



Production of Biodiesel from Algal Biomass : *Spirogyra* spp.,

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KEYWORDS	ABSTRACT
Chlorophyceae, Biofuel, transesterification, <i>Spirogyra</i> spp., Glycerin, biomass, LCMS.	<p>In this investigation algal oil was used as a raw material for biodiesel production. The decreasing fossil fuel resources cause both insufficiency in providing demand and increase in prices and it triggers the structural change in energy production and resources. In this context, the innovations in encouraging the use of renewable energy sources will make it possible to manage the passage from an unsustainable structure to a more sustainable structure. The necessary conditions for the world oil supply can be said to enter into a new era with the increasing demand pressure. In the present investigation, naturally occurring algal sample of Chlorophyceae member was collected from waterlogged area near Muttinakoppa, N R Pura (Tq) Chikkamagaluru (Dist.). The collected algal samples was identified as <i>Spirogyra</i> spp., and inoculated into the selective media, which favor the growth of algae, and the lipid was extracted from dried algal samples by ground method by using hexane as solvent. The extracted oil was converted into biofuel by transesterification process and estimates the FAME by using LCMS. The result indicates that the biofuel can be produced from <i>Spirogyra</i> spp.,</p>

1. INTRODUCTION

Biodiesel is an alternative liquid fuel for diesel engines that is produced by transesterification of vegetable oil or animal fat sources. Biodiesel is made by chemically reacting of oil or animal fat with alcohol in presence of catalyst producing fatty acid alkyl ester along with co-product glycerin. According to National Biodiesel

Board, biodiesel is as a mono-alkyl ester (Schneider, D 2008). The depletion of petroleum diesel reserves has caused an increase in demand and price of diesel. Biofuels are referred to liquid, solid and gaseous fuels derived from organic matter, they generally divided into primary biofuels such as fuel wood are used in an unprocessed form primarily for heating and electricity

production, secondary biofuels such as bioethanol and biodiesel are produced by processing biomass and could be used in vehicles and industrial processes. The idea of using algae as a source for biodiesel is not new, but now it has been taken seriously due to the increase in population, and rising price of petroleum. The basic sources of energy are petroleum, coal and natural gas. The continued use of petroleum sourced fuels is now widely recognized as unsustainable because of the depletion supplies and the contribution of these fuels to accumulation of carbon dioxide in the environment leading to increase of global warming. Algal biofuels offer great promise in contributing to the growing global demand for alternative sources of renewable energy (Ranjith Y and Parameswara Naik T 2021).

An innovative and attractive life style of human is fulfilled by primary energy source fossil fuel. The energy demand flies higher due to increasing population and industrialization. The world may face the challenges like rising prices of petroleum fuel, energy security, deforestation and growing global warming. Hence researcher seriously focused on the renewable energy sources as key solution for replacement of fossil fuel (Ranjith Y and Parameswara Naik T 2021). The bioenergy is becoming increasingly relevant as a possible and potential alternative to fossil fuel. Biofuels are liquid or gaseous fuels produced from biomass resources and used in place of, or in addition to diesel and other fossil fuel for transport, stationary, portable and other applications. Biofuels are derived from renewable biomass resources like agriculture, forestry and aquatic environment (Weissman, J.C. and D.M. Tillett 1992). These sources are taken in good consideration as feedstock producer for making the biofuel such as biodiesel, bioethanol, bio-oil and biogas. The utilization of renewable biomass energy in large extent provides sustainable development which link to global stability, economic growth, innovation in local market, reduces Green House gas emission and meeting the energy needs of vast rural population to get quality of life (Sanjaykumar N. D., et al., 2013).

Transesterification or alcoholysis is the reaction of a lipid with an alcohol to form esters and a by-product, glycerol. This reaction actually converts highly viscous raw lipid/oil into low molecular weight molecules in the form of fatty acid alkyl esters which can be used as an alternative fuel for diesel engines. Biodiesel is a term

used to describe "fuel comprised of monoalkyl esters of long-chain fatty acids that are derived from vegetable oils or animal fats" The demand for energy is increasing day by day due to the rapid growth in population and industrial development. The basic sources of energy are petroleum, natural gas, coal. The continued use of petroleum sources fuels is now widely recognized as unsustainable because of the depletion supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment leading to increase of global warming. Biodiesel from oil crops, waste cooking oil and animal fat cannot realistically satisfy even a small fraction of the existing demand for transport fuels. Recent researchers involved not only the existing renewable sources available from land plants, but also those coming from aquatic systems. The idea of using algae as a source of fuel is not new, but it is now being taken seriously because of the increasing price of petroleum and more significantly, the emerging concern about global warming that is associated with burning fossil fuels (Bangboyc A.I. and Hansen A.C. 2008).

2. MATERIALS AND METHODS

Collection of algal Samples:

The sample was collected from waterlogged area near Muttinakoppa, N R Pura (Tq) Chikkamagaluru (Dist.). and brought to the Department of P.G. studies and Research in Environmental Science, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Shivamogga, The collected algal sample was observed under microscope and identified with the help of standard literature and monographs: Smith (1950), Fritsch (1935).

