Biodiesel production from *Caulerpa racemosa* (macroalgae) oil

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In the present investigation, non-edible toxic oils from marine macroalgae *Caulerpa racemosa* species, richly available in India and composed of high calorific value and high FFA, have been selected as a feedstock for the making of biodiesel. Initially, oil extraction was carried out from the macroalgae biomass with different types of solvent systems and extraction steps. The algal oil was extracted with a solvent system consisting of 1 % diethyl ether and 10 % methylene chloride in n-hexane using ultrasonic pre-treatment technique. The algal oils were characterized by Gas Chromatography-Mass Spectrometry for composition analysis. The biodiesel was produced by transesterification method. The produced biodiesel was characterized and the conversion was calculated by Fourier-transform infrared spectroscopy (FTIR) analysis and Response Surface Methods. The fuel properties of obtained biodiesel were examined as per the American Standard Test Methods specifications in order to assess the potential of proposed biodiesel as an alternative fuel. Thus, marine macroalgae serve as a potential renewable raw-material for biodiesel production.

[**Keywords**: Extraction, Harvest, Macroalgae, Property, Transesterification]

Introduction

Marine algae are categorized into two groups depending on their size and morphology namely, macroalgae and microalgae, respectively. As the name suggests, majority of microalgae are microscopic photosynthetic organisms and unicellular. On the contrary, macroalgae are comprised of many cells that regulate into constructions like the stems, leaves and roots of developed plants[1]. Unlike microalgae, macroalgae have cells structured into constructions similar to the foliage, origins and also stem from increased crops[2]. Marine macroalgae is a great source of marine biomass as well as possible alternative for major supply of renewable energy source[3]. Large marine photosynthetic plant life can be observed in existence without the aid of any microscopic organisms. Macroalgae are multicellular vegetation growing in both fresh and saltwater. They are categorized into 3 groups depending on their coloring: (1) brown algae (Phaeophyceae); (2) green algae (Chlorophyceae); and (3) red algae (Rhodophyceae)[4]. The average photosynthetic efficiency of marine biomass is 6-8 % higher as compared to the earthly biomass.

Macroalgae are a group of quickly growing plant life that can grow up to considerable sizes in both freshwater and marine environment. The expansion of algal biomass is relocated to all-natural groups of sea grasses and also increased crops[5]. Similarly, macroalgal blooms result in transformations in the primary biogeochemical cycles of C, N, S and P. Owing to the damaging effects and eutrophication to the ecosystem, macroalgal species have to be washed around seaside areas. Thus, it is crucial to learn how anyone can work with macroalgal biomass as a replacement of alternative fuel source and to safeguard the ecosystem[6]. The most effective method in utilizing hazardous marine macroalgae is the generation of biodiesel. Algae for fuel production received global interest in the past decades, due to their yearly production rate, higher growth rate and efficient CO_2 fixation. As an outcome, the utilization of marine macroalgae for biodiesel generation offers dual benefits; (i) it can serve as a good source of biomass for the production of biofuels (biodiesel); and (ii) it saves the environment from their own adverse effects. Macroalgae biodiesel releases only a little quantity of NOx[7].

Caulerpa racemosa, rightly named because of its appearance as oval ocean grapes seaweed. This particular macroalgae is like a bunch of small grapes. Figure 1 reveals the picture message perspective of *C. racemosa* algae. It is green seaweed belonging to the class Chlorophyta. It is mostly available over tropical regions and invades rocky areas. It is also found in association with other seaweed that exists in the Mediterranean Sea region. This intense event is usually assessed as probably the most critical within the story of species created into the Mediterranean Sea. *Caulerpa racemosa* hasn't caused just as much interest as the famous' killer alga' *Caulerpa taxifolia*. The literature reports about the existence basically of alkaloids, terpenoids, and steroids. The group consists of several eco-friendly algal courses, of which *C. racemosa* is probably the most common edible variety. The bio-chemical scientific research on various species of genus *Caulerpa* has shown the generation of tri-terpenoids, acetylenics squirter penoids and di-terpenoids in addition to the nitrogenous compounds. Furthermore, *C. racemosa* species have been equipped with enzymes of palmitic acid & sterols. The primary goal of the present study is to acquire the oil from marine macroalgae biomass and convert the same into biodiesel.

