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Impact of Nutrient Conditions on Growth and Lipid Accumulation in *Scenedesmus obliquus* and *Chlorococcum humicola* as Biofuel Sources

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Abstract: This investigation utilizes two indigenous microalgal Species, *Scendesmus obliquus* and *Chlorococcum humicola*, to evaluate their lipid production capabilities as a potential biodiesel source under nutrient deprivation conditions, specifically nitrogen limitation at 50% (-N), phosphorus limitation at 50% (-P) and a combination of nitrogen and phosphorus starvation at 50% (-N) 50% (-P). The results indicated that lipid accumulation in *Scendesmus obliquus* was significantly more effective than that observed in *Chlorococcum humicola* at (-N50%), with a lipid content of 42.9% of dry weight, in contrast to a minimal lipid content of 15.7% observed in the control, and lipid accumulation in *Chlorococcum humicola* at (-N) 50% reached to 38.9% of dry weight compared with the control's 12.5% of dry weight. Furthermore, carbohydrate content elevated from 16% in the control to 41% at (-N) 50% + (-*P*) 50% treatment in *Scendesmus obliquus*, whereas the highest protein content recorded was 34% of dry weight in the control condition. In the case of *Chlorococcum humicola*, carbohydrate content was 40.5% of dry weight in the 50 % nitrogen deprivation treatment, with the highest protein content recorded being 29.8% of dry weight in the control condition.

Keywords: Microalgae; Nutrients; Lipid content; Biodiesel sources.

1. Introduction

Microalgae represents a diverse group of photosynthetic microorganisms with critical importance in global carbon cycling [1] and have attracted considerable attention as potential sources of renewable biofuels, nutraceuticals, and other high-value bioproducts [2]. Microalgae provide numerous benefits for biodiesel production over conventional biofuels [3]. Their rapid growth allows for superior photosynthetic efficiency, with biomass doubling occurring within 24 hours [4]. Microalgae's cultivation is versatile, thriving in various environments with minimal resources, and enhanced growth can be achieved through nutrient addition and aeration; they also fix significant amounts of carbon dioxide, contributing to carbon mitigation [5, 6]. The oil yield from microalgae exceeds that of traditional biofuel crops by 10-20 times per hectare, with the ability for frequent harvesting [2, 7]. Their high lipid content, ranging from 20% to 80% of dry biomass, positions microalgae as a viable biodiesel source, primarily in the form of triacylglycerols (TAGs) [8, 9]. Microalgae can be cultivated on land that is not arable, and thus does not compete for resources that food crops need in order to thrive [10]. Biodiesel obtained from microalgae has characteristics that resemble those of petroleum diesel, including its flash point and viscosity [11].

Biotic and abiotic factors are variable and influence the growth of microalgae and the accumulation of lipids. Autotrophic growth medium components such as phosphorus, carbon, nitrogen, and other salts are key components. An increase or reduction of this component can thus be influenced by the amount produced and the lipids produced by the microalgae [12]. Nutrient availability, particularly nitrogen (N) and phosphorus (P) concentration, plays a critical role in regulating the metabolic balance between growth and lipid accumulation in microalgae [13]. They build up organic compounds intracellularly as they develop photosynthetically, utilizing CO_2 as a carbon source [14]. In nutrient abundance, microalgae prioritize cell division and protein synthesis, while nitrogen or phosphorus deficiencies typically trigger stress responses that divert carbon flow toward fat and carbohydrate storage pathways. However, the magnitude and timing of this metabolic shift vary depending on the species [15]. Among various genera, *Scenedesmus obliquus* and *Chlorococcum humicola* are recognized for their high adaptability to different environmental conditions and their ability to accumulate significant amounts of lipids under stress [16]. *Scenedesmus obliquus* is a chlorophyte species characterized by high growth and a marked ability to accumulate Triacylglycerides under controlled nutrient stress. Recent experimental research has detailed the Fatty Acid Methyl Esters (FAME) patterns of *S. obliquus* strains and confirmed their suitability for biodiesel production when subjected to optimized feeding regimens [17]. *Chlorococcum humicola* has recently attracted attention as a species capable of producing large quantities of biomass and lipid under specific nitrogen and nitrogen/phosphorus ratios [18]. Although lipid accumulation under nutrient stress is common, the main challenge remains: finding a cultivation system that optimizes the productivity of both biomass and fat, rather than maximizing one at the expense of the other. Indeed, a high fat content does not necessarily translate to high productivity unless the biomass remains substantial. This study aims to enhancing and evaluation fatty acid from the two microalgae *Scenedesmus obliquus* and *Chlorococcum humicola* by starvation of nitrogen and phosphor, enhanced fatty acid biosynthesis under nutrient deficiencies improves the suitability of microalgae biomass for biodiesel production, providing a low-carbon alternative to fossil fuels. By promoting carbon sequestration and reducing greenhouse gas emissions, microalgae-derived biofuels contribute to climate change mitigation and support the transition to sustainable and environmentally friendly energy systems.

2. Materials and Methods

2.1. Sample Sampling and Collection of Microalgae

Freshwater algal specimens were procured from aquatic bodies situated in the Al-Husayniyah District of northeastern Baghdad. These specimens were gathered utilizing aseptic containers (100 ml capacity), meticulously annotated with the date and location of collection, and subsequently conveyed to the laboratory for incubation under optimal parameters (268 $\mu E/m^2/s$, light: dark cycle 16:8, and temperature 25±2 °C) [19].

2.2. Algae Isolation and Purification

Two techniques were used for algae isolation, streaking on plate agar and serial dilution method [20]. The first involved exposure on solidified agar plates. Chu-10 medium was prepared and solidified with 1.5% agar, then sterilized in an autoclave. After sterilization, the medium was poured into Petri dishes at 45–50 °C and allowed to solidify. Using a sterile loop, algal samples were streaked in straight lines on the agar surface.

The plates were then incubated under continuous illumination in a lighted, refrigerated incubator, maintained at approximately 25 ± 2 °C, with a light intensity of about 268 μE/m²/s and a photoperiod of 16:8 h (light: dark) for 10–14 days [19, 21]. The serial dilution technique was employed in the second method. Each of the ten test tubes held nine milliliters of sterile Chu-10 growth medium. After adding a sample of algal suspension to the first test tube and carefully mixing it, 1 ml was transferred to the next, and so on. In the end, each test tube was kept in the growth chamber for two weeks [19, 22].

2.3. Media Preparation and Sterilization

Certified culture media (modified Chu-10) were used for the growth of green algae (Chlorophyta) [21]. which their components described by [23] shown Table 1.

Table 1: Stock Solutions Component of Chu-10.

