Mapana Journal of Sciences
2018, Vol. 17, No. 4, 37-51
ISSN 0975-3303 | https://doi.org/10.12723/mjs.47.4

Sustainable Production of Algal Biodiesel Using *Chlorella Pyrenoidosa*

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Abstract

Production of sustainable, alternative fuel sources and their efficient utilisation is important for the global fuel supply and the environment. The microalgae, *Chlorella Pyrenoidosa* with high lipid content were cultured and the extraction of the oil-rich constituents was done by sonication followed by transesterification. The characterisation of the fuel based on saponification value, TLC, GC analysis, and the temporal profile of the biomass and the oil were performed. The oil extracted from *C. Pyrenoidosa* was found to be efficient for biodiesel production in large scale.

Keywords: *Chlorella Pyrenoidosa*, Biodiesel Production, Transesterification

1. Introduction

Large-scale depletion of fossil fuels has demanded research into renewable, environment-friendly alternatives for fuel. These are

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usually obtained from biological sources like food crops, although microalgae and their high lipid content are also desirable. Biodiesel, an alternative to fossil fuels [1] is a non-toxic, renewable, and biodegradable fuel [2], [3]. It is the mono-alkyl ester of long-chain fatty acids derived from renewable feedstocks, such as vegetable oil or animal fats [4]. Petroleum Derived Fuels (PDFs) are unsustainable due to the increase in Greenhouse Gases [5], [6], [7], the accumulation of untreated wastewater [8-11] and climate change. Algae trump food crops in the production of biofuels as they occupy lower space, can be cultivated anywhere and don't threaten food security in the region [13], [14]. They also have a higher oil [6] [15-17] content than food crops.

Biodiesel can be used to immediately replace conventional diesel in the transportation market and has many environmental benefits over other fuels, such as, helping in the reduction of the carbon footprint. It has the potential to substitute a portion of the oil consumed by automobiles because of the pre-existing diesel distribution infrastructure and vehicle fleet. Compression-ignitio diesel engines in the transportation sector can operate on biodiesel with little or no costly alterations. One specific source for biodiesel production that holds much potential is microalgae (National Biodiesel Board, 2009).

Biodiesel offers various advantages such as prolonged engine life, safety, biodegradability, higher flash point, lower exhaust emissions, comparable performance and engine durability, non-flammable and non-toxic, and reduces tailpipe emissions, visible smoke and noxious fumes, and odours [18-19]. Short-term problems of using biodiesel occur due to cold weather, plugging and gumming of filter lines and injectors, and engine knocking. Long-term effects include the coking of injectors and carbon deposits on the piston and head of the engine and failure of the proper functioning of the engine lubricating oil due to polymerisation. Most of them can be overcome by timely maintenance [12].

Microalgae-based biodiesel [6] [15-16] is a potentially renewable resource for displacement of liquid transport fuels derived from petroleum. Microalgae are easy to culture and require less space for cultivation. They convert carbon dioxide to potential biofuels,

valuable bioactive compounds such as carbohydrates, proteins, lipids and pigments. They contribute around 40 to 50 per cent of the oxygen in the atmosphere and consume carbon dioxide to grow photo-autotrophically and can be converted into several different types of renewable biofuels such as Green Diesel, Jet Fuel, Methane Biogas, Ethanol, and Butanol. Green Algae (Chlorophyceae), Diatoms (Bacillariophyceae) and Golden Algae (Chrysophyceae) are the three most important classes of algae. Green Algae, belonging to the genera *Chlamydomonas, Chlorella, Haematococcus and Dunaliella* are widely utilised. As aquatic relatives of plants, microalgae flourish in aerated, liquid cultures where the cells have sufficient access to light, carbon dioxide [20], and other nutrients.