Materials and Methods

Harvesting and extraction of oil from *Caulerpa racemosa*

The marine algae, *C. racemosa* were gathered from the Mandapam coast region, Ramanathapuram, Tamil Nadu, India. The collected algae were washed with seawater at first to clear away macroscopic epiphytes and sand particles. Finally, adhering salt present in the algae was eliminated with fresh water then shade dried for five to six times followed by oven drying at 55-70 °C for twenty two hours. It was then converted

Fig. 1 — *Caulerpa racemosa* macroalgae

in to powder form. Hexane is popular for oil extraction due to its great corrosiveness, low greasy recurring effects, boiling point, and high stability. The highest oil removal yield of 7.24 % was attained from *C. racemosa* biomass through the most helpful solvent mixture composing of 10 % CH_2Cl_2 and 1 % Diethyl Ether $(C_2H_5)_2O$ in n-hexane. From the literature, it was noticed that an identical solvent combination was employed for the extraction of triglycerides working with the bond elute process[8].

Approximately 6.58 % of petroleum extraction yield was attained utilizing n-hexane as a solvent. The marine algal oil extraction yielded 6.38 % using n-hexane-ether in solvent combination. While applying n-hexane-ethyl alcohol and n-hexane 2-isopropanol[9] as a solvent mixture, the oil extraction yield was witnessed to be 5.23 % and 5.57 %, respectively. Hexane is essentially the most widely used solvent for the removal of petroleum obtained from microalgae and macroalgae[10]. It is much less dangerous, has higher selective towards basic lipid fractions, and also minimal affinity towards non-lipid contaminants. Hexane has been used as a solvent for oil restoration from freshwater algae for biodiesel production. Table 1 shows the gas removal yield (%) with various solvent systems. Gas Chromatography-Mass Spectrometer analysis showed the oily structure of *C. racemosa* algal oil provided in Table 2. From the analysis, the saturated essential fatty acids have been discovered to be higher than the unsaturated essential

Table 1 — Oil extraction yield (%) with different solvent systems

Sr. No	Solvent/ Solvent Mixture	Ratio /Percentage	Oil extraction yield (%)
1	Hexane	--	6.58±0.08
2	Hexane : Ether	3:1	6.38±0.12
3	Diethyl Ether and Methylene Chloride in Hexane	1% and 10%	7.24±0.10
4	Hexane : 2 Isopropanol	3:2	5.57±0.05
5	Hexane: Ethyl alcohol	1:1	5.23±0.05

Table 2 — Fatty acid profile of oil

Sr. NO	Name of the fatty acid	Number of carbon atoms	Relative %
1	Lauric acid	12:0	0.095
2	Palmitic acid	16:0	41.52
3	Palmitic acid	16:0	41.59
4	Palmitoleic acid	16:2	13.39
5	Elaidic acid	18:2	15.58
6	Behenic acid	22:0	0.91
7	Oleic acid	18:1	8.12
8	Stearic acid	18:0	9.72
9	Arachidic acid	20:0	1.73

fatty acids. Among the saturated oily acids, palmitic acid was driven as visible methyl esters of 41.59 % within algal crude oil.

Results and Discussion

Transesterification

The process of altering the microbial oil into methyl esters is called transesterification. Technically, transesterification describes neutralizing the FFA (Free Fatty Acids), substituting a fatty acid particle or perhaps the triglyceride by glycerol and alcohol esters in the presence of a catalyst. The glycerol is found at the bottom layer whereas the biodiesel occupies the top-layer during this reaction as shown in Figure 2. The transesterification reaction was carried out using sodium hydroxide (Methanol and NaOH) as a catalyst. The methanol (20 %) is mixed with 5 % sodium hydroxide to create salt methoxide (CH_3ONa) along with 350 ml of algal oil.