	A-macronutrient nutrients	Concentration g/L		B-micronutrient nutrients	Concentration g/L
1.	Sodium Meta Silicate	5.8	8.	EDTA. Na_2	1.00
2.	$NaNo_3$	57.56		$H3BO_3$	2.86
3.	K_2Hpo_4	10		$MnCl_2.4H_2O$	1.81
4.	$MgSo_4.7H_2O$	25		$ZnSO_4.7H_2O$	0.222
5.	EDTA. Na_2	4.36		$NaMoO_4.5H_2O$	0.390
6.	$FeCl_3.6H_2O$	3.15		$CuSO_4.5H_2O$	0.079
7.	Na_2CO_3	20		$Co(NO_3)_2.6H_2O$	0.0494

Ten ml of stock solution No. 1 and one ml of stock solution No. (2, 3, 4, 5, 6 and 7) of macronutrients were taken and mixed with 1 ml of solution No. 8 of micronutrients and then added to one liter of distilled water. Then the media was sterilized using autoclave, except for stock solution K_2Hpo4, which was sterilized separately, pH of the media was set to 6.4 using sodium hydroxide and hydrochloric acid solution (0.01N) and then sterilized using an autoclave at 121°C, 15 bar, 15 min [24].

2.4. Algae Cultivation for Biomass

A flask containing 100 ml of a suitable growth medium for the isolated algae was prepared. The mixture was then incubated for 14 days after 10 ml of the isolated algal culture was added. After this period, the cultured algae were transferred to 500 ml of fresh growth medium and incubated for another 14 days. then transferred again to 1,000 ml of growth medium [25].

2.5. Determination the Growth Curve

The growth curve of alga was graphed on the basis of absorbance at a wavelength of 540 nm by UV-V is spectrophotometer and doubling time. All the measurements of this experiment were performed in triplicates. The growth rate (G) was calculated on the basis of the following equation [26]:

$$K = \frac{(Log\ ODt - Log\ ODo)}{t} \times 3.322 \quad (1)$$

$$G = \frac{0.301}{K} \quad (2)$$

Where t: time (days), K: growth rate, G: doubling time, OD_t: Growth after (t) days, and OD_0: algal growth at zero time.

2.6. Algae Stimulation for Lipid Production

Many variables can affect algal metabolism; in this case, the choice of variable of interest is nutrient deficiency, or more specifically, nitrogen and phosphorus deficiency. This is one of the most important variables that trigger lipid synthesis in algae [27]. Nitrates were represented as the nitrogen source in this study and were used for the isolated algae The nitrate $NaNO_3$ concentration is 57.56 g/l for modified Chu-10 culture media the control for the experiments. In this study, the phosphorus source utilized in the media for both isolated algae was potassium phosphate ($K2HPo_4$). K_2HPo_4 has a concentration 10g/l for modified Chu-10 media culture that is considered to be the control for the experiments [28]. Three nutrient treatments were used (N 50%, P 50 and (N 50% + P50%) 50% on two types of algae (*S. obliquus* and *C. humicola*).

2.7. Lipid Extraction

One gram of dried weight of microalgae was put in the thimble tube and later transferred to the designated cylinder in the Soxhlet apparatus. A solvent of 250 mL (hexane) was put in the flask. This took about 3-4 hours, after which the solvent in the cylinder turned from green to colorless, signifying that the solvent was extracted. The extracted samples were later dried in the rotary evaporator at 40°C for some minutes. The samples were poured in clean dishes and allowed to reach room temperature 25 °C for the whole night. The samples were later put in test tubes for analysis [29].

2.8. Lipid Analysis

Samples were analyzed by gas chromatography: aglint technologies (7820A) GC Mass spectrometer (5977E) USA in Ministry of Industry and Minerals/ IBN Al-Baytar state company.

2.9. Determination of Protein and Carbohydrate

The method in reference [30] was used to determine the protein, and reference [31] was used to determine the carbohydrate.

2.10. Statistical Analyses

Analysis of the data involved the use of the t-test for the assessment of the data obtained from the experimental groups. The data is expressed as mean values ± SD. One-way ANOVA with the least significant difference test was applied for the determination of significant differences between the means of the groups. The levels of significance of the data obtained from the analysis of the data are expressed by letters (A, B, C, D), with 'A' being the highest and the other letters being of progressively less significance. The values of $p > 0.05$ were considered to be statistically non-significant, while $p \leq 0.05$ were considered statistically significant. The analyses of the data were all done using the software package SPSS version 24.

3. Results and Discussion

After leaving the isolated samples, prepared using the two previous methods, in the laboratory incubator, a green color was observed. The results of the diagnostic study of these green algae showed that they belonged to two types of green microalgae: *Scenedesmus obliquus* and *Chlorococcum humicola*, shown Table 2. The density of the growth was measured for each stage of the growth phases, starting at time zero (daily) to twenty-one days to determine the best stage for harvesting. In this case, the harvesting stages for the two isolated algae are different since they grow at different rates.

Table 2. Scientific Classification of Isolated Algae.

Division	Class	Order	Family	Genus	Species
Chlorophyta	Chlorophyceae	Sphaeropleales	Scenedesmaceae	*Scenedesmus*	*obliquus*
Chlorophyta	Chlorophyceae	Chlorococcales	Chlorococcaceae	*Chlorococcum*	*humicola*

3.1. Nutrient effects on Scendesmus obliquus growth curve

For the control, the cells were in the lag phase for one day, then moved into the logarithmic phase on the second day and continued until the sixth day, then moved into the stationary phase. The stationary phase continued until the sixteenth day, when there was a reduction in the number of cells as shown in Figure 1.

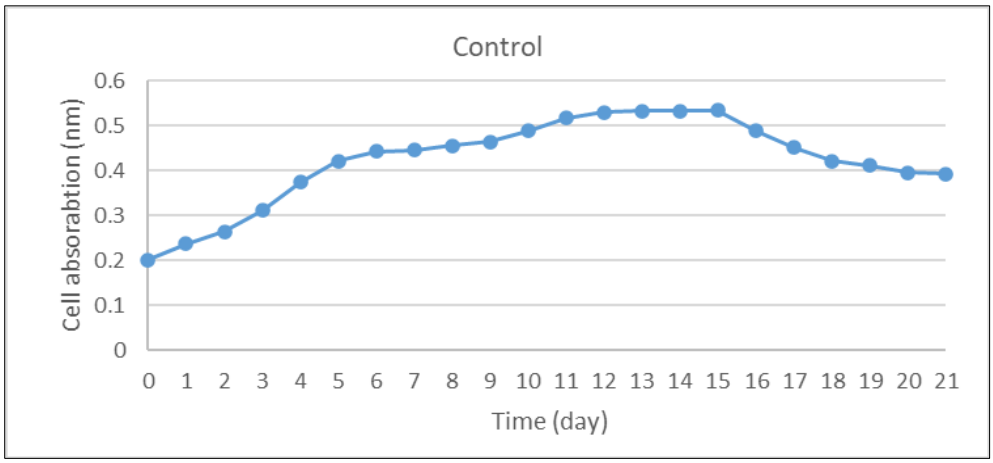

Figure 1. Growth curve of *Scendesmus obliquus* at control treatment.

In the -N50% treatment, the culture was in the lag phase for two days, after which the logarithmic phase started, lasting until the seventh day. The culture then proceeded to the stationary phase, which lasted until the nineteenth day, after which the decrease in the number of cells was coupled with the color transition from green to yellow-green as shown in Figure 2.

