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Production of Marine Microalgal Biomass for Biodiesel and Study the Efficiency of its Blends in Diesel Engines

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ABSTRACT

Microalgae are considered as raw materials for biodiesel production as a part-substitute for diesel. Eight microalgal strains such as, Tetraselmis sp., Dunaliella sp., Chlorella sp., Synechocystis sp., Nannochloropsis sp., Gloeocapsa sp., Synechococcus sp. and Oscillatoria sp. having lipid accumulation potential were used in the present study. Estimation of Chlorophyll a and carotenoid contents in algal strains grown under red fluorescent light revealed that there was an increase in biomass and oil yield respectively. Dunaliella sp. and Tetraselmis sp. cultured in tubular photobioreactor showed a maximum biomass of 1.38 g/l and higher percentage (72.6 %) of lipid accumulation respectively. Fatty Acid Methyl Ester (FAME) analysis of algal biodiesel showed the presence of saturated fattyacids peaks. AO10D was found as best algal biodiesel blend among algal biodiesel blends tested for their fuel properties, performance in engine and emission characteristics.

Introduction

Dependence on crude oil based fuels is unsustainable due to rising of fuel costs and declining crude oil resources [1]. India is also facing an unprecedented energy demand and the present import of crude oil is around 180 million tonnes per annum [2]. During 2013, diesel consumption has been hiked about 5.9 % at 12.5 million tonnes as against 11.8 million tonnes in 2012. India is the third-largest oil consumer in Asian region [3] and the fourth largest consumer of primary energy in the world [4], whereas its production rate is negligible and it relies only on the oil import. Diesel consumption has been increased by 4.2 per cent year-on-year (y-o-y) in 2013 to 6.2 million tonnes (MT), according to data compiled by the Petroleum Planning and Analysis Cell (PPAC) of the Ministry of Petroleum and Natural Gas, Govt. of India. Diesel consumption rate is faster than other petroleum products [5]. Hence, non-polluting renewable energy from biomass has been attracted more and more consideration from the academic and industrial sector [6].

Biodiesel is a renewable, environmental friendly, non-toxic, biodegradable and does not require significant modification to the existing engine technology [7]. Biodiesel production from edible oil resources is almost impossible in India because already 43 % of edible oil imported for catering the domestic needs [8]. Microalgae is recognized as a raw material for biodiesel production [9] and also used for harvesting many high-value products [10]. Microalgae are photosynthetic microorganisms which convert sunlight, water and CO2 to sugars, from which macromolecules such as lipids and triacylglycerides (TAGs) are synthesized and higher amounts of lipids accumulated more rapidly when compared to terrestrial plants because of their faster growth rates. Moreover, energy storage of microalgal lipids were two fold higher than terrestrial plants [11].

The TAGs are the promising and sustainable feedstock for biodiesel production. Many micro algae have the ability to produce substantial amounts of triacylglycerols (TAGs) (20-50 %) as storage lipid under photo-oxidative stress or other adverse environmental conditions [12].

Accumulation of the desired products in microalgae could be achieved by changing environmental factors such as temperature, illumination, pH, CO_2 supply, salt and nutrients [13]. Photobioreactors could be conveniently designed and used to control the critical variables such as light, nutrients supply and temperature during algal culture. Numerous studies have demonstrated that, sufficient supply of nitrogen promotes high growth rates in microalgae but with low oil content, whereas the nitrogen deficiency leads to reverse results [14]. Microalgal biodiesel has properties similar to petrodiesel including density, viscosity, flash point, cold flow and heating value. These properties make microalgal biodiesel as a suitable substitute for petrodiesel [15]. In the present study, tubular and flat plate photobioreactors have been designed and used for faster algal growth. Moreover, biodiesel extracted from algal oil was tested for engine performance and emission characteristics.

Experimental

Strain and culture conditions

The algal strains viz., Tetraselmis sp., Dunaliella sp., Chlorella sp., Synechocystis sp., Nannochloropsis sp., Gloeocapsa sp., Synechococcus sp. and Oscillatoria sp. were obtained from Central Marine Fisheries Research Institute (CMFRI)-Thoothukudi, Tamil Nadu, India. All the algal strains were cultured in sea water collected from Elliot's beach, Chennai, filtered and enriched with salts viz., $NaNO_3$ (0.1 g/l) and $Na_2HPO_4.2H_2O$ (0.01 g/l), sterilized by autoclaving at 121 °C at 1 atm for 15 min.

