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PRODUCTION OF BIODIESEL FROM ALGAE: A BIOFUEL FOR THE FUTURE THAT DEGRADES NATURALLY

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KEYWORDS

Macroalgae,
Lipid extraction,
Transesterification,
Biodiesel.

ABSTRACT

Algae have emerged as a promising renewable feedstock for biodiesel as the quest for alternatives to fossil fuels continues. Macroalgae are the macroscopic, multicellular variety of algae, whereas the Charophyta, a class of green algae that includes, Spirogyra and the stone-worts, are the most intricate freshwater forms of algae. By utilizing CO_2, sunlight, sugar, N_2, P, and K as nutrients, self-rejuvenation and rapid growth enable the production of lipids, proteins, and carbohydrates in enormous quantities over a short period of time. Recent technological developments have increased the efficiency of the growing, harvesting, pre-treatment, lipid extraction, and transesterification processes, bringing macroalgae biodiesel closer to being commercially viable. Although algae are the best alternative, production costs, and biofuel yield remain difficult to achieve. The algal biodiesel/green diesel has the potential to develop into a substitute supply of biodiesel to meet the rising global energy demand, it can be said. The benefits of algae-based biofuels for economic development are numerous. Many socioeconomic benefits that contribute to an outcome that can be maintained by the general public can be obtained by developing an algae cultivation-based biofuel sector.

1. INTRODUCTION

Non-renewable fossil fuel reservoirs will be depleted < 50 years due to their higher demand and over-exploitation. The urgent need for alternative fuel sources has been justified by the rapidly growing global population, ongoing energy crises, the quick depletion of non-renewable energy sources, the explosive growth in-vehicle use, the pollution risks from fuel emissions, and the related health diseases (Rajamani Raman *et al.*, 2019). Over the ensuing two decades, global energy consumption is anticipated to continue to increase. Coal, petroleum-based products, and natural gas are the main sources of fossil fuels that today meet the majority of the world's energy needs. When it became clear that there is a very finite supply of fossil fuels and that burning them causes a number of additional environmental issues, including global warming, the demand for these alternative fuels increased (Abhishek Maharishi *et al.*, 2005, Buran *et al.*, 2003). Biodiesel is a sustainable fuel that can replace petroleum-based diesel and lowers CO_2 emissions (Xiaodong Deng *et al.*, 2009). Any fuel that is produced using plant, algae, or animal waste biomass is referred to as biofuel. Biofuels are regarded as the most environmentally friendly natural energy source (Somerville C. *et al.*, 2007). India consumes almost five times more diesel fuel than gasoline, whereas, almost all other countries in the world use more gasoline than diesel fuel. India needs 200 billion gallons of biodiesel annually at the current consumption rate without using petroleum fuel. The Bioenergy Road Map Vision 2020" to develop new technologies and policies for biofuel production in India. Due to the fact that many algal strains have the capacity to accumulate lipids and that they grow their biomass at a faster rate and produce more photosynthetic energy than their terrestrial counterparts, algae have also become recognized as a potential feedstock for the production of biofuels (Sharma PK *et al.*, 2018). Producing this much biodiesel, require algae to be grown over an area of 13 million acres (5.4 million ha) i.e. only 2% of India's geographical area. Marine algae are a good option for biodiesel production in India because we have a coastline of 7,517 km. Marine algae have greater lipid productivity, are easy to mass produce, and produce lipids with high purity (Banerjee, A *et al.*,2002). Alternative diesel fuel known as biodiesel is produced from sustainable biological sources like vegetable oils and animal fats. It has low emission profiles, is harmless and biodegradable, and is therefore good for the environment. Because it is derived from renewable resources and has positive environmental effects, biodiesel has recently gained popularity. According to theirsources, biofuels are typically divided into three generations. First-generation biofuels, such as those produced from biomass made up of sugar, starch, vegetable fats, and oils, are those that are acquired straight from the food source (Priya DPS *et al.*, 2021). The second generation of biofuels is those that arecreated from plant biomass, which is primarily made up of lignocellulosic materials, as this creates the majority of the abundant and cost-effective non-food chemicals that are available from plants (C, Yan J *et al.*, 2015, Naik SN *et al.*, 2010). Previous studies have outlined the major capabilities of green algae inferred biomass for the production of a better kind of third- age biofuels, which have been sought after aspracticable and practical alternatives to non- renewable energy sources (Behera S. *et al.*,2015). When biodiesel is the desired result, either extracted oil is further refined via the chemical process of transesterification or oil produced through thermochemical means is further processed to produce biodiesel (Aresta, M. *et al.*, 2005). For macroalgae, concurrent transesterification and oil extraction has also been attempted (Maceiras et al. 2011).). Macroalgae and microalgae are the two basic classifications of algae. Microalgae are minute and unicellular, whereas macroalgae are the macroscopic, multicellular variety of algae.In the coastal regions of the world's oceans and seas, macroalgae are common and fall under the category of plants. Macroalgae are divided into three groups based on the color of the pigments in their biomass: green algae (Chlorophyta division), red algae (Rhodophyta division), and brown algae (Phaeophyta division). However, the three types of macroalgae also differ in terms of the quantity and kind of lipids, proteins, and carbohydrates in their structure in addition to the color of the pigments. Macroalgae typically include 80–90% water in addition to 50% carbohydrates, 1% lipids, and 7–38% minerals in their dry weight (Guiry, M. D. *et al*., 2012). Midway through the 19th century, the first biodiesel plant in the world advocated using algae as a source of both food and energy. During the Second World War, large-scale *Chlorella* algae farming was started in Japan, England, and Israel. The idea of employing these algae to produce energy was diverted to the production of food staples due to the abundance of fossil fuels (Yi-Feng C *et al*., 2011). Recently, there has been a lot of interest in the idea of using algae to produce alternative fuel. Algal biomass is mostly composed of lipids/natural oils, proteins, and carbohydrates. Algae are the only focus of the biodiesel industry since the majority of the natural oil produced by them is tri-glycerol, the ideal type of oil for creating biodiesel. Because of their quick biomass output and relatively high oil content, algae have long been considered potential promising sources for biofuel production. Algal mass culture can be carried out on non-arable areas utilizing non-potable saline water and wastewater since algae grow far faster than terrestrial crops do (Zhiyou Wen et al., 2019).