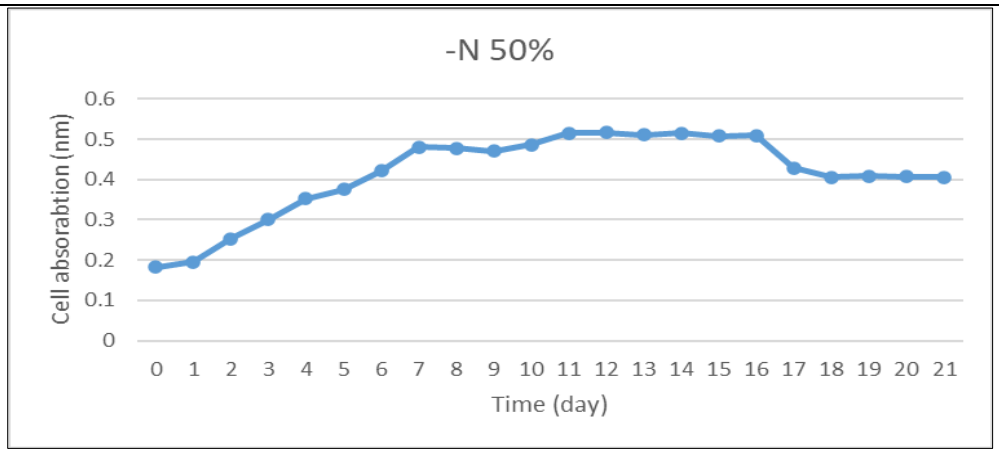

Figure 2. Growth curve of *S. obliquus* at –N50% treatment.

During the -P50% phase, the cells were in the lag phase for three days before the initiation of the logarithmic phase, which continued up to day seven. Then the cells entered the stationary phase from day seven today sixteen, and after that, there was a reduction in the number of cells with the color change from green to yellowish-green as shown in Figure 3.

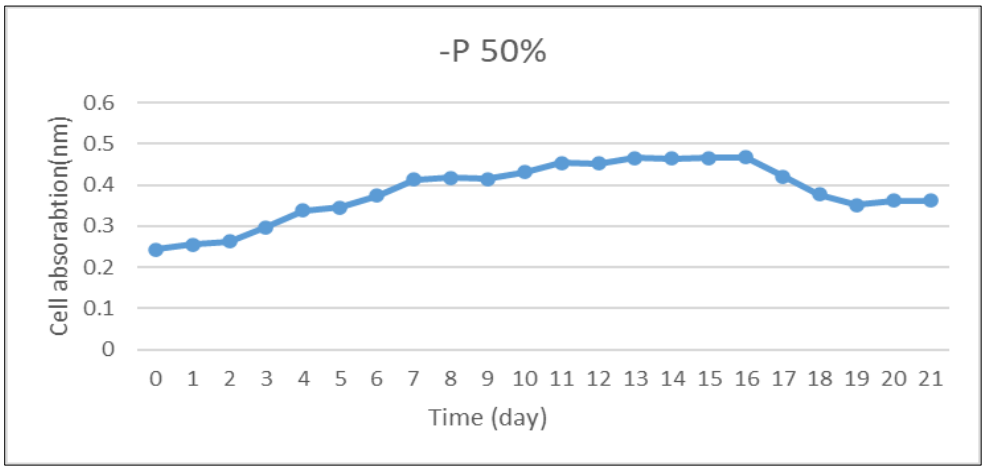

Figure 3. Growth curve of *S. obliquus* at -P50% treatment.

Lastly, the treatment involving (-N 50%) + (-P) 50% also stayed at the lag phase for five days before the logarithmic phase began. This went to the stationary phase, where it ended on day nineteen, and then the decrease in the number of cells and the color change from green to yellowish-green occurred as shown in Figure 4.

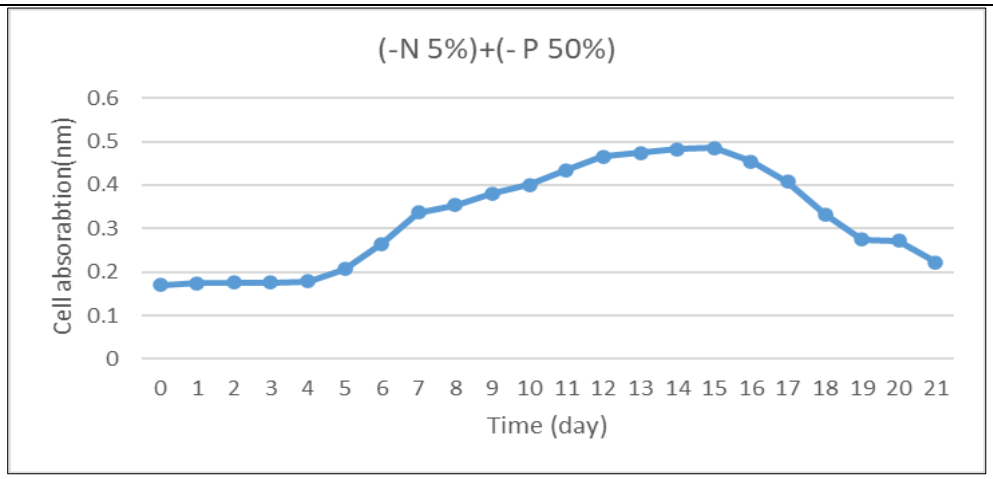

Figure 4. Growth curve of *S. obliquus* at (-N 50%) + (-P50%) treatment.

3.2. Effect of nutrient on growth curve for Chlorococcum humicola

From the results, it can be seen that the proportion of nitrogen affects the growth rate of *C. humicola* biomass. In the control experiment, the culture was in the lag phase for one day, followed by a logarithmic phase until day ten, and then a stationary phase until day seventeen, when there was a reduction in cell count as shown in Figure 5.

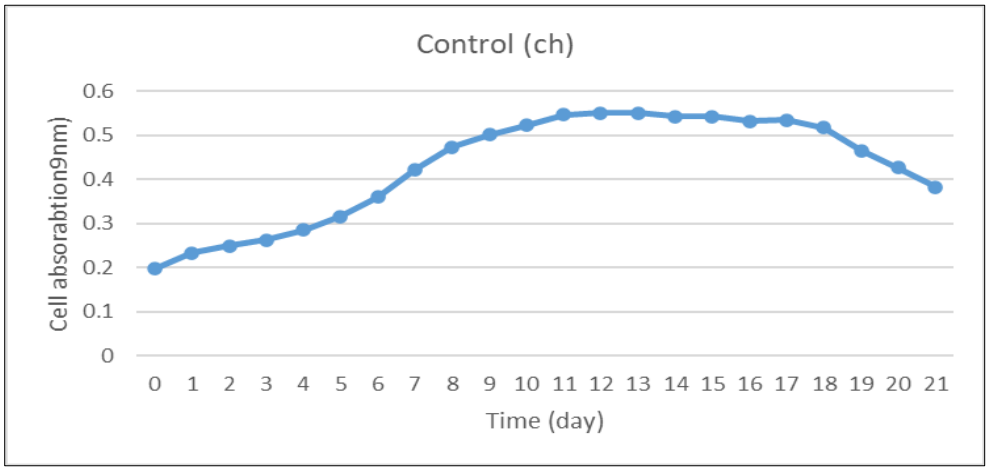

Figure 5. Growth Curve of *C. humicola* at Control.

