Investigation of the blue-green algae based bio diesel using n-hexane and n-hexanol solvents: diesel engine emission parameter and characterization studies

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Abstract. The present study utilizes the species namely *Spirulina platensis* for extracting the biodiesel using different solvents using trans-esterification process. The gas chromatographic analysis was carried out to determine the chemical compounds present in the algal extract. The selected species were cultivated in the open pond and subsequently, algae oil has been extracted using n-hexane and n-hexanol solvent. Further, the comparison of n-hexane and n-hexanol based bio-diesel in the BD-20 blended form is done. Additionally, the extracted biodiesel is tested for emission parameters in the diesel engine. These results are compared with the emission parameters of commercial diesel which shows that n-hexane and n-hexanol based bio-fuel produces lesser emissions at different load conditions. Hence the selected *Spirulina platensis* species is found to be the promising alternate energy source for fuel extraction. Moreover, they are prone to exhibit lesser toxic emissions in the air environment than the commercial sources and found to be a sustainable source.

1. Introduction

The gradual increasing global human population and usage of fossil fuel result in ozone depletion and harmful emissions. This requires alternative energy sources to meet the future demands as well to reduce the emissions. So to overcome the environmental problems associated with fossil fuel based emissions, the use of biodiesel using microalgae is proposed in the present study. Moreover, biodiesel is one of the viable alternatives which is easily available, technically feasible, and economically competitive.

Algae can be generally classified as macro and micro algae. Macro algae are commonly seaweeds which are also referred as marine algae which have a vital role in the growth of coral reefs. Some examples of macro algae include red, brown-kelps, Sargassum etc which are visible to the naked eye.
On the other hand, microalgae are the minor creatures typically found in freshwater and marine systems that absorb carbon dioxide as nutrients from the surrounding environment and convert sunlight to energy. They also produce lipid, proteins, carbohydrates, fat, and other valuable organic compounds as pigments [1, 2, 3]. The use of algae is widely exploited for the production of biogas, bio-hydrogen, bio ethanol, biodiesel etc. It is to be noted that the use of biomass extracted from algae also has a vital application in pollution remediation [4]. The presence of lipids, proteins, and alcohols in the algae play a vital role in the preparation of bio-fuels [5]. Algae being a renewable source limit ozone depletion and control harmful gaseous emissions. Additionally, bio mass (dried yield or fresh yield) products derived from these algae can also be used in nutrient supplements and naturals medicines [6] with oil content up to 20–50% in dry weight of biomass [7]. As prominent yields of biomass are produced from these algae within short periods, the present study proposes microalgae based species (Spirulina platensis) for extracting the biodiesel [8]. Further, apart from biodiesel production algae is also widely known for CO₂ capture [9].

In order to have the mass cultivation of algal sources for biodiesel production, strain selection and photosynthetic efficiency of the species are considered as significant factors [10]. Hence in the present study the Spirulina platensis, a unique blue green algal species which have a solar energy utilization and photosynthetic efficiency of 18% and 43% [11] respectively are chosen. It should be noted that Chlorella algae is reported with a photosynthetic efficiency up to 34% which is less than Spirulina platensis [12]. Moreover, this species can grow rapidly and the cultivation procedure is easy and convenient as well. Further, the growth of these species can occur at wide pH range of 8.6–9.5 and at moderate temperatures [13, 14].

In general, production of bio diesel involves the use of a solvent for extraction of one or more compounds from the mixture. Some examples of solvents include n- hexane, ethanol and alcoholic substances. They help in the easy extraction of oil from different biomasses such as Jatropha curcas, sunflower, vegetable wastes and different species of algae. Based on the polarity the solvents are classified as polar and non polar solvents. Common functional groups present in polar solvents include alcohols, ketones, carboxylic acids, and amides respectively.

Specifically, solvents help in extracting proteins, fats, lipids, glycerol [15] etc. Further, the selection of solvents plays an important role in extraction processes [16]. Mechanical pressing and sterling techniques are some of the methods used for extraction of oils [17]. However, there are chances for insufficient separation and reduced yield in the case of mechanical pressing. Hence sterling technique is considered as the best option for better separation. Trans-esterification is one of the commonly adopted techniques in biodiesel extraction. The process acid-catalyzed trans-esterification was used to convert diglycerides, fatty acid, triglycerides, and monoglycerides to fatty acid methyl esters [18].

It is understood that the major problems associated with the use of fossil fuel based energy sources are the high emission of air pollutants such as NOx , SOx , CO, and CO₂ [19]. Thus the bio diesel based energy sources are considered as one of the renewable sources of energy which produces less emission than conventional petrol based sources [20]. In addition to the extraction of biodiesel from Spirulina platensis, the study also aims at identifying the potential solvents for the extraction of the fuel. The study also aims at simulating the batch results in the diesel engine and studying and emission parameters on par with diesel engines using appropriate engine characterization techniques.