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Biomass and lipid estimation

Eight algal strains were grown in a culture vessel (500ml) under three fluorescent lights such as white, blue and red separately under 22 ºC for 40 days at a distance of 30 cm. The biomass was quantified by estimating the chlorophyll and carotenoid pigments level for every week. Chlorophyll was estimated from the dried algal biomass (500 mg) by methanol extraction followed by absorbance at 663 nm in a spectrophotometer. The carotenoid level was estimated based on Whyte method [11] and the absorbance was recorded at 450 nm spectrophotometrically. The lipid extraction was carried out based on Folch method [16].

Culture maintenance and Harvesting

Tubular photobioreactor (0.58 m X 0.098 m) containing culture medium (6 l) was inoculated with 500 ml of algal inoculum in late exponential phase. The reactor was illuminated with red fluorescent light on both sides at 30 cm distance. The culture was properly mixed using a sub-merged pump. 0.5 psi of CO_2 was bubbled through air sparger (3 mm) to maintain pH of 8.2-8.7. Similarily, growth of the algal strains was also studied in the Flat plate photobioreactor (0.08 m wide, 0.30 m length and 0.30 m height) containing 2.5 litres of culture media and 300ml of late exponential phase inoculum exposed to red fluorescent light [17]. The cultures in the reactors were incubated till maximum growth was obtained (for 15 days).

Lipid extraction and transesterification

The algal biomass was harvested by centrifugation at 10,000 rpm and the cells were dried using a desiccator. The algal oil was extracted from dried biomass by homogenization using chloroform:methanol mixture in 2:1 ratio [16] and the extract was continuously washed by centrifugation to remove the excess solvent. The colloidal solution obatained after washing was allowed for phase separation in order to collect algal oil from lower phase. The collected algal oil was subjected to transesterification process with methanol to yield algal methyl esters and the reaction was catalyzed by sodium methoxide. The reaction was enhanced by elevating the temperature to 55 ºC and biodiesel produced was separated from methanol by distillating at 65 ºC. The dissolved glycerol in the biodiesel phase was removed by continuous washing with hot water [17]. Fatty Acid Methyl Esters in the produced biodiesel was analyzed by GC-MS (FAMEs) (IITM, Chennai).

Evaluation of physico-chemical properties

The properties, such as, Density, (IS1448 part 16), Viscosity (IS1448 part 25), Aniline Point (IS1448 part 3), API gravity and Cetane No. (IS1448 part 16 and part 9), Flash point and Fire point (IS1448 part 20) were calculated for five algal biodiesel blends *viz*., AO10D(Algae oil [A.oil] 100+Diesel 900), EO5AO5D (Eucalyptus oil [E.oil] 50+A.oil 50+Diesel 900), AO5E5D (A.oil 50+ EtOH 50 + Diesel 900), EO5AO5E5D (E.oil 50+A.oil 50+ EtOH 50 + Diesel 850) against Q-diesel using standard protocol of Bureau of Indian Standards 1448 [18].

Performance of blends in diesel engine

Kirloskar 4 stroke (140 mm) single cylinder diesel engine with compression ratio 18:1 at 3.75 KW rate power @ 1500 rpm was used in the study [19]. The engine loading was carried out with an electrical dynamoter. The experiment was carried out at a steady state with different loads of 0 %, 20 %, 40 %, 60 %, 80 % and 100 %. Initially engine was run on diesel fuel and then switched over to algal blends under similar conditions. DIGAS444-AVL Exhaust Gas Analyzer and AVL smoke meter was used for measuring Carbon dioxide (CO_2), Carbon monoxide (CO), Nitrogen oxides (NO_x), Hydrocarbon (C_xH_y) and Opacity.