The combination is preserved in a rotating agitator for 16 hours at 200 rpm. Settling period of 24 hours is permitted for the splitting up of biodiesel, pigments, and glycerol. The glycerol is meticulously removed utilizing an inverted separator[11]. The transesterification process is carried through 2 phases. First phase is carried out by focusing sodium hydroxide as well as various other catalysts by varying their methanol focus at an elevated temperature. Second phase of the process is space heating of solution (at 50 ^{o}C), when methanol focus was kept continuous. Here, 180 ml of algal methyl ester was acquired when the response rate was approximately 50 %. Then the heat was elevated up to 55 ^{o}C. During that transition, development of 20 h vapor particle was decreased and the response rate increased to a value ≥ 75 % (240 ml of algal methyl ester was extracted). The focus of NaOH also played a crucial role in the development of methyl ester. At 50 ^{o}C, 5 % of NaOH was put into methanol to form CH_3ONa together with algal oil. This resulted in the development of over 90 % of oily acid methyl esters. During the transesterification impulse, it's mentioned that the algal oil has almost 10 % of FFA. It was determined from past literatures that the existence of cost-free fatty acids brings down the ester formation. Hence, it was reduced to 1 % with the addition of 1 % sulphuric acid targeted on the algal oil throughout the transesterification reaction[12].

Fourier-transform infrared spectroscopy (FTIR) analysis

Figures 3 and 4 show the FTIR spectra of *C. racemosa* algal oil and biodiesel, respectively. It can be observed from Figures 3 and 4 that the absorption bands are detected at about 2926 cm^{-1}. This corresponds to H-C = group, between 2922 cm^{-1} and 2852 cm^{-1} for the –CH_2- group, about 1734 cm^{-1}
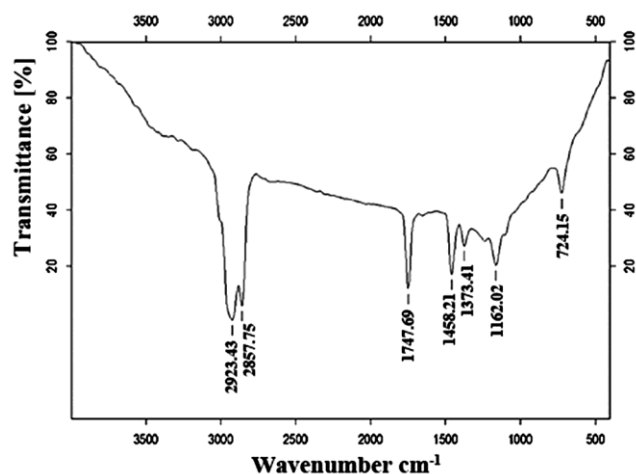

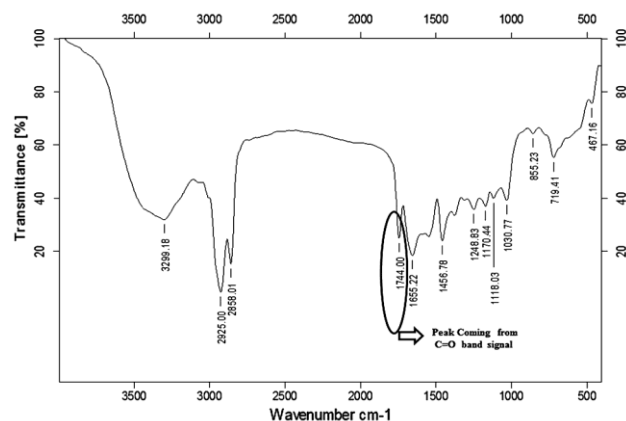Fig. 3 — A typical FTIR Spectrum of *Caulerpa racemosa* algal oil

Fig. 2 — *Caulerpa racemosa* algal oil and biodiesel (Source: Kannan & Christraj, 2018)