Algae have high lipid content [21], about 10-200 times more oil when compared to oilseed crops [22-23]. Microalgae are efficient biological factories capable of taking zero-energy forms of carbon and synthesising it into a high-density liquid (natural oil) and, are capable of storing carbon in the form of natural oils or as a polymer of carbohydrates. The lipid content of planktonic algae is considerably higher than of terrestrial plants. Some types of algae are made up of about 40% fatty acids based on their overall mass. *Chlorella Pyrenoidosa* is a non-flagellated, spherical, single-celled, freshwater, autotropic microalga and belongs to phylum Chlorophyta.

Triglycerides, a major neutral lipid can be used for the production of biodiesel from algae. The chain lengths of fatty acid vary from C-10 to C>20 depending on the species. Neutral lipids basically consist of hydrocarbons and triacylglycerols (TAGs). TAGs can be used as a feedstock for the production of biodiesel.

Production of biodiesel has been done primarily in four ways - direct use and blending, microemulsions, thermal cracking (Pyrolysis) and transesterification. Most commonly, production from microalgae occurs by base catalysed transesterification with alcohol [24]. Excess alcohol is added to drive the equilibrium toward the product's (biodiesel) side. The simplified transesterification reaction is

Triglycerides + free fatty acids + alcohol → alkyl esters + glycerin

Reaction 1.1 Generalized Transesterification Reaction

Figure 2.1: Generalised transersterification reaction

Methods such as mechanical extraction using hydraulic or screw, enzymatic extraction, chemical extraction through different organic solvents, ultrasonic extraction, and supercritical extraction [25] using carbon dioxide are used for oil extraction. The ultrasonic extraction of algal oil involves intense sonication of liquid which generates sound waves that propagate into the liquid media resulting in alternating high-pressure and low-pressure cycles. In brief, many parameters including lipid content, growth rate, fatty acid composition and cultivation conditions should be considered to identify the most promising microalgae species.

Various strains of the microalgae have been reported [26]. The production of the algal biofuel using the microalgae is not robust since it lacks many protocol standardisation, consistent production and its uses. In the present investigation, the appropriate strain selection for the production of the algal oil was carried out by culturing the algae, extracting the oil, converting the oil to the biofuel, and standardising the produced biofuel.

2. Materials and Methods

The flow diagram (Fig 2) displays the methods that were used in the production of biodiesel from *Chlorella Pyrenoidosa*. Based on the literature survey for the production of biodiesel, the suitable microalgae strain (*Chlorella Pyrenoidosa*) having high lipid content was selected. Culturing of species was done for their effective growth in laboratory conditions using a suitable medium. The biomass was harvested and the oil was extracted. The extracted oil was characterised to know the free fatty acids and transesterification was carried out to obtain the biodiesel.

2.1 Culturing of Green Algae

The selected strain *Chlorella Pyrenoidosa* was collected from NCIM, Pune, India. The procured *Chlorella Pyrenoidosa* (Green Algae) was kept at a low temperature (27°C). The growth of *Chlorella Pyrenoidosa* was suitable in Fog's media for culture methods and growth measurements. Fog's media was sterilised at 121°C for 15 minutes and transferred to 250 ml conical flasks. Collected cultures were inoculated to conical flasks. *Chlorella Pyrenoidosa* was cultured to obtain visible biomass at a pH of 7.5 using the following three methods.

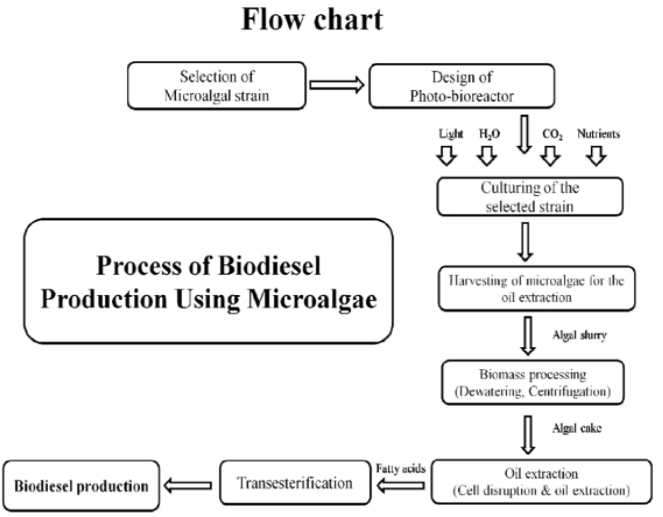

Figure 2.2: Production of Biodiesel

2.1.1 Petri plate and Test Tube Culture

The collected strain was cultured on solid Fog's agar medium in a hot air oven for 7-8 days; growth was observed and preserved for future use.