Results and Discussion

Flask study

Significant variation in the algal growth rate was observed individually under three different lights at 30 cm distance. The growth rate was depicted by analyzing the Chl *a* (µg/ml) content of the strains [20]. Growth rate of algal strains under white light increased gradually whereas, the strains exposed to blue light showed slower growth rate even after two weeks of culture and the algal strains grown under red fluorescent light was found directly proportional to the lipid content in these strains (Fig. 1A). To know whether the light intensity influcenced the growth of microalgal strains, the light intensities were measured using lux meter. The lux recorded for blue, white and red fluorescent light were, 960, 1020 and 1840 respectively. As the depth and cell concentration increases the light intensity must be increased to penetrate through the culture. Appropriate lux for Erlenmeyer flask was 1000 lux to avoid photo-inhibition and overheating whereas for higher volumes it could be above 5000 lux [21]. Similar results were reported for the red algae *Halymenia floresii* which showed different growth pattern under white, green, red and blue lights. The growth rate was found high for green light [21].

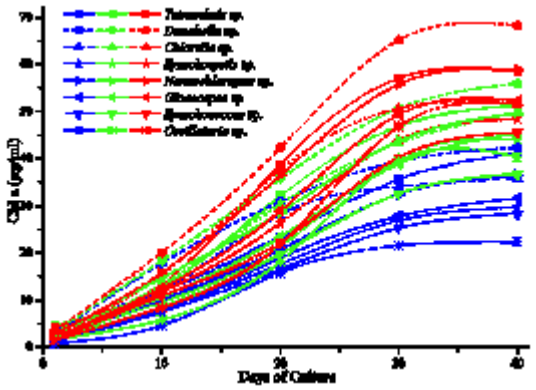


Fig 1A. Chlorophyll contents of microalgae cultured under blue, white and red fluorescent lights as a function of age.

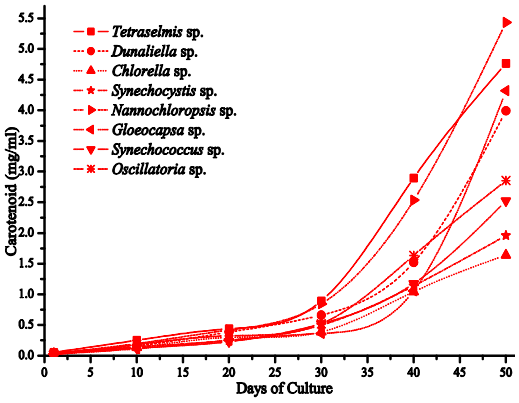


Fig. 1B. Carotenoid contents of microalgae cultured under red fluorescent light as a function of age.

Microalgae cells need nitrogen for production of amino acids [22]. Addition of nitrogen salts *viz*., $NaNO_3$ and $Na_2HPO_4.2H_2O$ contributed positively in algal growth and lipid accumulation as already reported nitrogen content in the medium was directly proportional to growth rate and inversely proportional to lipid content [23]. These results revealed that the seawater enriched medium with red fluorescent light (1840 lux) was suitable for algal growth.

Estimation of carotenoid was performed only for the algal cultures grown under red fluorescent light due to better biomass productivity and the results are represented in Fig. (1B). Comparatively, the strains grown under red fluorescent light showed steep increase in growth and carotenoid content, right from the initial days of culture. Algal cells grown under higher light range with nitrogen depletion, resulted in an increase in carotenoid content and accumulation of triacylglycerols (TAG) [24]. Three fold increase of lipid content was observed in Chlorella vulgaris when $NaNO_3$ concentration was reduced from 1.500 g/L to 0.375 g/L [25].

