

SUSTAINABLE BIOFUEL PRODUCTION FROM FRESHWATER MACROALGAE CURRENT STATUS AND FUTURE SCOPE

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ABSTRACT

Environmental, economic and political pressures have driven the interest towards the search of sustainable feedstock for bio fuel production. At present, macroalgae (green, brown and red macro algae) is getting growing consideration as an alternative resource for sustainable biomass to produce bio fuels, biochemical and food. The unique chemical composition and wide variation in the availability create various opportunities and also challenges for bio-based energy production. Recently, numerous studies have taken place in the exploitation of seaweed as carbon sources for the bio ethanol production. Thus, this paper attempts to highlight the characteristics, processing techniques and potential applications of the macroalgae. The Current study fresh water macroalgae as an alternative raw material for the biodiesel production. The obtained results show that biodiesel production from oil extracted from fresh algae is feasible by transesterification. Oil extraction can be carried out simultaneously with the transesterification. To investigate the optimum reaction conditions, the reaction was carried out at various methanol to oil molar ratios, catalyst concentrations and reaction temperatures. Moreover, the properties of macro algae transesterification residue after transesterification were analyzed, concluding that it is a suitable material for fuel manufacturing.

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INTRODUCTION

The gradual reducing of fossil fuels, it is now more vital than ever to explore for fuels that can be used as alternatives to crude oil-based fuels such as gasoline and diesel fuel. The conversion of oils into chemicals is identified as long chain mono alkyl esters, or biodiesel. Biodiesel can be formed from a variety of fats and agricultural commodities which consist of oilseeds such as canola and soybeans, rendered animal fats, used restaurant cooking oil, and palm oil. It can also be made from biomass such as from algae blooms. Biodiesel can be utilized in internal combustion diesel engine in its pure form or in any concentration with conventional diesel. The use of its pure form (B100) may necessitate certain engine modifications to avoid maintenance and performance problems. However, it is most frequently found mixed at a ratio of 20% biodiesel to

80% normal diesel (Allen 1998). The use of biodiesel in a conventional diesel engine results in significant reduction of unburned hydrocarbons, carbon monoxide and particulate matter. Biodiesel has no biodegradable and increase lubricity of diesel fuels. This means biodiesel is compatible with the next generation of diesel engine pollution reduction appliances. The recent investigation of the use of alternative, nonfood related feedstock such as oil from algae is become popular. Algae have the capability to convert carbon dioxide to biomass that can further be processed downstream to produce biodiesel, fertilizer and other useful products (Araujo *et al.*, 2017). The truth that algae grow in aqueous suspensions enables algae for more efficient access to water, carbon dioxide and other nutrients. This enables the potential for the making of more oil per unit area than other crops presently used (Bozbas 2008). Biodiesel manufacturers can decide to efficiently take care of wastewater while at the same time produce biodiesel. The chemical composition of algae differs based on species. Thus, algae have several characteristics that enable them to be a (primarily, methanol) in the presence of catalyst. The transesterification reaction can be catalyzing either using homogeneous catalyst

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(acid or base) or heterogeneous catalyst. The use of homogeneous catalyst especially sodium and potassium hydroxide provide higher reaction rate and conversion than acid catalyst for the transesterification of triglycerides (TG) to biodiesel. These alkali (sodium and potassium hydroxide) catalysts are more popular and most preferred in the commercial production of biodiesel for their low cost and availability. Major problem with the use of heterogeneous catalyst such as solid metal oxide and enzymatic catalysis in the production of biodiesel is the higher cost of the catalyst. Diesel engines are widely used as power sources in medium and heavy-duty applications. It is because diesel engines have lower fuel consumption and lower emission of carbon monoxide (Manikandan and Senthil Kumar 2014) (CO) and hydrocarbons (HC) compared with gasoline engines. In 1930s and 1940s fresh vegetable oils were used as diesel fuel when petroleum supplies were expensive or difficult to obtain. As the fossil fuels have been exhausted rapidly nowadays, there is a need to find out an alternative fuel to fulfill the demand of the world. Biodiesel as an alternative diesel fuel has recently attracted huge attention worldwide for its good exhaust emission, sustainability and biodegradability. These gaseous referred to greenhouse gases (GHG) that cause global warming. GHG such as carbon-dioxide (CO₂), carbon monoxide (CO), nitrogen oxide (NO_x) and sulfur oxide (SO_x) causes environmental pollution. Exhaust emission of diesel engines operating on neat biodiesel and its blends with diesel fuel have been reported in numerous studies. Many researchers investigate that increasing of biodiesel percentage in the blend will reduce the CO, SO_x, HC and particulate matter (PM) emission and smoke. However, the emission of NO_x depends on the biodiesel sources. In this study algal oil was extracted from macroalgae, algal oil from macroalgae was used to produce biodiesel through transesterification reaction. The engine performance at different engine speeds was compared between biodiesel blends and ordinary diesel. The formation of CO, NO_x and HC were also investigated and discussed, feasible biodiesel feedstock that deserves further research. Macroalgae are large multi cellular plants that are similar to vascular plants but lack the complex array of tissues used for reproduction (Briggs 2008). Macroalgae usually grow and attach to solid substrates such as coral skeletons, shells and rocks. They can be divided into three groupings: green algae- Division Chlorophyta, brown algae Division Phaeophyta and red algae Division Rhodophyta. Microalgae have high oil content but are hard to be cultivated and harvested in a cost efficient way. On the other hand, macroalgae provide low-cost cultivation and harvesting potential (Can *et al.*, 2008).