2.1.2 Shake Flask Culturing of *Chlorella Pyrenoidosa*

Culturing of *Chlorella Pyrenoidosa* was done in 500 ml of the sterilized media which were inoculated with the samples and the flasks were kept in a rotary shaker for 15 days at a rotation speed of 115 rpm. A visible biomass growth was seen in the flasks after 8-9

days of culturing. The contents in the conical flasks were centrifuged in order to obtain biomass of *Chlorella Pyrenoidosa*. Pellets were separated from the supernatant. These pellets were transferred to fresh media and kept in a rotary shaker for 15 days.

2.1.3 Bioreactor Culture

A horizontal glass PBR was set up which was provided with a source of light, aeration, thermometer, inlet and outlet. Sterilised Fog's media was added to the bioreactor. 250 ml of Inoculum was added. The temperature was maintained at 28°C. A visible biomass growth of *Chlorella Pyrenoidosa* was observed.

2.2 Harvesting of *Chlorella Pyrenoidosa*

Chlorella Pyrenoidosa attained the stationary phase at 15th day and started accumulating the oil in the cells. The microalgae biomass was harvested by centrifugation for 10 minutes at 10,000 rpm. The supernatant was discarded and pellets were taken in a pre-weighed tube. The sample was then crushed in pestle and mortar. The weight of tubes along with the sample was taken and tubes were sealed. The Petri plates were stored in a refrigerator.

2.3 Extraction of Oil from *Chlorella Pyrenoidosa* Biomass

The Algal biomass obtained was placed in a mortar pestle, grounded and sonicated for 3-5 minutes. 27.8 grams of sample was dissolved in (90%) of ethanol. Sonication was carried for 15 minutes. The sample was centrifuged twice at 10,000 rpm for 10 minutes. Pellet was discarded and the supernatant was taken in a beaker. The supernatant was transferred to centrifuge tubes. The solvent (Ethanol) evaporated leaving behind the oil. The characterisation of crude oil was carried out based on three of the following parameters.

2.3.1 Saponification Value

Saponification value is expressed by Potassium Hydroxide (KOH) in milligrams required to saponify one gram of fat. Saponification index for the crude oil obtained was experimentally determined by treatment with alcoholic KOH solution, followed by titration of the KOH excess with HCl, according to the standard protocol. (Romanian Standard SR EN ISO 3657: 2005).

Saponification value is given as:

SV = [(blank–test) * 0.028 * 1000 * N_{HCl}] / (wt. of oil * N_{KOH})

2.3.2 TLC Method for the Identification of Lipid in the Oil

TLC was carried out to separate triglycerides from oil using the solvent system.

Chloroform: Hexane: Methanol was taken as a solvent in the ratio of 8:6:1. The yellow colour on the TLC sheet confirmed the presence of lipid in the algal oil.

2.3.3 GC Analysis of Free Fatty Acids

GC analysis was carried out to determine free fatty acids content of oil obtained from pestle and mortar and sonication. A DEGS-15% stainless steel column of 3m and 3mm ID having a Chromosome W solid support was used. The run was conducted for 30 minutes at a temperature of 180°C, 220° C, 230°C, 210°C for the column, injector, detector, and ageing respectively.