Culturing of microalgal strains in photobioreactor

Biomass and oil content of all the algal strains such as, *Tetraselmis* sp., *Dunaliella* sp., *Chlorella* sp., *Synechocystis* sp., *Nannochloropsis* sp., *Gloeocapsa* sp., *Synechococcus* sp. and *Oscillatoria* sp. cultured in tubular and flat plate photobioreactors were studied. Biomass harvested was recorded in g/l and lipid content was calculated in percentage (Fig. 1C). The results showed that *Dunaliella* sp. had a maximum biomass yield of about 1.38 g/l whereas, *Gloeocapsa* sp. had a minimum of 0.95 g/l and other strains had yielded 0.96 to 1.29 g/l. *Dunaliella* sp. has the capacity to grow under extreme salinity conditions showed better biomass production in photobioreactors [26]. Moisture content in biomass results in lower FAME yield [27]. However, *Tetraselmis* sp. had higher percentage of oil yield when compared to other strains. *Tetraselmis suecica* is considered as one of the best lipid producers in tubular reactor in the outdoor condition [28]. Tubular photobioreactor showed better algal growth/biomass than the flat plate photobioreactor (Fig. 1C), this might be due to ideal mixing achieved in tubular photobioreactor which was lacking in the flat plate photobioreactor. The biomass yield reported in vertical photobioreactor was about 25–70 % higher than horizontal system [29].

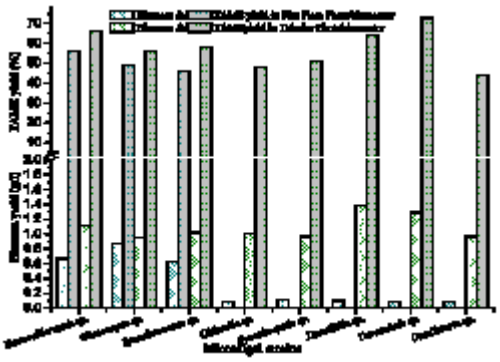


Fig 1C. Comparison of biomass and oil yields (%) in flat plate and tubular photobioreactors.

Gas Chromatography-Mass Spectroscopy (GC/MS) Analysis

Characterization of Fatty Acid Methyl Esters (FAME) content is an important component of high quality biodiesel; hence the biodiesel produced from each algal strain was analyzed under GC-MS. The GC/MS chromatogram with different peaks clearly depicted the typical structure of the FAMEs (Table 1). The fatty acid profiling of microalgae ultimately decides the quality of the biodiesel. The carbon chain length of saturated and unsaturated fatty acids affects properties of biodiesel, such as cetane number, oxidative stability and cold-flow.

Physico-chemical property test

Physical and chemical properties of alglal biodiesel should be essentially known before using them as fuel.

Algal biodiesel obtained from 8 algal strains were pooled together as the volume of algal biodiesel from individual species obtained was less than the required quantity to perform further analysis and then blended with Q-diesel and subjected to different property tests (Table 2). Fuel atomization and distribution in engine was controlled by viscosity. Too high viscosity (value) leads to additional heat generation in the injection part, attributes shear in pump plunger and cylinders. All the Q-diesel blends, Cetane number denotes the fuel ignition delay time and combustion quality. High cetane index guarantees the good cold start and minimizes the white smoke formation. The cetane number of AO10D mixture was found maximum 31.20 which is a desirable property for a transportation fuel. Konthe et al [30] observed that high cetane number for the esters of saturated fatty acids like palmitic and stearic acids. High flash point (84) was observed in all algal biodiesel blends when compared to other oil blends (data not shown) because of Eicosane content in algal oil. The presence of saturated fatty acids ranges from C16 to C20 in the algal biodiesel again confirms its fuel property. As the quality of biodiesel depends on the chain length of the FAMEs [31], the presence of fatty acids in the blend AO10D from C16 to C20 reflects its high quality.

Table 1. GC/MS analysis of algal biodiesel extracted from marine algae.

Microalgae	FAME
Tetraselmis sp.	Octadecanoic acid-4-hydroxy-methylester Methox acetic acid-4-tridecyl ester Eicosane
Nannochloropsis sp.	Methox acetic acid-4-tridecyl ester
Dunaliella sp.	Pentadecanoic acid-13-methyl-methyl ester 16-Octadecanoic acid-methylester
Chlorella sp.	Hexadecane, Eicosane
Synechococcus sp.	Methox acetic acid-4-tridecyl ester
Gloeocapsa sp.	16-Octadecanoic acid-methylester
Synechocystis sp.	Octadecanoic acid-4-hydroxy-methylester
Oscillatoria sp.	Methox acetic acid-4-tridecyl ester

Table 2. Fuel properties of algal biodiesel blends.