In (-N 50%) of the treatment, the lag phase lasted four days, then the logarithmic phase began until the seven day, his was followed by the stabilization phase, which began on the eighth day and continued until the fifteenth day, then it entered a decline phase that lasted until the sixteenth day, then a change in color from bluish-green to yellowish-green was observed, as shown in Figure 6.

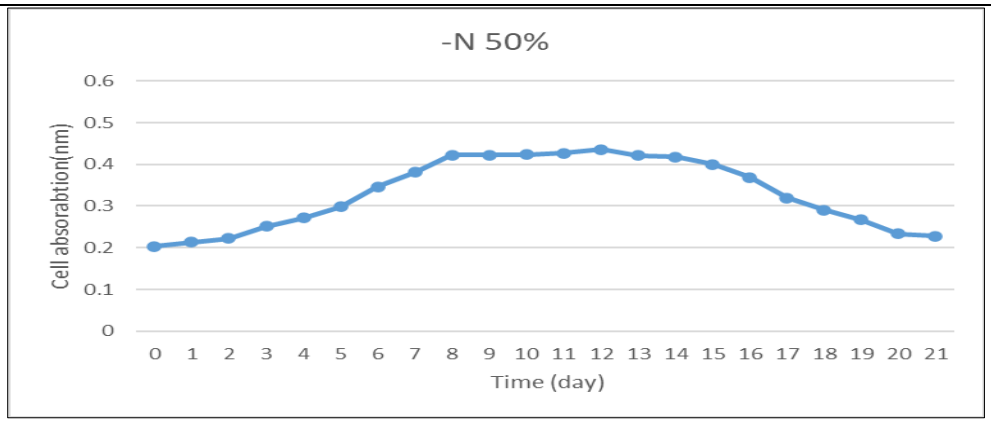

Figure 6. Growth curve of *C. humicola* at (-N 50%).

In the -P 50% treatment the microalgae spent three days in log phase t phase, then the logarithm phase began until the six days, then it entered the stationary phase until thirteen days and entered the decline phase, as shown in Figure 7.

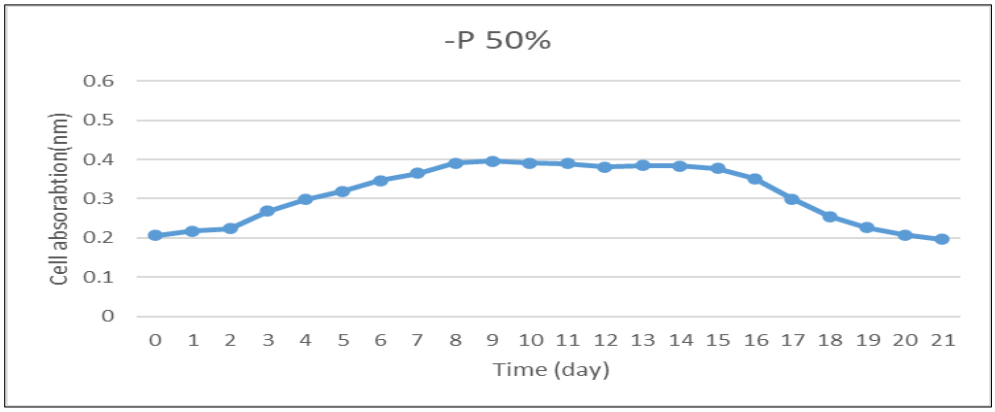

Figure 7. Growth curve of *C. humicola* at -P 50%.

Finally, for the (-N) 50% + (-P) 50% treatment, the culture entered the lag phase for five days, after which the logarithmic phase started, lasting until the seventh day, followed by the stationary phase, which continued until the nineteenth day. Then came the decrease in cell count, as well as the color change from green to yellowish-green, as shown in as shown in Figure 8.

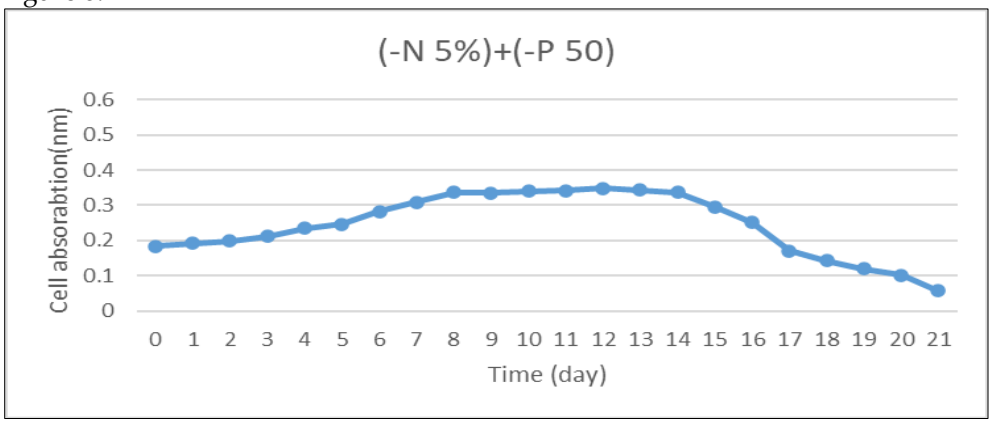

Figure 8. Growth curve of *C. humicola* at (-N) 50% + (-P) 50%.

The results showed a relationship between growth rates and doubling time for both algal species in all treatments. In the control treatment for *S. obliquus*, the growth rate (k) ranged from 0.343 days in the control treatment to 0.142 days at (-N) 50% +(-P) 50% as well as, and also showed a decrease in growth rate in ether treatment as it recorded (0.241 and 0.298) for each of the treatment(-N) 50% and (-P50%). Respectively as shown in Table 3 and Figure 9.

Table 3. Effect of different percentages of nutrient on growth rate (K) for both algae *Scendesmus obliquus* and *Chlorococcum humicola*.