2.4 Transesterification

Transesterification was carried out to obtain bio-diesel from the crude oil. The alkali catalysed method using NaOH or KOH and methanol was used for the transesterification of algal oil to create biodiesel. The algal oil was added to the beaker and placed on a water bath at 55° C for 2 hours. The methanol NaOH/KOH was added to the beaker and stirred until dissolved. The mixture would then phase separate as Glycerin has a higher density and is insoluble in biodiesel. The biodiesel phase was extracted and washed with Magnesol to remove any impurities.

3. Results and Discussion

In this study, Fog's media was used for the growth of *Chlorella Pyrenoidosa*. In Fog's media, visible growth of microalgae was seen in 15 days after inoculation. It was observed that the growth of *Chlorella Pyrenoidosa* in three culturing techniques was dependent on factors such as surface area, a number of inoculums added, the temperature at which it was maintained, the degree of aeration and so on. Table 3.1 shows time taken for visible growth by different

culturing techniques. Fig 3.1 shows the growth of *Chlorella Pyrenoidosa* in solid media.

Table 3.1: Culturing of *Chlorella Pyrenoidosa* using three different methods

Methodology No.	Culturing Technique	Growth (in days)
1	Petriplate and test tube cultures	10-12
2	Shake flask method	11-14
3	Photobioreactor	16-22

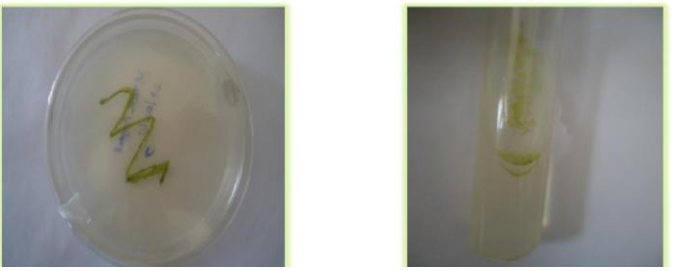

Figure 3.1: Growth of *Chlorella* in solid media

The extraction process was carried out and the supernatant was discarded. The pellets were taken in a pre-weighed tube. The amount of oil obtained from sonication and the pestle method is as shown in Table 3.2.1 and 3.2.2.

Table 3.2.1 Extraction of oil from Algal biomass by sonication

Time of Sonication	Weight of Eppendorf (g)	The weight of Eppendorf + Weight of the sample (g)	The weight of oil (g)
5 min	1.02	1.55	0.53

Table 3.2.2: Extraction of Oil from Chlorella pyrenoidosa Biomass by Pestle and Mortar

The weight of Eppendorf Tube	1.02 g
The weight of Eppendorf + Sample	1.025 g
The weight of Eppendorf oil	0.005 g

3.3 Characterisation of Algal Oil

3.3.1 Saponification Value

The saponification value of oil extracted was estimated according to the method of British Pharmacopoeia. The titration carried out, yielded a saponification value of 13.127, ultimately giving 0.367g of Oleic acid. The estimation resulted in 0.41g free fatty acids in 1.42g of oil.

3.3.2 GC Analysis for Free Fatty Acid Present in the Oil

The qualitative free fatty acids were estimated using a Gas Chromatogram. Table 3.3.1 shows the major free fatty acid compounds present in the oil extracted. Fig 3.3 represents the GC analysis of the Chromatogram and free fatty acids. From the GC analysis, Palmitic and Stearic acid were found to be the major fatty acids present in the oil extract. The high enrichment of Palmitic acid and Stearic acid could be desirable for designer biodiesel fuels. Table 3.3.2 gives the profile of free fatty acids of the oil samples obtained by sonication and the Mortar-Pestle method.

