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# ENHANCEMENT OF TRIACYLGLYCEROL IN *CHLORELLA PYRENOIDOSA* BY UV IRRADIATION

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## ABSTRACT

Microalgae with rich oil content are the promising alternatives for the depleting fossil fuel reserves, but there needs a great deal of work to be done in improving the lipid content of the microalgae strains. In this study, strain improvement in *Chlorella pyrenoidosa* was carried out for enhancing the production of neutral lipids mainly constituting triacylglycerol (TAG) which is the major source of biodiesel using UV radiation. The microalgal culture was exposed to different UV irradiation for different time periods and the results showed an increase in both biomass and TAG yields. A highest increase of 2.22 fold in biomass and 2.5 fold in TAG over control was observed for UV irradiation of 25 mins and 20 mins respectively. Gas chromatographic analysis of neutral lipid fractions from total lipids of UV irradiated samples showed an increase in percentage of some of the monounsaturated fatty acids which is considered as one of the preferred property of the biodiesel[1].

**KEYWORDS**: **Triacylglycerol, *Chlorella pryenoidosa*, Biodiesel, Microalgae, UV radiation.**

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## INTRODUCTION

Mechanical energy can't be accomplished effectively without petroleum, normal gas, coal, hydro power and atomic energy; and they turned into the fundamental regular hotspots for the energy. The requirement of petroleum and its by-products are increasing day by day because of the increment in population and industrialization. The world is entering a time of declining non-renewable energy assets, prominently known as 'peak Oil', while energy concern is expanding. The world's oil production is expected to decline in the middle of one and ten decades [2]. As a consequence of this approaching energy emergency, both government and private industries are looking at options of energy. In the most recent 10 years, numerous studies have been directed on biofuels for substituting fossil fuels and lesser the discharge of greenhouse gases (GHG) which is in charge of an unnatural weather change[3]. This excess use of petroleum has almost depleted the natural sources of diesel, which has led to increase in petroleum prices in everyday life and it will keep increasing until an alternative is adapted for it. Biodiesel, in the recent years became renowned everywhere in the world because of its accessibility, renewability, non-toxicity, improved gas emissions and its biodegradability. Biodiesel is converted into liquid fuels so that it can be used by automobiles and also for heating purposes. Biodiesel is produced by mixing a vegetable oil or animal fat with a short-chain alcohol, such as methanol, ethanol, or butanol and a catalyst[4]. But these sources are also limited, therefore scientists came up with an idea of producing biodiesel with natural sources like plants, vegetables, etc. Soybean oil, sunflower oil, palm oil etc can be used for the production of biodiesel. It also has some disadvantages like the amount of biodiesel produced with a huge quantity of these oils was very low. Therefore biodiesel production from microalgae came into existence[5].The microalgae for biodiesel are aquatic unicellular algae. These algae are photosynthetic and have high growth rate and population density. Under optimum conditions, algae can grow and double its biomass in less than 24 hours. Microalgae consist of large amount of lipid in it, which is approximately 50 %[6,7].Some species of algae like *Chlorella sp*. contains 28-30% of lipid, *Nitzschia sp. contains* 45 – 47% of lipid, *Nannochloropsis sp. contains* 31 – 68% of lipid and *Schizochytrium sp. contains* 50 – 77% of lipid[6] .Microalgae produce and store neutral lipids as unsaturated fats, phospholipids, glycolipids and it can be utilized as feedstock's for biodiesel production. Hence, in the present study, the growth, oil content and biodiesel production from microalgae *Chlorella pyrenoidosa* were examined. Previous research work has reported only the increase in biomass and total lipid using UV irradiation which did not report any increase in TAG production. Hence in the present research work an attempt was made to increase the TAG production through UV irradiation.

## MATERIALS AND METHODS

### *(i) Culturing Chlorella pyrenoidosa*

*Chlorella pyrenoidosa* (NCIM Accession No. 2738) was procured from NCIM-Pune, India. The culture was allowed to grow in FOG medium[8] at 25°C for 4 days under fluorescent light illumination, with the aeration of 2lpm. The experiment is repeated twice.

### *(ii) UV irradiation*

*Chlorella pyrenoidosa*, was subjected to mutational analysis for the enhanced production of TAG. Randomly generated mutants were screened for TAG production500ml of the culture with continuous stirring on magnetic stirrer was exposed to UV light for different time intervals (5 mins, 10 mins, 15 mins, 20 mins and 25 mins). The inoculums were taken out at respective time intervals and inoculated into 1litre of FOG medium (pH-7.5) and grown for 4 days at 25°C.