Treatment	Growth rate (day)	
	S. obliquus	*C. humicola*
Control	0.343	0.358
(-N) 50%	0.241	0.133
(-P) 50%	0.289	0.245
(-N) 50% + (-P) 50%	0.142	0.129

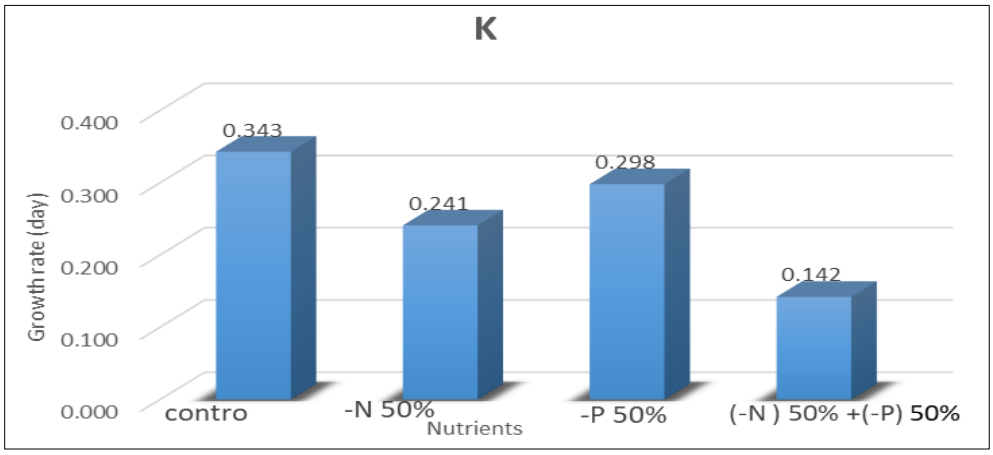

Figure 9. Growth rate *S. obliquus* at different nutrient.

The shorter doubling time rescored 0.88 at control, while the longer doubling time at treatment (-N) 50% +(-P) 50%, was rescored 2.30, also showing increased doubling time for either treatment, as it recorded 1.33 and 1.03 days for each of the treatment (-N) 50%, and (-P) 50%, respectively as shown in Figure 10.

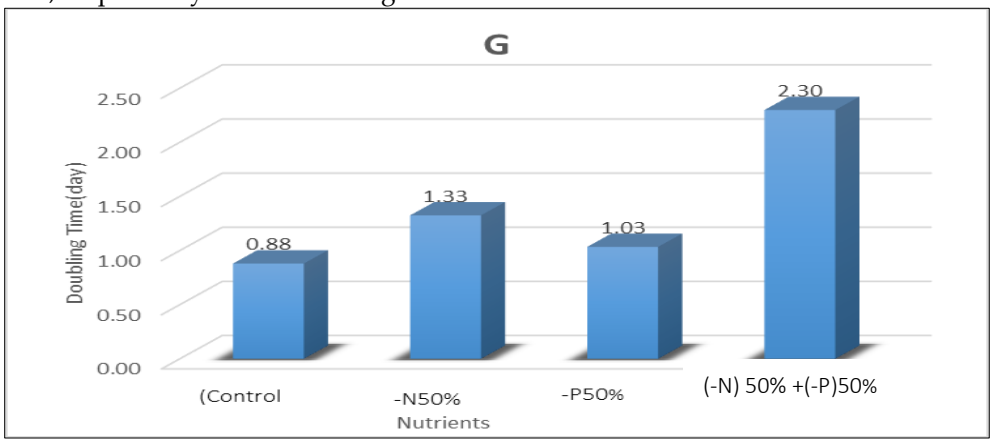

Figure 10. Doubling time of *S. obliquus* at different nutrient.

In this study it is shown that the K of *C.humicola* decreases for all treatments except the control the growth rate decreased from 0.358 at control to 0.129 (-N50%) + (-P50%), as well as, showing a decrease in growth rate for the rest of the treatments,as it recorded 0.133 and 0.245 for each of the treatments, (-N) 50% and (-P) 50% respectively as shown in Table 4 and Figure 11.

Table 4. Effect of different of nutrient on doubling time (G) for both algae *S.obliquus* and *C. humicola*.

Tested Algae	Control Mean±SD	-N 50% Mean±SD	-P 50% Mean±SD	(-N)50%+(-P)50% Mean±SD
Chlorococcum humicola	0.86±0.08D	2.27±0.02B	1.26±0.1C	2.69±0.6A
Scendesmus obliquus	0.88±0.03D	1.32±0.2B	1.03±0.1C	2.3±0.4A
P value	0.91	0.01	0.05	0.04

The Least Significant Difference (LSD) test was used to determine significant differences between the tested means. The letters (A, B, C, and D) represent the levels of significance, with the highest level being (A) and decreasing to the lowest level (D). Values of p≤0.05 were considered significantly different, P≤ 0.05 is considered statistically significant.

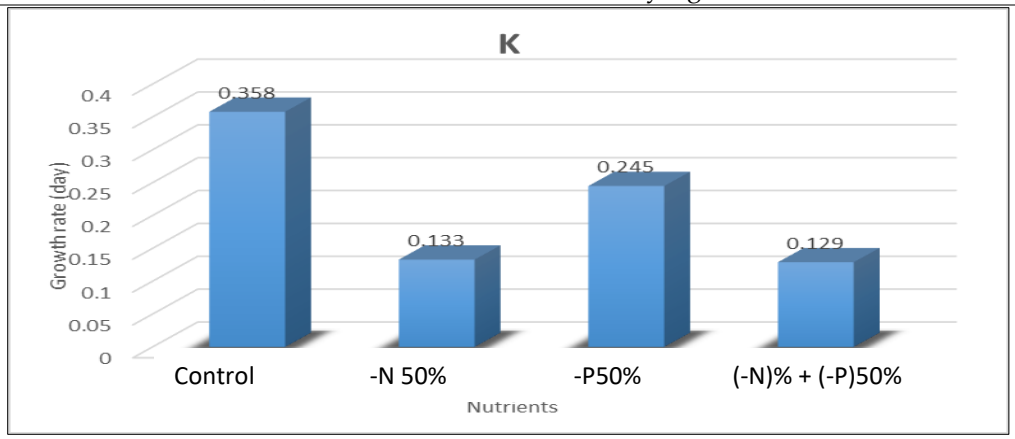

Figure 11. Growth rate of *C. humicola* at different nutrient.

The shortest doubling time was recorded at 0.86 days at the control, while the longest doubling time at (-N50%) + (-P50%) was recorded at 2.69 days. It also showed an increase in the doubling time of the ether treatment, where it recorded 2.27 and 1.26 days for each of the (-N) 50% and (-P) 50% treatments, respectively as shown in Figure 12.

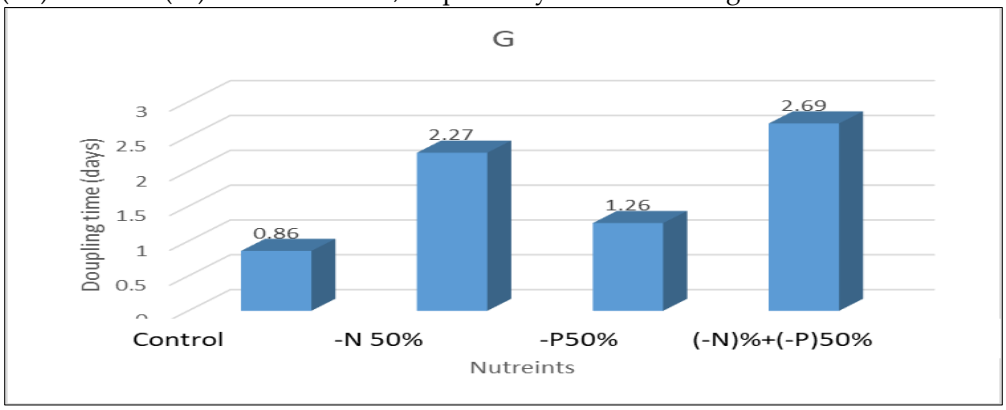

Figure 12. Doubling time of *C. humicola* at different nutrient.