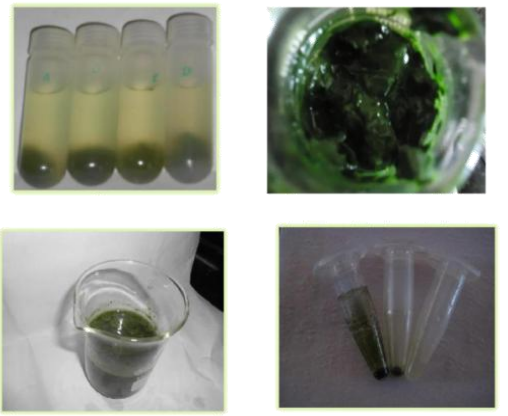

Figure 3.2: Algal pellets obtained (a) and crude oil obtained after extraction (b)

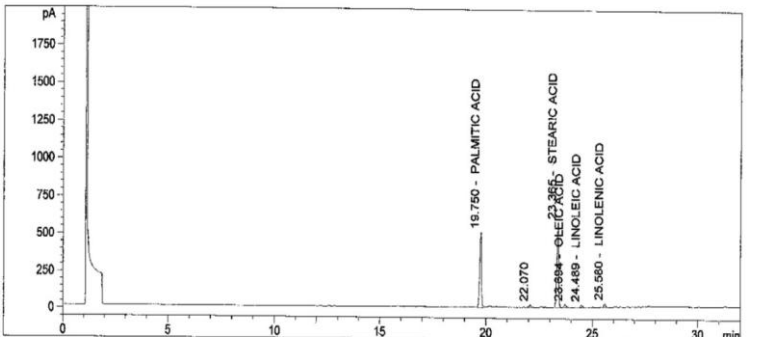

Figure 3.3: GC Chromatogram and fatty acid composition of *Chlorella Pyrenoidosa*

Table 3.3.1: Gas chromatogram of oil sample extracted by Sonication Method

Compound Name	Park	Width (min)	Height	Area (PA*S)	Area%
Palmitic Acid	6	0.10	505.96	2952.06	42.61
Stearic Acid	8	0.10	596.62	3476.94	50.18
Oleic Acid	9	0.13	17.46	139.01	2.01
Linoleic Acid	10	0.11	14.84	100.28	1.45
Linolenic Acid	11	0.10	24.25	138.39	2.00

Table 3.3.2: Free fatty acids profile of oil sample obtained by Sonication and Mortar Pestle method

Fatty Acid	Percentage	RT(min)
Palmitic Acid	42.61	19.75
Stearic Acid	50.18	23.36
Oleic Acid	2.01	23.61
Linoleic Acid	1.45	24.48
Linolenic Acid	2.0	23.58

3.4 Transesterification of *Chlorella Pyrenoidosa*

The obtained microalgal oil was subjected to a transesterification process to obtain biodiesel.

Fig 3.4.1 represents the production of biomass obtained by *Chlorella Pyrenoidosa* after providing the different levels of media. This study was made to understand the nutrition levels, which can support the growth of algae. The trend line shows that the there is an

exponential growth of the biomass with respect to the increase of the media/nutrition. The 'r' value was found highly positive representing the significant positive relationship between algal biomass productions with the increase of the media. The temporal distribution of the growth of the algal biomass for regular intervals of 15 days revealed an increase in the biomass (Fig 3.4.2).

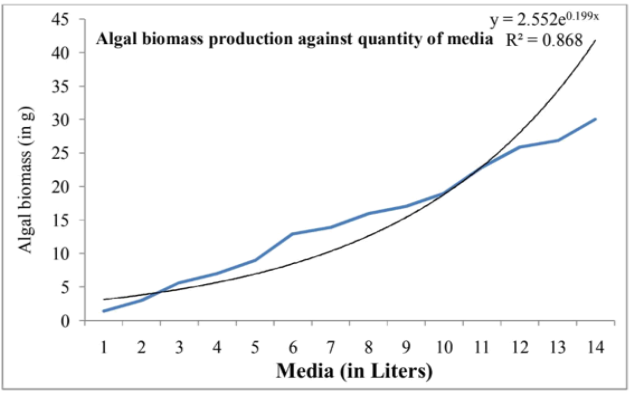

Figure 3.4.1: Prediction of *Chlorella Pyrenoidosa* Biomass obtained corresponding to quantity of Media