Fig. 4 — A typical FTIR Spectrum of *Caulerpa racemosa* algal oil biodiesel

for the carbonyl group and at 765 cm^{-1} for –(CH$_2$)n- "the sequence of aliphatic chains of fatty acids". The FTIR spectra of *C. racemosa* algal oil and biodiesel showed almost similar appearances because they have nearly the same chemical compounds. But slight differences were observed. In the spectrum of *C. racemosa* algal biodiesel as shown in Figure 4, the (C=O stretch) band of methyl ester is observed at 1733 cm^{-1}. The band that has been observed in the spectrum of *C. racemosa* algal biodiesel was at 1175-1232 cm^{-1}, which is attributed to methyl and nearby carbonyl groups. The other characteristic peak was observed at 2917 cm^{-1}, which is the characteristic of FAME[13].

Response surface methods

The Design Expert 8.0 software was utilized to cultivate the experiments for Response Surface Methodology. The style for the elements used in the transformation of methyl ester was obtained from the Response surface technique, with 2 level and 3-factor central composite designs. The elements selected are response heat within the assortment of 50–60 ^{o}C; NaOH focuses within the range of 3–7 mg; and response time in the span of 30-90 min. The variety of NaOH, response temperature as well as response time was selected based upon the preliminary scientific studies conducted in the test facility. The conversion element was selected as the result. In this particular research, a pair of 25 experiments counting the 2^3 factorial experiments, 6 middle points and 6 star factors coded are taken out. Response surface plots were developed using the repaired polynomial situation attained as a result of the regression evaluation. The plots 5 and 6 were developed with one independent variable (constant values) and other 2 dependent variables. The fit and quality of the polynomial style equation have been analyzed through the coefficient of R^2, along with its regression coefficient. F test with a buoyancy level of 95 % was followed. All charts are of curvilinear in shape following the quadratic model. Figure 5, shows the outcome of NaOH and response time over the transformation of the methyl ester with continuous temperature of 55 ^{o}C. The transformation of methyl ester was developed largely with the simultaneous expansion of NaOH concentration and response time. It reaches an optimum value at intermediate level for NaOH of 5 mg, along with 60 min response time. After that, reduction with the expansion of response time and NaOH concentration were observed. This is

due to the presence of additional NaOH concentration, as it can develop blends. Also it is tough enough to sort out the methyl ester from glycerol[14]. It is also easy to attain larger yield with the expansion of response time at less availability of NaOH. Thus, response time was arrived to be the major factor in obtaining higher transformation of methyl ester. Precisely the same declaration can also be made from Figure 6 which shows the outcome on the response time and reaction heat over the transformation of the methyl ester (with a fixed awareness NaOH of 5 mg). From the comparison of outcomes provided in Figures 5 and 6, the optimum values are found to be 5mg NaOH concentration, 60 min reaction time and 550 ^{o}C reaction heats for the optimum yield of 93 %. The transesterification response of algae oil has attained the best success at reaction time of 90 min and response heat of 500 ^{o}C.
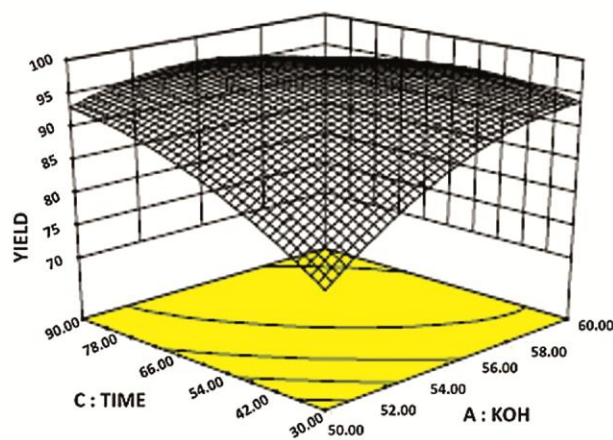