Nitrogen is an important nutritional element required by microalgae, regulating the synthesis of proteins and growth-related metabolites [9]. Nitrogen also helps in the formation of photosynthetic cells, which are found in the chloroplasts [32]. In the case of nitrogen deficiency within the culture medium, microalgae have been known to store high lipid levels, but nitrogen deficiency restricts the synthesis of sufficient protein, thereby affecting cell mass production [2]. When there is a lack of nitrogen, carbon flux in the cell is channeled to the formation of lipids instead of proteins and other compounds that contain nitrogen. The consequence of this is an increase in the production of fatty acids and TAGs, which are the main constituents of algal lipids [33]. Based on our results, the growth rate increased, but the doubling time decreased in the control group because most of the growth medium provides the inorganic component that makes up the algal cell. This is consistent with the study [12] nitrogen deficiency leads to a significant inhibition of microalgae growth kinetics, resulting in reduced growth rates and decreased biomass productivity [18]. Also this finding is in concordance with research conducted by [17] which indicated that nitrogen deficiency exerts a significantly adverse impact on *Scenedesmus obliquus* growth rate, culminating in a markedly diminished biomass production, a reduction in cellular density, a decline in growth performance, and a metabolic transition from growth to lipid accumulation. Phosphorus is indispensable for microalgal proliferation and metabolic processes. Although it comprises approximately 1% of biomass, phosphorus serves as a pivotal growth-limiting determinant in microalgal biotechnological applications; moreover, phosphorus accessibility considerably affects biomass composition, especially lipid and carbohydrate accumulation [9]. The research findings that have been acquired concerning the element phosphorus exhibit a remarkable consistency with the observations made by [34] which specifically indicates that the replenishment of phosphorus, commonly referred to as P repletion, generally serves to optimize and maximize the growth rate of the organisms in question, as well as significantly enhance the final biomass yield.

3.3. The Effect of different percentages of nutrients on lipids

The collection of biomasses from both isolated algal species was conducted at the conclusion of the exponential growth phase for the purpose of analyzing lipid, carbohydrate, and protein content. This study shows increased lipid content in all treatments when compared with the control, in Table 5 shows the lipid content for S. obliquus increased from 15.8% at control to 42.9% in the (-N 50%) treatment, and the other treatment lipid contents have shown varying proportions, and they were 33.3% and 30.4% at the treatment (-P) 50% and (-N50%) + (-P50%) %), respectively. The identical pattern demonstrated an elevation in lipid content across all treatments when juxtaposed with the control for *C. humicola*, although the observed lipid content remained inferior to that of *S. obliquus*. The lipid content exhibited an increase from 12.5% in the control to 38.9% in the (-N)50% treatment, while the lipid contents in the other treatments displayed varying proportions of 32.7% and 36.8% at the treatment (-P) 50% and (-N50%) + (-P50%) %) respectively, as shown in Figure 13.

Table 5: Effect of different nutrient concentration on lipids Content (%) for *S. obliquus* and *C. humicola.*

Nutrient	*S. obliquus*	*C. humicola*
Control	15.8%	12.5%
(-N) 50%	42.9%	38.9%
(-P) 50%	33.3%	32.7%
(-N) 50% + (-P) 50%	30.4%	36.8%

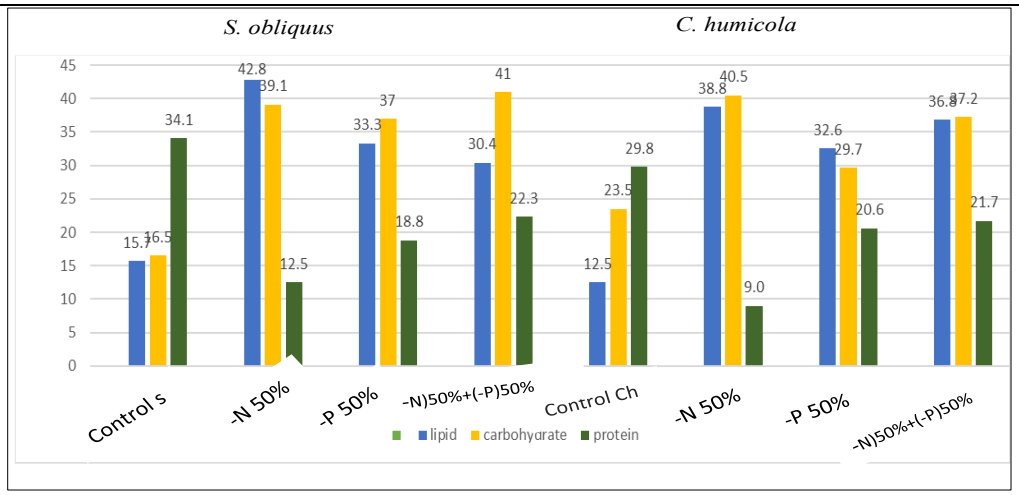

Figure 13. Total lipid, carbohydrate and protein of *S. obliquus* and *C. humicola* at different nutrient concentration.

3.4. Effect of nutrient concentration on protein and carbohydrate content for both algae S. obliquus and C. humicola

The implications of restricting nitrogen and phosphorus on the biochemical composition of the microalgae species *S. obliquus* and *C. humicola* were investigated. A notable enhancement in the accumulation of lipids and carbohydrates was documented conversely, a decline in protein content per cell was observed across all experimental treatments. Current results showed, the effect of different percentages of nutrients on *S. obliquus* that the highest percentage of protein at the control and the lowest percentage at -N 50% treatment reaching from 34.1% to 12.5%. On the other hand, the influence of varying proportions of nutrients on *C. humicola* that the highest percentage of protein at the control 29.8 % and the lowest percentage at -N 50% treatment Reaching to 9%. Protein levels in both microalgal species declined markedly under all treatments compared to the control. Overall, *S. obliquus* exhibited higher protein content than *C. humicola*, and statistically significant differences were observed among the treatments for both species. Nitrogen insufficiency directly diminishes the accessibility of nitrogen essential for the biosynthesis of amino acids and proteins; cellular mechanisms decelerate or cease protein synthesis and decompose certain intracellular proteins to furnish crucial metabolic pathways with nitrogen, thereby reducing the proportion of protein in the dry mass [35]. while phosphorus insufficiency diminishes the cellular capacity to produce adenosine triphosphate (ATP), synthesize nucleic acids such as ribosomal RNA (rRNA) and messenger RNA (mRNA), as well as membrane phospholipids, which ultimately results in a reduction in protein synthesis (fewer ribosomes and diminished translation) and a reallocation of carbon and nitrogen towards energy reserves rather than towards the formation of protein structures [36]. This is consistent with reference [29] where the protein percentage was highest in control treatment *Chlorella vulgaris* and *Chroococcus minor*. This investigation also elucidates that an augmented carbohydrate concentration in *S. obliquus*, when subjected to varying conditions, resulted in diminished levels of phosphorus and nitrogen nutrients across all treatments. The study examined the influence of disparate nutrient percentages on the carbohydrate levels, revealing that the highest concentration was observed at the treatment of (-N) 50% + (-P) 50%, which was quantified at 42%, as shown Table 6. Conversely, in *C. humicola*, an increase in carbohydrate concentration upon exposure resulted in reduced phosphorus and nitrogen nutrients across each treatment.