MATERIALS METHODS

Algae sample collection

The fresh water Macro algae in the well, Sample were collected from, Mayanur Check dam, Tamil Nadu, India. Latitude and longitude of sample collected 11.2342° N, 78.8807° E.

Culture and maintenance of Protist-filament algae

Isolated Protist-filament algae was inoculated in Erlenmeyer flask having BG11 Medium and incubated at room tem-

perature under continuous dark and sunlight period for 15-20 days, for their growth.

Assay of total lipids

Total lipids were extracted from fresh macroalgal biomass using a slightly modified method of Bligh and Dyer (Iverson *et al.*, 2001). The lipids were extracted with chloroform-methanol (2:1, v/v) and then separated into chloroform and aqueous methanol layers by the addition of methanol and water to give a final solvent ratio of chloroform: methanol: water of 1:1:0.9. The chloroform layer was washed with 20 mL of a 5% NaCl solution, and evaporated to dryness. Thereafter, the weight of the crude lipid obtained from each sample was measured gravimetrically. Experiments were carried out in triplicate, and data are expressed as mean SD.

Oil extraction

The collected macroalgal species were grind with pestle and mortar and dried for 20 min at 80 °C in incubator. Hexane and Ether (20:20 ml) solution were mixed with dried algae. The mixture was kept for 24 hrs for settling.

Biomass collection

Biomass of two macroalgal species were collected by filtration and its percentage were calculated.

Evaporation

The extracted oil was evaporated in Vacuum to release hexane and ether

Mixing of Catalyst and Methanol

0.25g NaOH was mixed with 24 ml methanol and stirred properly for 20 min.

Biodiesel production

The mixture was poured in algal oil in conical flask and shaker for 3 hrs by electric shaker at 300 rpm for the process of Transesterification.

Settling

The mixture was kept for 16 hrs for settling of biodiesel and sediment.

Lipid estimation by Vanillin method Principle

Lipids react with sulfuric acid to form carbonium ions which subsequently react with the vanillin phosphate ester to yield a purple complex that is measured photometrically at 540 nm. The intensity of the colour is proportional to the Total lipids concentration. Chloroform, Methanol, cholesterol, H₂SO₄, sulfo-phosphoric-vanillin acid agent, 96 well plate was purchased from Tarson, India and cholesterol standard stock 10 mg/ml.

Standard preparation

Standards	S1	S2	S3	S4	S5	S6	S7	S8
Chloroform and Methanol (2:1)	-	500µl	500µl	500µl	500µl	500µl	500µl	500µl
Serial dilution of cholesterol	1ml from the stock	500µl	500µl	500µl	500µl	500µl	500µl	500µl
Conc. of standards	10 mg/ml	5mg/ml	2.5mg/ml	1.25 mg/ml	0.625 mg/ml	0.312 mg/ml	0.156 mg/ml	0.078 mg/ml



Prepare the standard sample solution

1. Prepare the solvent, chloroform: methanol 2:1
2. Mix cholesterol in solvent at predetermined concentration, for instance 5mg/ml or 10 mg/ml.
3. Vary volume of the standard sample to assign different amount of cholesterol in different tubes.

Prepare the samples

1. Dissolve the samples in water at a predetermined concentration;
2. Vary the volume of mucins to assign different amount of samples in different tubes.

Measure background absorbance

1. Add 100 µl concentrated sulfuric acid into each tube and incubating at 90°C for 10 min (on a dry heating bath).
2. Cooling at room temperature and measuring background absorbance at 540nm.

Measure the absorbance after color development

1. Prepare the sulfo-phosphoric-vanillin acid agent: 0.2 mg vanillin per ml 17% phosphoric acid) for color development;
2. Add 50 µl sulfo-phosphoric-vanillin acid agent for color development;
3. Measuring absorbance at 540 nm after 5 min of color development.