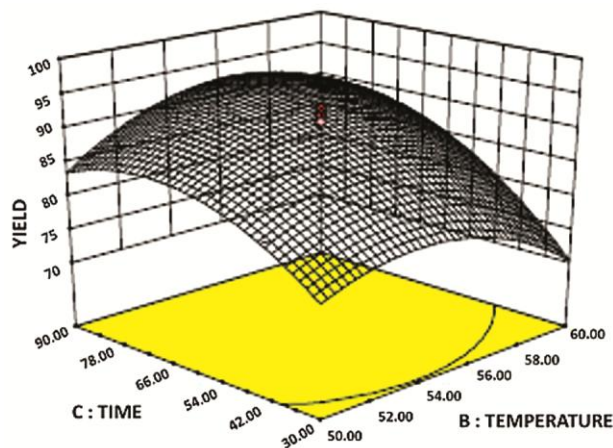Fig. 5 — Response surface of yield of biodiesel in % with catalyst and reaction time

Fig. 6 — Response surface of yield of biodiesel in % with catalyst and reaction temperature and reaction time

Table 3 — Physicochemical characterization of biodiesel

Properties	Units	biodiesel	ASTM limits	ASTM method
Density at 15 °C	kg/m^3	868	N/A	D 4052
Kinematic viscosity at 40 °C	mm^2/s	4.30	1.9-6.0	D 445
Flash point	°C	155	> 130 min	D 93
Pour point	°C	-4	N/A	D 975
Cloud point	°C	-4	N/A	D2500
Cetane number	--	58.23	47 min	D 613
Water and sediment	vol%	0.010	0.050 max	D 2709
Acid value	mg NaOH/g	0.13	0.8	D 664
Copper strip corrosion	--	1	No.3. max	D 130
Distillation temperature	°C	335	360	D 1160
Free glycerine	mass %	0.0080	0.020	D 6584
Total glycerine mass	mass %	0.092	0.240	D 6584
Phosphorus	mass %	0.00031	0.001	D 4951
Sulphur mass	mass %	0.00089	0.05	D 5453
Carbon residue mass	mass %	0.0073	0.050	D 4530
Sulphated ash mass	mass %	0.0091	0.020	D 874

Fuel properties

The flashpoint of *C. racemosa* biomass was observed at 155 °C. The viscosity was observed as 4.30 mm^2/s and the density was determined as 868 kg/m^3 (Table 3). The kinematic viscosity is useful for monitoring the quality of biodiesel during storage and the viscosity has been noted in the literature leads to lower viscosity at higher density[15]. The cetane number is the measure of a fuel's auto-ignition quality characteristics. A higher cetane number is preferred for better fuel consumption. *Caulerpa racemosa* biodiesel cetane number was measured as 58.23. This was comparatively higher than that of conventional feedstock biodiesel[16]. The cloud point and pour point were determined as -4 °C and -4 °C, respectively. The free glycerine and total glycerine were detected as 0.0080 % and 0.092 %. Very small amounts of sulphur and phosphorus were found in the obtained biodiesel. The acid value was measured as 0.13 mg NaOH/g and it is comparatively very less when compared to other biodiesels. As a result, the properties of *C. racemosa* algae biodiesel were in good agreement with the ASTM D 6751 standards specifications (Table 3).

Conclusion

In this current study, marine macroalgae had been selected and refined, making them appropriate for biodiesel production. The created biodiesel from the selected macroalgae species was used in testing. The pretreated algal oil was changed into biodiesel by the transesterification impulse using basic catalyst. The transesterification reaction conditions have been optimized to boost the transformation of biodiesel. FTIR analytical method was utilized for the depiction of chemical structure of biodiesel. Effects of various details such as kinematic viscosity, pour point, flash point, heating value, etc., have been investigated. The type of fuel quality is typically in comparison to the ASTM biodiesel standard format. Therefore, it was determined that *C. racemosa* oil methyl esters could be inexpensive with reduced procedure fees and a boost to biodiesel quality.

Conflict of Interest

No conflict of interest was found under any category.

Author Contributions

MB is the principal & corresponding author carried out the research work with the support of KL and PS. The authors KR, NBT & VV helped in preparation and completion of the article. Author VV extended the support of submission and updating the article according to reviewer comments.

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