2. MATERIALS AND METHODS

2.1 COLLECTION OF ALGAL SAMPLES

Microalgae and macroalgae water samples were taken aseptically from locations in Thoothukudi, Kumbakonam, and Karur that seemed to have algal growth. The macroalgae were manually gathered. A Mesh net was used to catch microscopic algae. The algae are allowed to settle and gather at the bottom of the container after the water samples were left overnight. Both the water sample of algae and the floating algae on the water were taken separately from the test site and placed in clean plastic containers with labels.

2.2 ISOLATION OF ALGAE FROM ENVIRONMENTAL SAMPLE

From ponds, lakes, and rivers, water samples with a visible algal population were taken. Whether marine or freshwater algae, cultures from collections, or native wild species are most suitable for large-scale production should be the determining factor in algae isolation. Standard platingtechniques were employed to divide algal populations from the field water samples in order to isolate single algal species. The colonies were isolated using several mediumrecipes. These diluted samples were placed in sterilized plastic petri dishes containing agar media.Throughout the course of 14 days, algae were allowed to grow. Instead of preparing TAP agar plates, the algal samples are directly put into the TAP media solution which is taken in a test tube. 100 microlitre of algal samples was let into the test tubes using Micropipette. Test tubes were kept at room temperature with proper airflow and light facility. Growth of algae in the test tubes can be detected after a few weeks.

2.3 IDENTIFICATION OF ALGAE

Algal cultures were split based on a morphological study of the colonies on an agar nutrient medium. The substance that has been isolated is kept on a tiny slide. At 40X magnification, slide observation is most effective. Using a fluorescence microscope, a high power of at least 40X to 60X is needed to observe species properly. Drawing or taking a photo of algae might help identify it. Following microscopic examination, these isolates were identified to the genus level based on the form of the individual cells.

2.4 SUSPENSION MEDIA FOR ALGAL GROWTH

Tap salt, Phosphate buffer solution, Trace elements, Tris Base, and Acetic acid are a composition of Tap Media solution. Weigh 0.484 g of Tris Base and put it in the bottle. Measure 5 ml of Tap salt and pour it into the bottle. Pipette out 0.075 ml of Phosphate solution into the bottle. Pipette out 0.2 ml of Trace elements into the bottle. Measure 200 ml of water and pour it into the bottle. Pipette out 200 ml of Acetic acid into the bottle. Shake well for mixing. Measure the P^H using a P^H meter. The P^H range should between 6.5 – 7.5. Autoclave at 121 degrees Celsius for 15 minutes.