Transesterification process

The transesterification process was conducted simultaneously with the extraction in order to avoid the previous step of oil extraction and purification of obtained oil. The experimental device is presented in Fig.1. In order to carry out the reaction, 1000 g of dry algae was mixed with 2.5 L of hexane and introduced in a thermostat reactor. The mixture was heated at 60 °C and after that methanol in which sodium hydroxide had been previously dissolved was added to the reactor. A refrigeration column is coupled at the top of the reactor in order to condense the evaporated reagents. Reaction was conducted at the same temperature for different reaction times with constant stirring. The reaction mixture, after the reaction, was cooled to room temperature. Then, the solid phase was separated by filtration using a Buckner funnel under vacuum. Then, the reaction mixture was introduced in a decantation funnel over 9 h, so the bottom layer, glycerine, was separated from the mixture biodiesel and hexane (top layer). After, the top layer was washed with water to remove the excess methanol and the traces of catalyst (Karaosmanoglu *et al.*, 1996 and Lang *et al.*, 2001). In order to obtain the crude biodiesel, it was necessary to remove the solvent by distillation. The solvent was reused in the next batch reaction.

RESULT AND DISCUSSION

Fresh water macroalgae analysis

The lipid analysis of Fresh water macro algae used during this experiment is presented in Table-1- 2.

Table 1 Lipid estimation

S.No.	Concentration	OD Value
1	10	0.09

2	5	0.08
3	2.5	0.06
4	1.25	0.05
5	0.625	0.03
6	0.312	0.02
7	0.156	0.01
8	0.078	0.01

Table 2 Lipid Estimation

Test Sample	OD	mg/ml	Mean value
Algae	0.465	55.25	60.79
	0.549	65.75	
	0.514	61.37	

Compare to the higher concentration to low concentration better results 60.79 mean value obtained for the Table – 2. *C. vulgaris* has been previously identified as a potential strain for use in domestic wastewater treatment by (Singh and Dhar 2007), treated domestic wastewater from a secondary system for microalgae growth. They achieved a maximum removal of 53.8% and 49.6% for nitrogen and phosphorus, respectively, using *C. vulgaris*. It is important to consider that nitrogen and phosphorus removal by microalgae will occur even in effluent from the same site. This is a consequence of the dynamics of the effluent wastewater, which will affect the initial concentration of nitrogen and phosphorus in the effluent from the secondary treatment system. Bioethanol production from *Sargassum spp.* was carried out by (Borines *et al.*, 2013) with a conversion yield rate of 89%. Using fermentation, *Gracilaria verrucosa*, red seaweed is used to produce bioethanol production with a yield of 0.43 g/g sugars was achieved (Kumar *et al.*, 2013). During GC-MS analysis more than 42 compounds present in the Protist filament species.

The final objective of this study is the use of algae residues accumulated on the river. For this reason, various macroalgae mixtures have been collected from the river, pond and the oil content of these mixtures has been determined. Moreover, the oil content of various macroalgae species has been analyzed with the purpose of finding the optimum species of macroalgae for the biodiesel production.

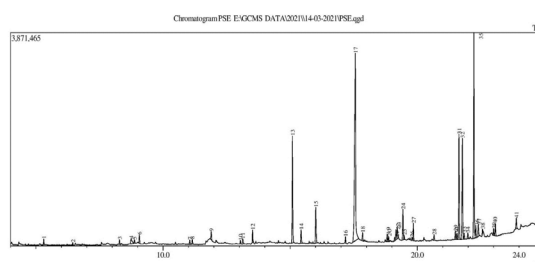
Conflict of Interest

There is no conflict of interest with regard to this Manuscript.

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Gas Chromatography-Mass Spectrometry (GC-MS) analysis

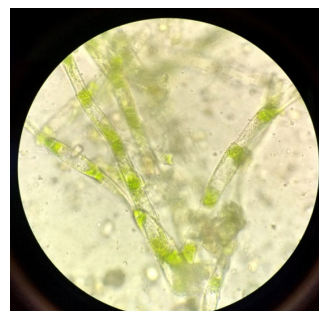


Sample collection

Protist –Filament Algae

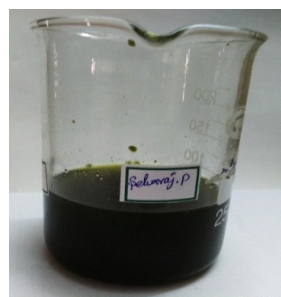
Compounds present in sample by GC-MS analysis