2. Materials and Methods

2.1. Materials.

The microalgea Spirulina platensis mother strain (Accession No: 5143) was purchased from National Chemical Laboratory (NCL), Pune. n- hexane and n- hexanol was procured from S D Fine Chem. Limited, Mumbai.
2.2. **Microorganism preservation.**
The mother strain has been preserved at room temperature at around 29 °C. It was kept in the test tube covered by the cotton plug-in and rapped with aluminium foils. This prevents them from exposure to sunlight and from the fungal infection which ultimately may lead to the death of the strain.

2.3. **Cultivation.**
Initially, *Spirulina platensis* was streaked (using a sterile nichrome loop) in 250ml conical flask and was inoculated in a 250ml distilled water and cultured under Blue Green 11 medium (BG11 medium). The chemicals required for the BG11 medium is given in Table 1.

| Sl no | Chemical     | Quantity |
|-------|--------------|----------|
| 1     | MgSO$_4$.7H$_2$O | 0.1      |
| 2     | K$_2$HPO$_4$  | 0.25     |
| 3     | NaCl          | 0.5      |
| 4     | CaCl$_2$.H$_2$O | 0.02     |
| 5     | NaHCO$_3$     | 5.0      |
| 6     | NaNO$_3$      | 6.0      |
| 7     | K$_2$SO$_4$   | 0.5      |
| 8     | Distilled water | 250 ml  |

As the significant growth of the culture is seen in the conical flask, the cultivation of the *Spirulina platensis* species is carried out in the large scale set up as shown in figure 1. This is carried out in Fiber Reinforced Plastic. It is 5 X 3 feet with the depth of 15 inches and 540 L capacity. The culture was added to the tank containing 50 L water. At lag phase, no color change is noticed in the aqueous medium. Later at the 5th day (Exponential phase), a thick green colored bed of algae is formed. This indicates the growth of *Spirulina platensis*. The addition of culture continued till the end of the stationary phase. The first day and fifth day of algae growth of *Spirulina platensis* are shown in figure 1 and figure 2.
3. Yield extraction from algae solution
At the end of the stationary phase, the algae containing solution was transferred into a new tube. Finally, after the complete growth of algae, flocculation technique is used to separate out the yield. This is carried out by adding 2g of sodium hydroxide with 15 ml of methanol to the tub containing algae. The algae finally precipitated and settled out at the bottom of the container. In order to remove the water content from the wet bio mass, vacuum drying has been carried out followed by air drying for 2 days. After the completion of the drying process, a thick green colored yield was obtained weighing around 30g. The images of the wet biomass and the powdered yield are shown in figures 3 and figure 4.

![Figure 3. Wet bio mass](image1)

![Figure 4. Powdered yield](image2)

4. Preparation of algal oil
The extraction of algal oil is carried out using the solvents namely n-hexane and n-hexanol and done in two phases. In the first phase, a known quantity of powdered algae is treated with 30 ml of n-hexane. Then, the solution was allowed to stir continuously for about an hour using magnetic stirrer. The resulted solution was finally filtered by Whatman filter paper (0.22 µm pore size; diameter 45 mm) and thus the algal oil is extracted. The excess solvent present in the algal oil was collected through collector flask using rotary vacuum evaporator (Yamato RE300). During this process, n-Hexane was evaporated at 65ºC (Boiling point 70 ºC). Finally, 50 ml of pure algal oil with lipid content was obtained. The collected solvents were re-used in other testing processes. In the second phase, the same process is repeated for the extraction of algal oil using n-hexanol. It is to be noted that n-hexanol is evaporated at 100 ºC (Boiling point 160ºC).

5. Preparation of bio - fuel
25ml of Methanol (CH$_3$OH) and 2.0g of NaOH was added to 50 mL of algal oil prepared using different solvents and allowed to stir for about 45 minutes using magnetic stirrer at a temperature of 50ºC - 55ºC. The resulted solution was then poured into the separating funnel and kept for 12 hours. After this process, a mixture of methyl esters and fatty acid is constituted at the top layer of the separating funnel and an aqueous layer is formed at the bottom. To remove the impurities present in the aqueous layer, 15% of distilled water was added to the mixture and washing is continued until the complete removal of impurities is ensured. The figure 5 and figure 6 shows the bio-diesel prepared using n-hexane and n-hexanol solvent respectively.
6. Characterization of biodiesel

The characterization of n-hexane and n-hexanol based biodiesel was carried out and compared with commercial diesel. To satisfy the standards of fuel the quality parameters are analyzed. The parameters include flashpoint, fire point, cloud point, pour point, kinematic viscosity and specific gravity.