Fuel property	Q-Diesel	AO10D	AO5, EtOH5D	AO5 EO5D	AO5EO5 EtOH5D
Viscosity (mm^2/sec)	1.9 – 6.0	0.17	0.13	0.16	0.17
Specific gravity (Kg/m^3)	0.87 – 0.90	0.812	0.810	0.814	0.814
Flash point°C	≥130	84	84	80	76
Aniline point°C	82	80	79	77	78
Cetane No.	≥47	51.20	50.12	49.59	50.01
API gravity	≥43	42.76	43.19	42.33	43.42

Performance of blends in diesel engine

The Engine performance such as Brake thermal efficiency, Total fuel consumption, Specific Fuel Consumption and emission characteristics viz., CO_2 emission, HC emission, CO emission, NOx emission and smoke opacity of Q-diesel and biodiesel blends were tested on the diesel engine and the results are described below:

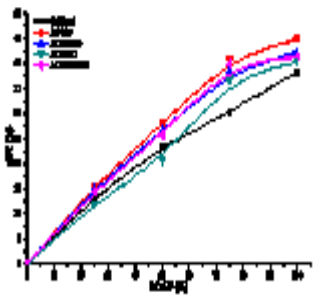
Brake Thermal efficiency (ηBT)

The brake thermal efficiency of diesel blends was calculated and a graph was plotted against break thermal efficiency and load (Fig. 2A). The brake thermal efficiency for algal oil-diesel blend (AO10D) and AO5EO5D showed higher efficiency of about 45 % and 40 % respectively at full load (Fig. 2C) whereas diesel oil has 37 % of efficiency. Higher efficiencies are attained mostly due to complete fuel

combustion [32]. Even though efficiencies of AO5E5D and E05A05E5D were similar to diesel, the decrease in their efficiencies implies incomplete combustion due to the disassociation characteristics of too many blends and improper mixing especially with EtOH.

Total fuel consumption (TFC)

Brake specific fuel consumption is a measure of how efficiently an engine is utilizing the supplied fuel to produce mechanical energy. The TFC for diesel and algal blends are plotted for comparison (Fig. 2B). It has been observed that TFC of AO5E5D, EO5AO5E5D and Q-diesel was increasing as a function of load. The brake specific fuel consumption of AO10D was the least at all loads. The brake specific fuel consumption of all blends varied from 0.55 kg/kWh at '0th' load to 0.22 kg/kWh at full load which indicated the complete combustion of algal diesel oil blends. TFC for algal biodiesel blends was found lower.



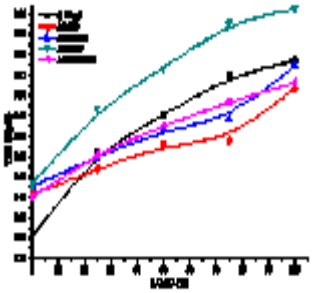
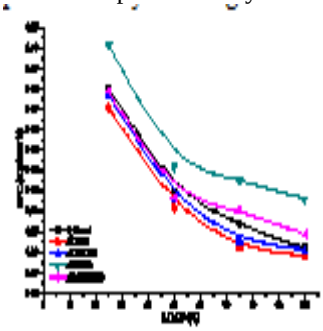


Fig. 2A. Load vs ηBT **Fig. 2B. Load vs TFC**

Specific Fuel Consumption (SFC)

The SFC was calculated by dividing the TFC rate by Brake pressure. Decrease in SFC on increasing load has been observed (Fig. 2C). The SFC of AO10D was the least but for AO5EO5D it was the highest. According to the equation of quantitative analysis on SFC which states that minimum SFC values are possible only with higher BP and lower TFC



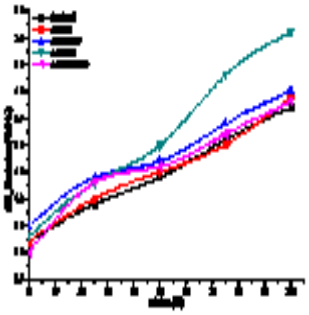


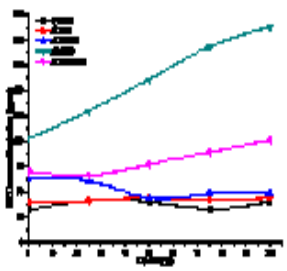
Fig. 2C. Load vs SFC **Fig. 2D. Load vs CO_2 emission**

