# Biodiesel Production from Algae Exposed to Stressful Conditions

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**A large use of petroleum sourced fuels is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment. Renewable, carbon neutral, transport fuels are necessary for environmental and economic sustainability. Biodiesel, as an alternative fuel, has many merits. Microalgae are unicellular biofactories that can make oil (TAGs) from sunlight and $CO_2$. Approaches for making algal biodiesel economically competitive with petrodiesel are being reported earlier. But better algae strains will be needed if algae-derived biofuels are to achieve their potential. There is a need 'to enhance growth rates, to reduce the cost of oil extraction and reduce the cost of nutrients'.An alga (*Cladophora glomerata*) isolated from Mutha-Mula complex (Sangam Bridge) in Pune (India) was subjected to various stressful conditions and studies were carried out to determine whether they are beneficial or detrimental to the amount of oil content present in the alga. The study showed out that mutation by exposure to UV radiation brought about certain enhancing changes with 5 min UV exposure. The exposure to electric current did not show any enhancing effects. It showed a gradual reduction in the algal oil content as compared to the standard reading. The biomass obtained was more in the electric current passage studies as compared to the biomass content in UV radiation studies.**

**Key words:** Algae, biodiesel, algal oil, lipids, Triacylglycerol.

Biodiesel is a clean-burning, oxygenated fuel (methyl-ester) made from vegetable oils or animal fats which can be burned in existing diesel engines.

Biodiesel is an alternative fuel for diesel engine that is gaining attention because of its primary advantages are that it is one of the most renewable fuels currently available and it is also non-toxic and biodegradable[1]. It can also be used directly in most diesel engines without requiring extensive engine modifications[2].

Biodiesel derived from oil crops is a potential renewable and carbon neutral alternative to petroleum fuels. Unfortunately, biodiesel from oil crops, waste cooking oil and animal fat cannot realistically satisfy even a small fraction of the existing demand for transport fuels. Algae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels[3]. Like plants, algae use sunlight to produce oils but they do so more efficiently than crop plants. Oil productivity of many algae greatly exceeds the oil productivity of the best producing oil crops. Unlike oil crops, algae grow extremely rapidly and many are exceedingly rich in oil.

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Oil content in microalgae can exceed 80% by weight of dry biomass[4]. Depending on species, microalgae produce many different kinds of lipids, hydrocarbons and other complex oils[5, 6,7].

Microalgae with high oil productivities are desired for producing biodiesel

Many microalgae have the ability to produce substantial amounts (e.g. 20–50% dry cell weight) of triacylglycerols (TAG) as a storage lipid under photo-oxidative stress or other adverse environmental conditions[8]. Under stress conditions, lipids formation occurs as the photosynthetic rate declines. Algae that produce large quantities of lipids generally demonstrate the ability to subsist under the stress for a prolonged period[9].

Current research is however focused on isolation and selection of commonly occurring algae, screening and evaluating the oil production by placing the algae under stressful conditions for lipid induction.

One method used was to expose the isolated algae to Ultra-Violet radiation and study the mutational effect if any.

Other method was the electro-system. The electro-system was developed and used for wastewater treatment and the effect of this very novel, improved process showed that the rate of bacterial generation was increased to double, i.e., the cell doubling time to half by application of pico-current[10].

## MATERIAL AND METHODS

Algal samples were collected from the banks of Mutha - Mula River, in Pune and stored at 4ºC until inoculated in the culture medium.

**Identification of strains**

The identification of the algae collected was done under the guidance of Dr. Chowgule, Head of Botany Department, at the University of Pune.

**Culturing of the selected strain**

Out of the 4 algal strains obtained, Cladophora was obtained in large quantity and therefore was physically separated at the R D Aga Research Technology and Innovation Centre, Thermax Limited, Chinchwad, Pune, under the guidance of Dr. V.K.Raman, Divisional Manager & Head.

The isolated alga was inoculated in sterile Modified CHU No. 10 media and was incubated at room temperature under the influence of 200 watts light for 3 weeks. The growth from the agar plates was subcultured into two 1-liter flasks for obtain bulk growth required for further studies.