S.No.	Name
1.	Pentane, 1,1-diethoxy-
2.	Propane, 1,1,3-triethoxy-
3.	Octanoic acid, ethyl ester
4.	5-Hydroxymethylfurfural
5.	Benzothiazole
6.	benzaldehyde, 4-(1-methylethyl)-
7.	hexadecanoic acid, ethyl ester
8.	Decane
9.	Piperazine-2,5-dione, 1,4-dimethyl-3,3'-bis-
10.	Glutaric acid, di(pent-4-en-2-yl) ester
11.	1,3-dioxocane, 2-pentadecyl-
12.	1,2-benzenedicarboxylic acid, diethyl ester
13.	benzene, ethylphenoxy-
14.	tetradecanoic acid
15.	benzene, ethylphenoxy-
16.	Hexadecanoic acid, methyl ester
17.	n-Hexadecanoic acid
18.	hexadecanoic acid, ethyl ester
19.	9,12-octadecadienoic acid (z,z)-, methyl ester
20.	9-octadecenoic acid (z)-, methyl ester
21.	Methyl stearate
22.	9,12-Octadecadienoic acid (Z,Z)-
23.	9-Octadecenoic acid, (E)-
24.	Octadecanoic acid
25.	Ethyl Oleate
26.	Heptadecane
27.	Eicosyl acetate
28.	Eicosane
29.	Eicosane
30.	Tetracosyl acetate
31.	Phenol, 2,4-bis(1-phenylethyl)-
32.	Phenol, 2,4-bis(1-phenylethyl)-
33.	1,3-Diphenyl-1-(2-hydroxyphenyl)butane
34.	Dodecanamide, N-ethyl-
35.	Phenol, 2,4-bis(1-phenylethyl)-
36.	Dotriacontane
37.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
38.	Bis(2-Ethylhexyl) Phthalate
39.	2-((2H-benzotriazo)-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol
40.	Hexacosane
41.	Hexatriacontane
42.	Dotriacontane



Macroscopic view of fresh water Protist –Filament Algae

Plate 2 Algae Extract Oil



Algae extraction



Evaporated algae (Bio- Diesel)



Bio diesel of fresh water macro algae

Plate 3

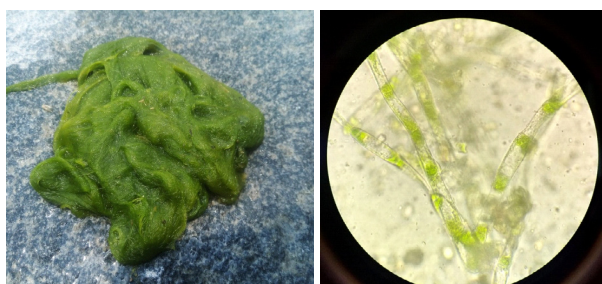


96 well plate



Lipid Estimation Eppendorf tube

Plate 1 sample collection and macroscopic view



References

- Allen, C.A.W. Prediction of biodiesel fuel atomization characteristics based on measured properties. Ph.D. Thesis, Faculty of Engineering, Dalhousie University, 1998; pp. 200.
- Araujo, K., Mahajan, D., Kerr, R., and Silva, M. da. Global Biofuels at the Crossroads: An Overview of Technical, Policy, and Investment Complexities in the Sustainability of Biofuel Development. Agriculture 2017, 7(4), 32.

3. Bozbas K. Biodiesel as an alternative motor fuel: production and policies in the European Union. Renewable and Sustainable Energy Reviews 2008, 12: 542–52.
4. Briggs. M. . Wide scale Biodiesel Production from Algae 2008, energybulletin.net.
5. Can H., Murat C., Ibrahim O., Yakup I., Adnan P., M. S. Salman. Performance Characteristics of a Low Heat Rejection Diesel Engine Operating With Biodiesel, Renewable Energy 2008, Vol. 33, pp. 1709-1715.
6. Manikandan, P.Senthil Kumar. Comparison of Biodiesel Production from Macro and Micro Algae, Int.J. Chem, Tech Res 2014, 6(9),pp 4143-4147.
7. Iverson, S.J.; Lang, S.L. Cooper, M.H. Comparison of the Bligh and Dyer and Folch Methods for total lipid determination in a broad range of marine tissue. Lipids 2001, 36, 1283–1287.
8. Singh NK, Dhar D.W. Nitrogen and phosphorus scavenging potential in microalgae. Indian J Biotechnol 2007, 6:52–6.
9. Karaosmanoglu F, Cigizoglu KB, Tuter M, Ertekin S. Investigation of the refining step of biodiesel production. Energy Fuels 1996, 10:890–5.
10. Lang X, Dalai AK, Bakhshi NN, Reaney MJ, Hertz PB. Preparation and characterization of biodiesels from various biooils. Bioresour Technol 2001, 80:53–62.
11. Borines MG, de Leon RL, Cuello JL. Bioethanol production from the macroalgae *Sargassum* spp. Bioresour Technol 2013. 138:22–29.
12. Kumar S, Gupta R, Kumar G. Bioethanol production from *Gracilaria verrucosa*, a red alga, in a bio refinery approach. Bioresour Technol 2013, 135:150–156.

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