Isolation and culture of algal samples:

Once the algae were identified, they were inoculated into the selective media, which favor the growth of algae. In case of more than one alga in a sample, serial dilution was performed followed to obtain uni-algal cultures. The samples were cultured in BG-11 media at 27-30°C for 21 days. of lac predators emerging from the caged samples were counted, and the percentage reduction in the incidence of predators over control was worked out.

Composition of BG - 11 medium

Sl. No	Stock	Grams / 1000 ml
1	NaNO ₃	15.0
2	K ₂ HPO ₄ ·3H ₂ O	4.0
3	MgSO ₄ ·7H ₂ O	7.5
4	CaCl ₂ ·2H ₂ O	3.6
5	FeC ₆ H ₅ O ₇ ·NH ₄ OH	0.6
6	Na ₂ CO ₃	2.0
7	Citric acid	0.6
8	EDTA Solution	0.745 gm Ethylene Diamine Tetra Acetic acid and 0.557 gm of FeSO ₄ ·7H ₂ O – 1ml in 1 Liter

Harvesting:

The algal culture was filtered with the help of filter paper then weighed separately. Then the filtrate was dried in Hot Air oven at 800C for 3hrs.

Oil extraction

The dried algae were ground with motor and pestle as much as possible. The ground algae were dried for 20 min at 800C in a incubator for releasing water. Hexane and ether solution (1:1 vol) were mixed with the dried ground algae to extract oil. Then the mixture was kept for 24h for settling. Then the biomass was collected after filtration and weighted.

Evaporation

The extracted oil was evaporated in vaccum to release hexane and ether solutions using rotary evaporator, and 0.25g NaOH was mixed with 24ml methanol and stirred properly for 20 min.

Biodiesel production

The mixture of catalyst and methanol was poured into the algal oil in a conical flask. The following reaction and steps were followed.

Transesterification

The conical flask containing solution was shaken for 3h by rotatory shaker at 300rpm. After shaking the solution was kept for 16h to settle the biodiesel and sediment layers clearly. The biodiesel was separated from sedimentation by flask separator carefully. Quantity of sediment was measured. Biodiesel was washed by 5% water until it was become clean. Biodiesel was dried by using dryer and finally kept under the running fan for 12h. And measured by using measuring

cylinder; pH was measured by using pH strips and stored for analysis.

FAME analysis and physical properties

LC-MS was used for the analysis of fatty acid. The Density, Viscosity value of biodiesel was calculated from their percentage.

RESULT AND DISCUSSION

Biomass productivity of algae for selected Media (mg ⁻¹)							
<i>Spirogyra spp.</i>	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
	2.00±0.04	3.45±0.02	6.11±0.02	8.65±0.03	12.21±0.25	16.00±0.03	20.01±0.02

The investigation was carried out to isolate and growth prospecting of algae for biofuel production. The *Spirogyra spp.* showed maximum dry biomass weight for BG-11 (20.01 gms). The remarkable growth rate was observed in BG-11, the lag phase on first 3 days, the exponential growth phase was seen on 20th day and stationary phase was observed on 21st day. The biomass productivity of *Spirogyra spp.* in BG-11 was observed 1.047 gl⁻¹ d⁻¹ and lipid extracted from biomass was 0.5714 gl⁻¹ d⁻¹, the 14.01% lipid content was obtained.

The result shows that the biofuel can produced from Chlorophyceae members *Spirogyra spp.*, Algae are simple autotrophic organisms and from simple inorganic molecules such as carbon dioxide they produce complex organic compounds using energy from light or inorganic chemical reactions. Lipids extracted from *Spirogyra spp.*, used for the biodiesel production. Biodiesel is produced with a process known as transesterification. Biodiesel produced using *Spirogyra spp.*, as lipid source. Glycerol is a byproduct of biodiesel production and it can be used in food industries, pharmaceutical industries and cosmetic industries. Amount of glycerol produced using the lipids of respective algal sample was recorded and the lipid extracted from *Spirogyra spp.*, was the best feed for glycerol production. Biomass is also a byproduct of algal sample and it can be used as a fertilizer or fodder.

The result showed 14.01 % amount of oil in algal sample, and also the Density of algal oil matches the density ranges of a biofuels given by EN 14214 and ISO 15607, and the viscosity range given by EN14214 and ISO

15607 is 4.9 mm²/s, thus our result matches to these standards. LCMS analysis of *Spirogyra* spp., showed the following types of lipids present: Myristic acid (C14:0), Palmitic acid (C16:0), Linolic acid (C16:3), Stearic acid (C18:0), Oleic acid (C18:1), Linoleic acid (C18:2).

Algal biofuels offer great promise in contributing to the growing global demand for alternative sources of renewable energy. However, to make algae-based fuels cost competitive with petroleum, lipid production capabilities of algae need to improve substantially and the use of alternate economic nutrient sources to cultivate algae is recommended for the reduction of production cost significantly.

Samples	% of Algal oil (w/w)	Density g/cm ³	Viscosity (mm ² /sec)	pH
<i>Spirogyra</i> spp.,	14.01	0.889	4.6	7

Conflict of interest statement

Authors declare that they do not have any conflict of interest.

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