### *(iii) Harvesting*

At the end of the growth cycle, the microalgal culture was harvested using flocculation method[9] by the addition of alum (hydrated potassium aluminum sulphate) and finally dewateration was done by centrifugation at 7000 rpm for 5 mins. After harvesting, the wet biomass was dried in hot air oven at 100°C overnight. The dried biomass were grinded to a fine powder using mortar and pestle and weighed.

### *(iv) Total lipid extraction using Folch method*

200 mg of biomass was incubated overnight in the mixture of chloroform and methanol (2:1). This solution was then subjected to phase separation in a 125ml separating funnel[10]. The bottom chloroform phase containing total lipids was collected into a beaker of known weight (W1). The collected chloroform phase was completely evaporated and final weight of beaker (W2) was taken. The total amount of lipids was estimated by calculating the difference in weights of the beaker .Weight of total lipid = W2 - W1.

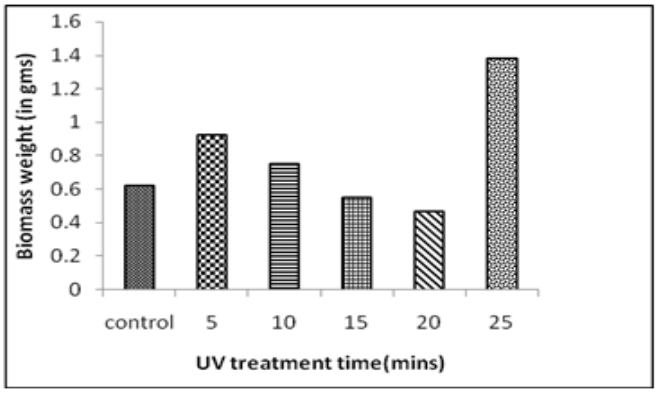
### *(v) Lipid fractionation using column chromatography*

Lipid fractionation was performed by following standard Frostegard method[11], The BIO RAD column (1.5 cm inner diameter and 20 cm length) with silica gel (230-400 mesh) was equilibrated with chloroform. The extracted total lipid sample was applied to the column in the chloroform solvent (1mL). The neutral lipid fraction was then eluted with chloroform (3 times the column volume), followed by glycolipids fraction elution with acetone: methanol (9:1) and phospholipids fraction elution with methanol. Solvent in all the eluted fractions was evaporated to1 ml for preparation of Fatty acid methyl esters (FAMEs) for GC analysis. Fatty acid analysis was performed by injecting 0.5 µL of the sample with nitrogen as a carrier gas. The following temperature program was adopted for detection of FAME: Initial temperature 100$^{o}$C, 1 min hold; ramp at 10$^{o}$C min$^{-1}$ until 180$^{o}$C with 1 min hold; ramp at 10$^{o}$C min$^{-1}$ until 240$^{o}$C, with a 2 min hold.

## RESULT AND DISCUSSION

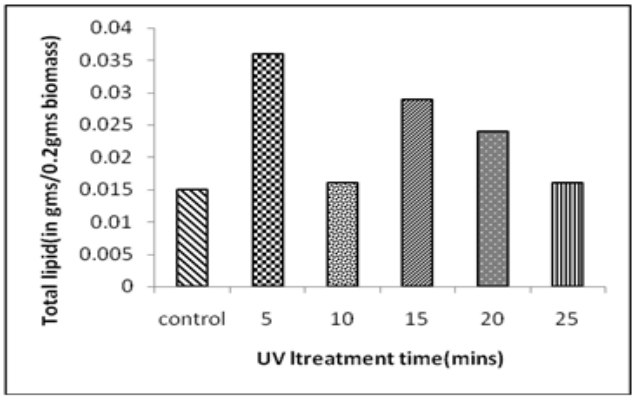
UV treatment for *Chlorella pyrenoidsa* showed the highest increase in biomass yield of 2.22 fold for the sample treated for a period of 25 mins, whereas the samples treated for 10 and 5 minutes showed an increase in biomass yield of 1.2 fold and 1.48 fold, respectively. The remaining two samples exposed to UV for 15 and 20mins has shown a slight decrease in biomass yield of 1.13 and 1.3 fold respectively when compared to control. The biomass yield for different UV exposure times was shown in Fig I.