TABLE 1

Tap salt composition

Chemicals	Conc.
Ammonium Chloride	3.2 g
Magnesium Sulphate Heptahydrate	0.8 g
Calcium Chloride Dihydrate	0.4 g
Deionized water	200 ml

TABLE 2

Phosphate Buffer composition

Chemicals	Conc.
Dipotassium Hydrogen phosphate	28.8 g
Potassium Dihydrogen Phosphate	14.4 g
Deionized water	100 ml

TABLE 3

Trace element composition

Chemicals	Conc.
$Na_2EDTA.2H_2O$	50 g
Zinc Sulphate Heptahydrate	22 g
Boric acid	11.4 g
Manganese Chloride Tetrahydrate	5.06 g
Cobalt (II) Chloride Hexahydrate	1.61 g
Copper Sulphate Pentahydrate	1.57 g
Ammonium Molybdate Tetrahydrate	1.10 g
Iron Sulphate Heptahydrate	4.99 g
Potassium Hydroxide	17 g
Deionized water	200 ml

2.5 CULTURING OF IDENTIFIED ALGAL SPECIES

Flame the mouth of the conical flask which contains the prepared suspension medium by rotating it in a circular motion. Flame the loop until it turns red and let it cool. Pick an isolated colony from the sample TAP agar plate using the inoculation loop. Immediately transfer the colony into the suspension media. Flame the loop and mouth of the conical flask again. Do not forget to seal the sample TAP agar plate with parafilm for future use. Place the conical flask in the incubator which has a lamp in it. Set the speed at 139 rpm and the temperature at 23.9 degrees Celsius. The growth of the algae in the suspension media can be detected after 7 days. Healthy algae should produce green color in the suspension media. The cultivated macroalgae is harvested manually.

2.6 DRYING OF HARVESTED WET ALGAL BIOMASS

Both the dry route and the wet route techniques require a large amount of energy to create biodiesel from algal biomass, but the end product is biodiesel of comparable grade. Energy is used extensively in the dry route process for both drying the biomass and extracting the oil (Aparna Gautam *et al.*, Pundlik R. Bhagat *et al*). The wet algal biomass was dried in the open air. Grind till the powder biomass is obtained.

2.7EXTRACTION OF OIL FROM ALGAL POWDER

Dried, crushed algae were combined with hexane in a test tube. The ratio of biomass to hexane was taken to be 1:2.Then the mixture was kept for 24 h for settling and for separation of the two layers in the test tube. The organic phase containing the algae oil was emptied into another test tube. The

Algal oil was separated from Algae biomass by filtration. The extracted oil was evaporated in a water bath to release hexane.

2.5 BIODIESEL CONVERSION BY TRANSESTERIFICATION PROCESS

1 gram of Sodium hydroxide was added to methanol. Methanol was poured into the algal oil. The ratio of algal oil to methanol was taken as 1:3. Fatty Acid Methyl Esters (i.e. Biodiesel) are obtained.

2.6 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS

The Fourier Transform Infrared Spectroscopy is a versatile technique for the evaluation of functional groups and the structure of chemical compounds. FTIR technique has wide applications such as the complex analysis of petroleum hydrocarbon, oil, heterogeneous catalyst, and complex organic compound (Khopkar S. M *et al*., 2008).

3. RESULTS AND DISCUSSION

3.1 IDENTIFIED ALGAL SPECIES

Under the Fluorescence microscope, the clearly identified macroalgal species is *Spirogyra*

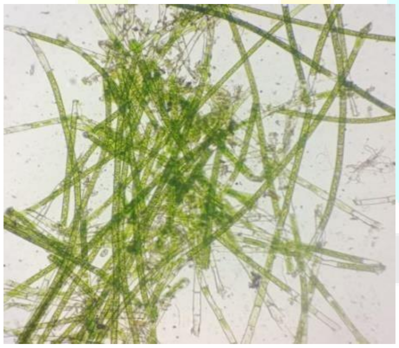

Figure1: Microscopic view of *Spirogyra*

3.2 ISOLATED SPIROGYRA SPECIES

The identified *spirogyra sp.* was isolated from the environmental sample by using a TAP media solution.

Figure 2: Isolated *Spirogyra* species

3.3 CULTIVATED SPIROGYRA ALGAE

The macroalgae *spirogyra* were cultivated in 100ml of Walne's media at room temperature under the light. The growthof the *spirogyra was* seen after 14 days.

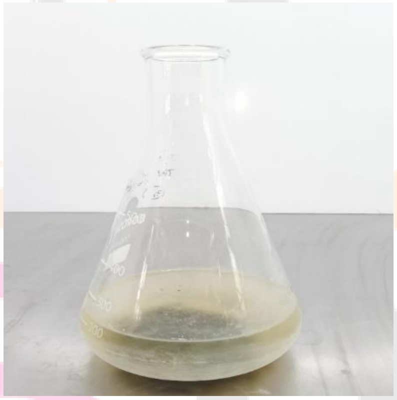

Figure 3: Cultivated *Spirogyra* sp.

3.4 DRIED SPIROGYRA ALGAE

The cultivated macroalgae *spirogyra* were harvested manually and dried in the open air/ Sunlight.

Figure 4: Dried *Spirogyra* sp.

The dried algal biomass was made into powder using mortar and pestle.