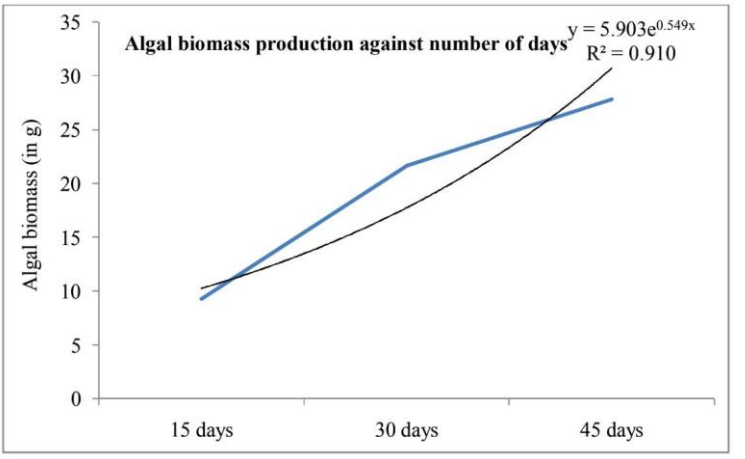

Figure 3.4.2: Temporal Distribution of *Chlorella Pyrenoidosa* biomass production

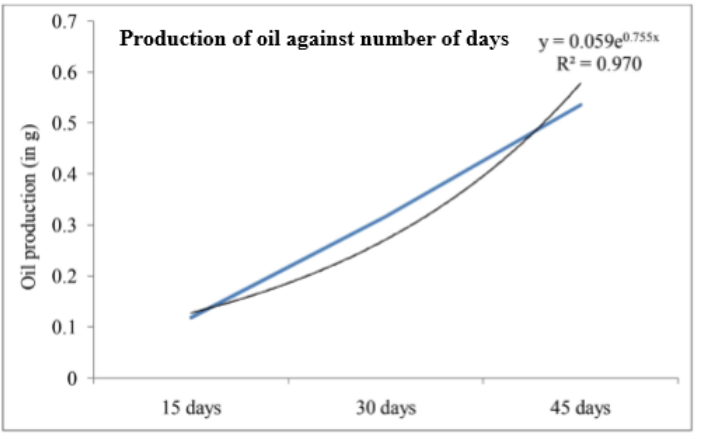

Figure 3.4.3: Temporal Distribution of Oil obtained from *Chlorella pyrenoidosa* Biomass

The growth of the *Chlorella Pyrenoidosa* for different durations has shown similar trends as that of the media. Figure 3.4.3 represents the temporal distribution of the oil obtained from the *Chlorella Pyrenoidosa* species. The temporal production of the algal biomass and the oil exhibits the significant positive relationship with the duration of the study.

From GC analysis the major fatty acids found are Palmitic (42.61) and Stearic acids (50.18), which could be desirable for biodiesel. Hence this species can be recommended for the production of biodiesel on a large scale.

4. Conclusion

The production of biofuel from microalgae has gained considerable attention due to the fact that they can be converted into several different types of renewable biofuels such as Green Diesel, Jet Fuel, Methane Biogas, Ethanol, and Butanol. Algal biodiesel is environment-friendly. They are not harmful to living organisms and also possess high nutritional values. Sonication and Mortar-Pestle method were followed to extract the oil from the *Chlorella Pyrenoidosa* biomass. Saponification value of 0.41g of free fatty acid was found and the obtained algal oil was analysed by GC.

The free fatty acid profile showed: Palmitic acid (42.61%), Stearic acid (50.18%), Oleic acid (2.01%), Linoleic acid (1.45%) and Linolenic acid (2.0%). Palmitic acid and Stearic acid were found to be the major fatty acids present in *Chlorella Pyrenoidosa* oil extract. By transesterification, process biodiesel was produced from the extracted oil. 1.5µl of biodiesel was obtained from 27.8 g of algal biomass. In view of the physico-chemical parameters of microalgae, oil extracted from *Chlorella Pyrenoidosa* can be efficiently used for biodiesel production on a large scale.

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