botryococcus* sp. lipid production levels are quite high, of hydrocarbons, which can become a valuable strain for biofuel production. The above statement can be supported by the work of Miao and Wu (2020), who found the same lipid production process in *Botryococcus* sp. and said that its lipid accumulation was 2 days = 2 corners over a wide range of environmental conditions; it is a good candidate for biofuel application.[27] In contrast, *Chlorella* sp. is reported to be economically viable in optimized temperature and light conditions, showing quite good lipid, but it is best in biomass productivity, which cannot be neglected in large-scale biofuel production. The reported study revealed that the fluctuations of lipid production in different microalgal strains are due to different ecological factors, e.g., temperature, pH, and light levels. In all experimental sets, *Botryococcus* sp. showed the highest lipid content formation about cultivation, which substantiates the fact it can be a major strain in sustainable biofuel production.[28] However, the conferring modulation of environmental parameters to the best cultures becomes crucial by checking previously published work concerning the increase of lipid yields and contributing to more effective algal biofuel systems.

The lipids obtained from microalgae (*Chlorella*, *Chlorococcum*, *Botryococcus*, and *Scenedesmus*) produced 4 major HPLC peaks and several minor ones resolved with baseline separation. HPLC analysis of *Botryococcus*showed the highest peak in the chromatogram which was separated within 33.9min through C18 column showed the available lipids. Eluted under isocratic conditions, these lipids were verified by their retention durations and the absorption spectra of the corresponding reference standards. Purity is determined by calculating the percentage of the peak area of lipids in relation to the overall area for each microalgae lipid synthesized.

By using the calculations of the Gas Chromatography-Mass Spectrometry (GC-MS) technique, it can be particularly noted that the portion of fatty acids in *Chlorococcum* got up to 30.63%, among which methyl 9, 12, 15-octadecatrienoate is along the line of being at the top-most position with the percentage of 11.57. They were carried out in the case of *Chlorella* and *Scenedesmus* species, which showed that the contents, mainly hydrocarbons and fatty acids, made up the largest part of the total lipid profiles and were inclined to agree more with the trend. There was significantly more presence of hydrocarbons (35.28%) and fatty acid methyl esters (29.34%) in *Chlorella*, and the prominent substances were undecane, dodecane, and tridecane among them. Hence, the information garnered to the effect that these microorganisms are real producers of hydrocarbons and can be the basis of biofuel production is supported. *Scenedesmus*, for its part, was observed to have a high hydrocarbon monocontainer, which was around 28.18%, with 24.14% of its lipids being fatty acids, which are the vital parts for making biofuels, so this finding can pinpoint its position in biofuels. In brief, the predominant proportion of hydrocarbons and fatty acids present in microalgal strains surely implies that they are quite suitable for biodiesel, as the hydrocarbons are the main ingredients for the synthesis of biofuels. The obtained results of these studies are the same as those of the previous ones that have shown that microalgae, including *Chlorococcum*, *Chlorella*, and *Scenedesmus*, can be highly efficient sources of biodiesel production because of their good lipid and hydrocarbon profiles.[29.30]

5. Conclusion

This study concluded that *Botryococcus* sp. was the most efficient strain for lipid production under all tested conditions, making it an ideal candidate for biofuel production. It consistently produced the highest lipid content, especially at 25°C, 4 lux light intensity, and pH 7.3. Other microalgae species, such as *Chlorella Chlorococcum* and *Scenedesmus* species, showed lower lipid yields but promising biomass production, with *Chlorella* sp. performing best at 35°C and *Chlorococcum* sp. at 30°C. Light intensity and pH significantly influenced lipid accumulation, with optimal results at 4 lux and pH 7.3. These findings emphasize the importance of optimizing cultural conditions for enhanced lipid production in microalgae, particularly for biofuel applications.

6. Ethical Committee Approval

Not applicable.

7. Source of Funding

None.

8. Conflict of Interest

None.

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