**Oil Extraction**

After obtaining adequate growth, the broth was filtered and the alga was recovered in a Petri dish. The wet weight of the alga was recorded and divided into two parts. One part was dried in a hot air oven at 80ºC for 40 minutes for releasing water. Dry weight was recorded. Aliquots of 20 grams of dried algae were ground with mortar and pestle as much as possible. Hexane and ether solution, in equal quantities (15 ml) were mixed with the dried ground algae to extract oil. Then the mixture was kept for 24 hrs for settling to obtain standard oil content reading.

**The remaining dried alga was taken for the following studies**

**Mutation by UV radiation**

UV radiation is one of the most important mutagenic agents. Four Petri dishes containing 20 grams of partially-dried alga each were exposed to UV radiation (260 nm) for time intervals of 5 mins, up to 20 mins. The algal oil from each of the dishes was obtained by the same method as above.

**Effect of electro-system**

Four trays of 20 grams of alga each was taken from the partially-dried filtered portion and subjected to electric current (50 volts) in an electrophoretic unit for time interval of 5 minutes. The algal oil from each tray of alga was obtained by the same method described above.

**Mixing of algal oil with catalyst and Methanol**

The algal oil obtained from both the methods above, is mixed with methanol (24mL) and a catalyst - Sodium Hydroxide (0.25gm) to produce Biodiesel. The mixture was kept overnight and later separated from sedimentation by flask separator. The above layer was collected and washed with 5% water.

## RESULTS

**The samples collected from the Mutha- Mula River were identified as follows**

a) Cladophora

b) Lyngbya
c) Spirogyra
d) Oedogonium

In this study, *Cladophora glomerata* was found to be in abundance but at the same time the remaining three strains were detected to be in their death phase. Therefore, physical separation was done to eliminate these three strains from the study.

**The alga inoculated on agar plates and broth showed the following observations**

1. The growth of the alga was confluent and did not cover the entire dish in the first week
2. The growth of alga in the second week was found to be more quantitative and without contamination.
3. The growth obtained was then inoculated in the broth flasks for better growth.

**Interpretation**

The interest in algal oil is not new, though the widespread interest in making Biodiesel from algal oil is more recent. Many experts believe that phytoplankton lipids are by far the most promising source for a bio-fuel (11). Enhancing Fatty acid composition of algal oils suitable for preparation of Biodiesel is of prime importance. Biodiesel (monoalkyl esters) is one of such alternative fuel, which is obtained by the transesterification of triglyceride oil with monohydric alcohols. Triacylglycerol (TAGs) production in most algae is correlated to several types of stress conditions. The development of algal strains that produce maximum storage lipids under conditions of maximal photosynthesis would be a breakthrough in the commercial development of algae.

In an effort to better comprehend how physical and chemical processes affect the biosynthesis of energy-rich lipid compounds in algae, the goal of this study was to expose the algae to UV radiation and electric current and oil extracted through repeated washing, or percolation, with an organic solvent such as hexane and ether.

The algal oil was obtained from both the methods described above and mixed with methanol (24mL) and a catalyst - Sodium Hydroxide (0.25gm) to produce Biodiesel.

After mutational studies were performed, it was found out that mutation by exposure to UV radiation brought about certain enhancing changes with 5 min UV exposure.

**Table 1.** Results of Mutation with UV radiation

| Plate No | Time of exposure (minutes) | Dry weight (gms) | Extracted oil (gmas) | Weight of Biomass (gms) |
|---|---|---|---|---|
| 0 Standard | Nil | 20 | 1.2 | 3.5 |
| 1 | 5 | 20 | 1.4 | 3.1 |
| 2 | 10 | 20 | 0.9 | 3.4 |
| 3 | 15 | 20 | 0.9 | 3.6 |
| 4 | 20 | 20 | 0.7 | 3.2 |

**Table 2.** Results of electro-system

| Plate No | Time of exposure (minutes) | Dry weight (gms) | Extracted oil (gmas) | Weight of Biomass (gms) |
|---|---|---|---|---|
| 0 Standard | Nil | 20 | 1.7 | 3.2 |
| 1 | 5 | 20 | 1.1 | 4.6 |
| 2 | 10 | 20 | 1.1 | 4.7 |
| 3 | 15 | 20 | 0.8 | 4.2 |
| 4 | 20 | 20 | 0.6 | 4.1 |

The exposure to electric current did not show any enhancing effects. It showed a gradual reduction in the algal oil content as compared to the standard reading.

The biomass obtained was more in the electric current passage studies as compared to the biomass content in UV radiation studies.

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