The research assessed the impact of varied nutrient percentages on carbohydrate content, with the maximum concentration recorded at the treatment of (-N) 50% + (-P) 50%, amounting to 40.5%. On the other hand, Carbohydrate content of both microalgae was increased among treatments. When algal organisms experience a deficiency in nitrogen or phosphorus, a notable physiological transition transpires within their energy metabolism pathway, culminating in a marked augmentation of carbohydrate accumulation [37]. A deficiency in nitrogen constrains the cellular capacity to synthesize proteins and chlorophyll, thereby diminishing vegetative biomass and compelling the cell to transmute photosynthetically derived carbon into sugars and starches as alternative storage substrates, consequently resulting in a substantial elevation of carbohydrate concentrations. A deficiency in phosphorus obstructs the biosynthesis of energy compounds such as ATP and NADPH and impedes the synthesis of nucleic acids and cellular membranes, which ultimately leads to diminished growth and cellular division [38]. Furthermore, excess carbon that remains unutilized is redirected towards carbohydrate storage pathways. In instances where nitrogen and phosphorus deficiencies coexist, growth inhibition intensifies, and the accumulation of intracellular carbon is expedited, yielding elevated carbohydrate levels due to the algal cells' propensity to convert the majority of photosynthetic byproducts into storage compounds rather than employing them for cellular growth or the synthesis of novel components.

Table 6. Effect of different nutrient concentration on protein and carbohydrate Content (%) for S. obliquus and C. humicola.

treatmeant	S. obliquus	C. humicola	S. obliquus	C. humicola
	proteins		carbohydrates	
Control	34.1	29.8	16.5	23.5
(-N) 50%	12.5	9.0	39.1	40.5
(-P) 50%	18.8	20.6	37	29.7
(-N) 50% + (-P) 50%	22.3	21.7	41	37.2

3.5. The Fatty Acid analysis by GC-MS

GC-MS analysis was performed to determine the qualitative and quantitative composition of fatty acids extracted from algae under different treatments. The results showed the presence of a range of saturated and unsaturated fatty acids, the most common of which were: Tetradecanoic acid also known as myristic acid (C14:0), Palmitoleic acid (C16:1), Pentadecanoic acid (C15:0), Stearic acid (C18:0), Oleic Acid (C18:1), Linoleic acid (C18:2), Linolenic acid (C18:3), In addition to playing an important role in the biofuel industry these substances are extensively recognized for their biochemical functions, antioxidants like oleic and palmitic acids, which reduce oxidative stress and affect cell proliferation and apoptosis, encompassing anti-inflammatory and antineoplastic characteristics, which may have facilitated the cytotoxic ramifications observed in this investigation [39, 40]. In the control treatment of *S. obliquus*, Palmitoleic acid constituted the highest percentage of fatty acids 9.17%, as shown Figure 14. Under the nitrogen deficiency treatment (-N 50%), an increase in Palmitoleic acid 16.63% and Oleic Acid 16.17% as shown Figure 15, Table 7. The phosphorus deficiency treatment showed a significant increase in unsaturated fatty acid particularly Oleic acid which reached 17.23% compared to the control. In the (-N) 50% + (-P) 50% treatment, the extracts exhibited the highest levels of total fatty acids, with Pentadecanoic acid (C18:1) being the most abundant 57.70%.

Regarding the *C. humicola* species, the gas chromatography (GC) results for the control treatment showed that Stearic acid had the highest percentage among the fatty acids reaching at 10. 83%. In contrast, an increase in oleic acid 2.64% was observed in the nitrogen deficiency treatment compared to the control treatment. As for phosphor deficiency –P50% treatment, oleic acid is reached to 1.11%. GC-MS analysis revealed significant changes in fatty acid composition under nitrogen and phosphorus deficiencies compared to the control sample, suggesting a metabolic reprogramming toward lipid accumulation under nutrient-deficient conditions [15]. Elevated levels of stearic acid, oleic acid, and palmitoleic acid indicate increased synthesis of stored lipids, particularly triglycerides, which are the primary raw materials for biodiesel production. Nutrient deficiencies are known to redirect cellular carbon flow from growth-related processes toward acetyl-CoA and fatty acid synthesis pathways, promoting lipid accumulation when cell division is restricted [11, 41]. The nitrogen and phosphorus deficiencies showed a clear effect on fatty acid production compared to the control, likely due to inhibition of protein and chlorophyll synthesis, and the resulting conversion of photosynthetic carbon into lipid-storing compounds. This metabolic shift typically favors the production of saturated and monounsaturated fatty acids, such as stearic and oleic acids, which are highly desirable in biodiesel due to their favorable combustion characteristics, oxidative stability, and Cetan number. Phosphorus deficiency also contributed to lipid accumulation, possibly through membrane lipid remodeling mechanisms that reduce phosphorus demand while increasing neutral lipid content, although its effect was relatively minor. From a biofuel perspective, the predominance of C16-C18 fatty acids observed in this study is particularly significant because the lengths of these chains are optimal for biodiesel quality, providing a balance between fuel stability and flowability. Therefore, nutrient stress not only enhances lipid quantity but also improves fatty acid composition, a crucial factor in determining biodiesel performance. These findings support the potential of controlled nutrient reduction strategies as an effective and cost-efficient approach to improving lipid productivity in microalgae for renewable energy applications, as shown in Figures 16 and 17.

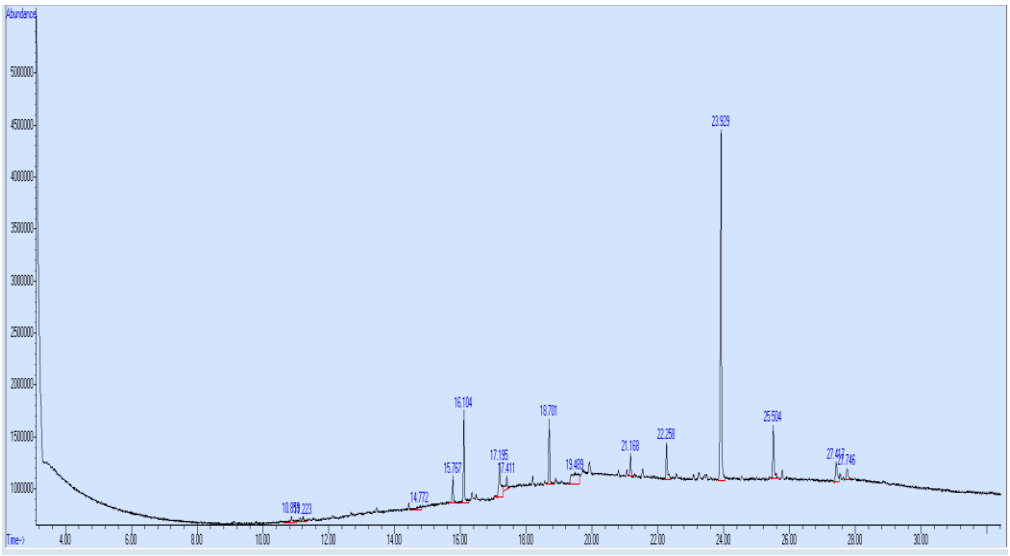

Figure 14. GC Analysis of *S. obliquus* at control treatment.