CO_2 emission

All the blends had shown some significant effect on CO_2 emission (Fig. 2D). At the maximum load, 6 % of CO_2 was emitted by AO5EO5D while 5.7 % was released by Q-diesel. The emission levels of blends (AO10D, AO5EO5D, AO5E5D and EO5AO5E5D) were found higher than that of diesel because of the non-homogeneity in the blended mixture [32]. CO_2 emission was lower due to late burning.

HC emission

The algal oil blends AO10D and Q-diesel had shown similar effect in HC emission whereas AO5E5D showed higher emission rate (Fig. 2E). HC emissions higher due to improper combustion of non-homogeneity exist between air and blend fuel. Godiganur et al. [33] found that the reduction in HC was linear with the addition of biodiesel for the blends.



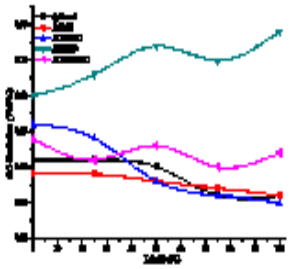


Fig. 2E. Load vs HC hexane Emission **Fig. 2F. Load vs CO Emission**

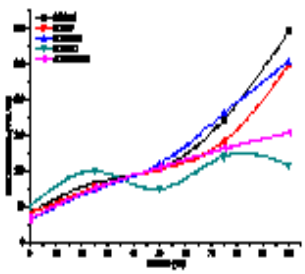
CO emission

At mid load the percentage of CO emission of the algal blends AO10D and AO5EO5D were comparable with Q-diesel (Fig. 2F). The other two blends had shown higher percentage of CO emission. CO emission is mainly due to lack of O_2, poor air entrainment, poor mixture preparation at incomplete combustion [34]. Generally CI engines operate with lean mixture emit lower CO whereas with rich mixture emits high CO. It could be concluded that blends are rich mixtures with high CO. Lower CO emission has been observed in AO10D sample.

NO_x emission

At full load the NO_x emission of the algal oil blends AO10D and AO5EO5D was lower than the Q-diesel whereas the emission rates of other blends were much higher (Fig. 2G).

At higher loads NOx emissions of diesel is more than algal oil blend (AO10D) whereas at lower loads (up to 60 %), the NOx emissions are comparable with Q-diesel which indicates that the rate of heat released is also comparable. The higher peak pressure inside the cylinder is the reason for higher NOx emission [35].



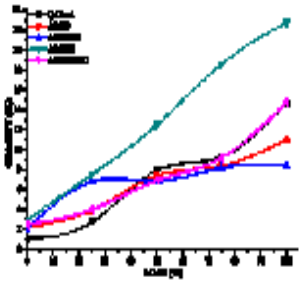


Fig. 2G. Load vs NO emission **Fig. 2H. Load vs Opacity**

Smoke Opacity

Smoke emission of the blends AO10D and AO5EO5D at full load was less when compared to Q-diesel whereas the blend AO5E5D had shown higher opacity than Q-diesel at mid load (Fig. 2H). Similarly at higher loads, the opacity of algal blends was found higher due to unavailability of supplied air and abnormal combustion [34, 35]. High loads imply that more fuel is injected into the combustion chamber and hence incomplete combustion of fuel is prominent. Reduction of smoke emission for AO10D leads to the improvement of its combustion quality when compared to diesel fuel.

Conclusion

Studies on algal growth, FAME analysis, Fuel and emission characteristics proved that algal biodiesel could be produced in a cost effective manner and the properties of algal biodiesel-diesel blend (AO10D) was found suitable as a transportation fuel. The FAME analysis confirmed the presence of essential saturated fattyacids in the biodiesel. Moreover testing of these algal oil blends in the diesel engine implies that the blend AO10D has a good engine performance and low emission characteristics at optimum load of 50 %.

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