Figure 5: Powdered *Spirogyra* algal biomass

3.5 OIL EXTRACTION AND BIODIESEL CONVERSION BY TRANSESTERIFICATION

Hexane was added to the dried algal biomass in the ratio of 1:2, for oil extraction. To that methanol was added in the ratio of 1:3, for biodiesel conversion by transesterification.

Figure 6: Oil extraction and Transesterification

After the transesterification reaction, Fatty Acid Methyl Ester (BIODIESEL) is obtained along with glycerol as a byproduct.

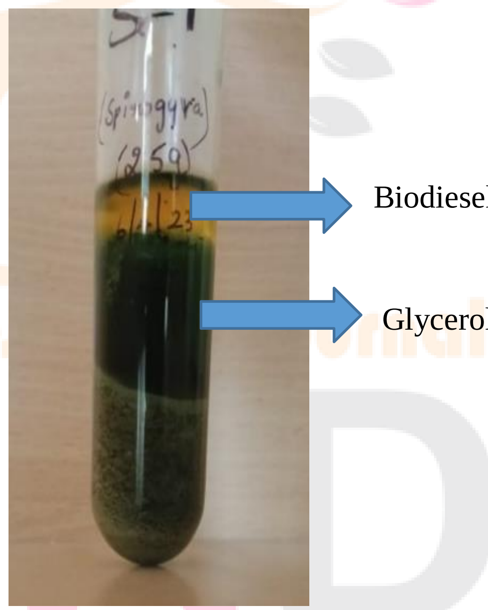

Figure 7: Transesterification

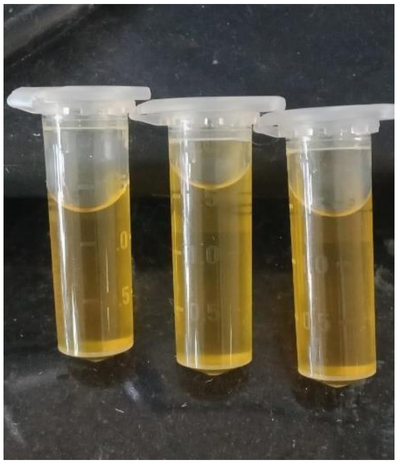

Figure 8: Separated Biodiesel

3.6 FTIR ANALYSIS

The main goal of the FTIR spectroscopy was to show how the transesterification process affected the infrared spectra of biodiesel made from Spirogyra sp. algal oil. The transesterification process most impacts the new signal at 1465.90 cm^{-1}, which is obviously the methyl ester group. The signal at 1728.22 cm^{-1} could be absolutely the ester (C = O) group. 2916.37 cm^{-1} assigned to alkanes (C-H); 2322.29 cm^{-1} and 1265.30 cm^{-1} assigned to C-O; 1643.35 cm^{-1} assigned to alkene (C = C); 1381.63 cm^{-1} assigned to isopropyl group; 1188.15 cm^{-1} assigned to C-O-C; 1080.14 cm^{-1} assigned to polysaccharides; 864.11 cm^{-1} assigned to aromatic groups; 725.23 cm^{-1} to 501.49 cm^{-1} assigned to Halogen compound (C-Cl); 401.19 cm^{-1} to 354.90 cm^{-1} assigned to Halogen compound (C-I) (Laurens *et al*., 2011).

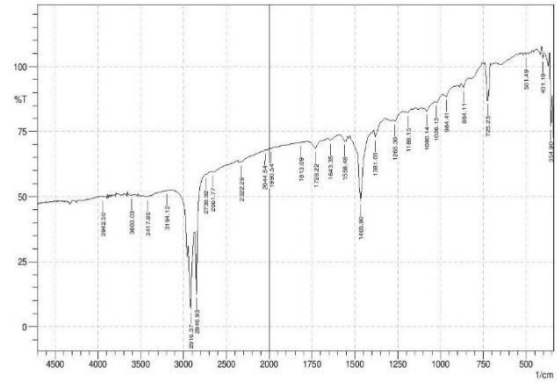

Figure 9: FTIR analysis of biodiesel produced from *spirogyra* with NaOHcatalyst.

4. CONCLUSION

The macroalgae *Spirogyra* species were chosen for the production of biodiesel. The isolated Spirogyra was cultivated using a tap media solution. The macroalgae *Spirogyra* were harvested and dried using the open-air method. Hexane, a solvent was used for the oil extraction process. Using a base-catalyzed transesterification technique, fatty acid methyl ester synthesis was completed. Using Fourier Transform Infrared Spectroscopy (FTIR), the oil and biodiesel esters were evaluated. A high absorption carbonyl frequency peak at 1728.22 cm^{-1} can be seen in the Macroalgae *Spirogyra* biodiesel's FTIR spectra. This analysis indicates that there is a good chance that *Spirogyra* will be used to create biodiesel on a commercial scale in the future.

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