**Figure I**
***Biomass yield for different UV exposure times***



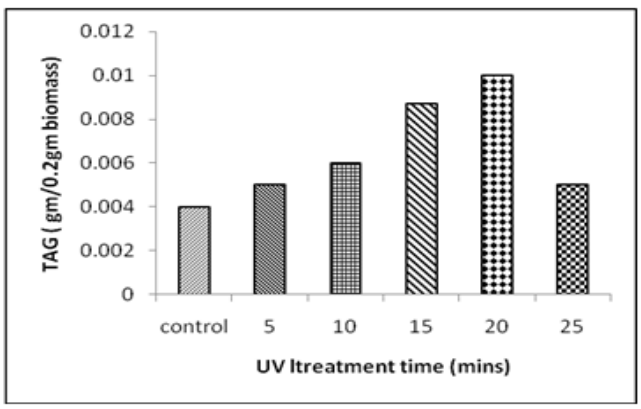

***Total lipid content enhanced with 1.6, 1.93 and 2.4 fold for UV treatment of 20, 15 and 5mins, respectively. The total lipid variation with different exposure time was given in Fig II.***

**Figure II**
***total lipid variation with different exposure time***




***Significant TAG enhancement of 2.5 fold and 2.17 fold was found in the samples with UV exposure time of 20 and 15minutes, respectively, whereas other exposure times showed insignificant increase in TAG. TAG yields for different UV irradiation are shown in Fig III.***

**Figure III**
***TAG yields for different UV irradiation***




The exposure time of 25 mins lead to significant increase in biomass but it did not show any effect on total lipid and TAG yield whereas exposure time of 20 mins and 15 mins reported an increase in total lipid as well as in TAG yield. UV irradiation for 5 mins shown drastic increase in total lipid over 15 and 20 mins but

that did not contribute to the increase in TAG yield as an increase in total lipid might be because of other two lipid fractions i.e glycolipids and phospholipids. The GC analysis of the neutral lipid fractions showed an increase in monounsaturated fatty acids like myristoleic acid and Cis 10 pentadecanoic acid, which is considered as one of the preferred property for biodiesel. The fatty acid analysis of the neutral lipid fraction for different UV irradiation times was given in table I.

**Table 1**
***Fatty acid analysis of the neutral lipid fraction for different UV irradiation times***

| Fatty acid | Control | 5 min | 10 min | 15 min | 20 min | 25 min |
|---|---|---|---|---|---|---|
| Myristoleic acid methyl ester(14:1) | 0.6 | 2.1 | 2.7 | 1.5 | 4.2 | - |
| Petadecanoic acid methyl ester(15:0) | 2.1 | 3.7 | 4.3 | 3.0 | 5.1 | 3.9 |
| Cis 10 pentadecanoic acid(15:1) | 0.8 | 0.8 | 3.9 | 1.2 | - | 1.8 |
| Palmitic acid(16:0) | 0.8 | 1.0 | 4.9 | 1.7 | 0.8 | 2.6 |
| Palmitoleic acid(16:1) | 13.9 | 10.7 | 6.8 | 12.8 | 11.8 | 10.2 |
| Heptadecanoic acid methyl ester(17:0) | 1.7 | 5.7 | 2.0 | 6.2 | 1.4 | 5.1 |
| Cis 10 heptadecanoic acid(17:1) | 0.8 | 2.4 | 0.8 | 3.0 | 3.0 | 4.4 |
| Oleic acid(18:1n9c) | 0.2 | 5.3 | 9.4 | 12.1 | 6.4 | 11.8 |
| Lenoelaidic acid(18:2n6t) | 5.3 | 5.7 | 6.7 | 6.3 | 4.6 | 4.3 |
| Lenoliec acid(18:2n6c) | 0.2 | 0.5 | 0.6 | - | 0.5 | - |
| Arachidic acid(20:0) | 4.2 | 3.3 | 5.6 | 5.3 | 2.6 | - |
| Cis 11 eicosenoic acid(20:1) | 0.3 | - | - | 0.4 | - | 0.5 |
| Linolenic acid(18:3n3) | 12.2 | 10.2 | 12.4 | 18.4 | 4.5 | 9.2 |
| Behenic acid(22:0) | 2.0 | - | 1.4 | - | - | 0.9 |

## CONCLUSION

In this study an attempt was made to enhance the production of TAG in *Chlorella pyrenoidosa* using UV irradiation was made. In this processes significant increase in TAG was observed under UV exposure of 20mins and 15mins with 2.5 fold and 2.17 fold rise in TAG yield respectively. The highest biomass yield was observed for 25mins but showed an insignificant increase in TAG. The effect of UV irradiation on *Chlorella pyrenoidosa genome* that lead to an increase in the yield of TAG which can be further investigated by whole genome sequencing.

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## CONFLICT OF INTEREST

Authors declare that no conflict of interest.

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