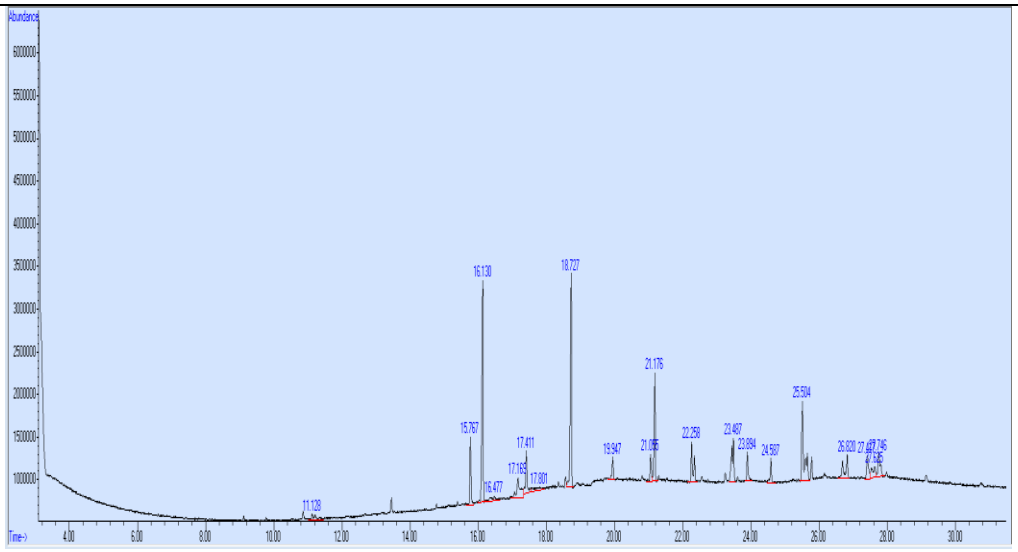

Figure 15. GC Analysis of *S. obliquus* at –N 50% treatment.

Table 7. The GC-Ms Analysis of S. obliquus.

Control treatment		–N 50% treatment	
Fatty acid	Area%	Fatty acid	Area%
Tetradecanoic acid	3.05	Tetradecanoic acid	4.81
Palmitoleic acid	9.17	Palmitoleic acid	16.63
Pentadecanoic acid	6.68	Pentadecanoic acid	4.68
Stearic acid	2.53	Stearic acid	3.93
Oleic Acid	6.21	Oleic Acid	16.17
Linoleic acid	7.14	Tridecanoic acid	1.77
Linolenic acid	1.75	Linolenic acid	7.00

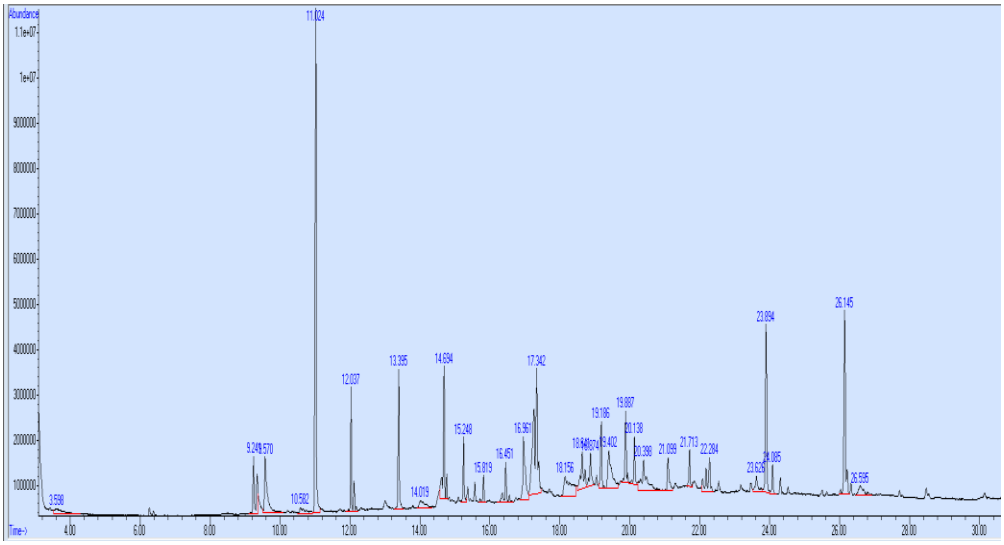

Figure 16. GC Analysis of *C. humicola* at control treatment.

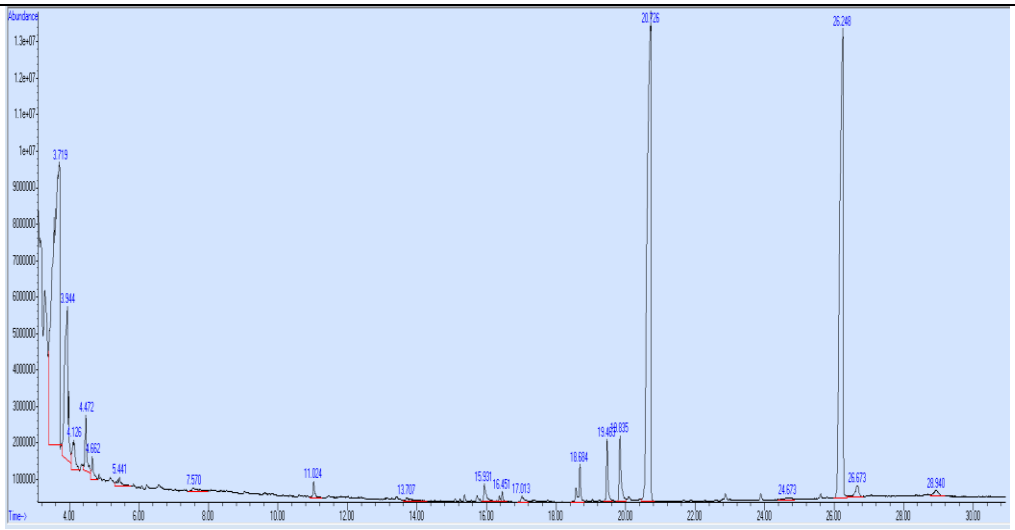

Figure 17. GC Analysis of *C. humicola* at –P 50% treatment.

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