Two strokes diesel engine (Lean combustion type) with the capacity of 7.5 HP at 5.59 kW was used for testing at different loads. CO$_2$, CO, unburned hydrocarbons (HC), and NOx were measured by Automotive Emission Analyzer (HORIBA MEXA-584L). Engine Tests were done at Automotive Industrial and Research Centre, Amrita Vishwa Vidyapeetham, Coimbatore. Commercial diesel was obtained from Bharat fuel distributor, Coimbatore, India and it was mixed with BD20 algae based biodiesel and labelled as the blended biodiesel. The diesel engine was gently started and warmed up at an idle condition of 30 minutes under no load conditions. Initially, commercial diesel was poured into the tank and the engine speed was set at 2300 rpm, and the readings were recorded for different loads i.e. 0, 3, 6, 8, and 10 Nm. The fuel consumption for every load at an interval of 10-seconds was noted as well. The same procedure was carried out for blended biodiesel.

7. Results and discussions

7.1 Bio-diesel quality parameters.

The parameters flash point, fire point, cloud point, pour point, kinematic viscosity and specific gravity are analyzed and the results are given in Table 2.

7.1.1. Emission characteristics of algae diesel.

7.1.1.1. Carbon dioxide emission characterization with different load condition (Nm).

The CO$_2$ emission at different loads was measured and the results are shown in Figure 7. From the curve, it is understood that the bio–diesel emitted less CO$_2$ compare to pure diesel. Among the two solvents based bio-diesel, n-hexanol based bio-diesel emitted less CO$_2$ compared to N-hexane based bio-diesel at intermediate load and a similar trend is observed for the rest of the loads.
Table 2. Bio-diesel quality Parameters

| Quality parameters               | Diesel     | Algae biodiesel | Bio-Diesel (n-hexane) | (Bio-Diesel (n-hexanol) |
|----------------------------------|------------|-----------------|-----------------------|------------------------|
| Flash point (° C)                | 55-96      | 95              | 94                    | 95                     |
| Fire point (° C)                 | >93        | 98              | 97                    | 97                     |
| Cloud point (° C)                | -28-(-7)   | 2               | 2                     | 2                      |
| Pour point (° C)                 | 6-11       | 1               | 1                     | 1                      |
| Kinematic Viscosity (mm²/s)      | 35.2-86.6  | 44.78           | 44.10                 | 43.10                  |
| Calorific Value (MJ/Kg)         | 45.28      | 36.38           | 36.35                 | 37.21                  |

Figure 7. Comparison of Load (Nm) Vs CO₂ emission %.

7.1.1.2. Nitrogen Oxide Emission characterization with different Load (Nm).

Among the various air pollutants, NOx causes most hazardous emissions while burning fuel in the diesel engine. From figure 8, it is inferred that n-hexane BD20 operating at lower load (0-5 Nm) emits lesser percentage of (2.5%) of nitrogen oxides than the commercial diesel and n-hexanol based biodiesel. The above results of carbon dioxide and nitrogen oxide emissions have clearly indicated that the algal based biodiesel exhibits minimal environment impact.

It is extensively studied that with a help of using different metal based additives, further reduction in the NOx and carbon dioxide emissions is possible which will also improve ignition and combustion efficiency, and stabilize fuel mixtures [21, 22]. In a recent study [23] bio-diesel extracted from jatropha is investigated for pollution minimization. In particular, they used a metal based cerium oxide additive mixed with fuel and found that the emission of nitrous oxides decreased up to 23.5%.

7.1.1.3. Carbon Monoxide Emission characterization with different Load (Nm).

In figure 9 it is observed that pure diesel and hexane based biodiesel are exhibiting same percentages of carbon monoxide emissions at lower loads. However relatively, at higher loads, CO emission increases slightly. But for n-hexanol solvent based bio-diesel the emissions were slightly increased and reached around 0.2%. Hence to reduce the carbon monoxide emissions the biodiesel content can be increased by mixing more oxygenated additives in biodiesel and cetane number will be higher [24]. The main reason for the reduction of CO emissions in the bio-diesel is the availability of oxygen which also helps in better combustion.
7.1.1.4. Hydrocarbon Emission characterization with different Load (Nm).

The figure 10 shows that in n-hexanol solvent based bio-diesel the emission of hydrocarbon drastically increases to 16% and 18% at lower and higher load respectively. This increase in hydrocarbon emission is because of the non-polar nature of the solvent.

It is to be noted that the incomplete combustion of fossil fuels and fuel evaporation from the engine could also contribute to hydrocarbon emission. This emission can be reduced by increasing the bio-diesel quantity [25] and by changing the air fuel ratio in the diesel engine. This is because when there is more oxygen mixed with fuel, it helps in good oxidation and complete combustion [26, 27].

7.1.1.5. Engine characterization for different Load (Nm) Vs Brake Specific Fuel Consumption.

The figure 11 shows the fuel consumption of engine versus different load (Nm). Brake specific fuel consumption (BSFC) is a parameter that indicates the efficiency of an internal combustion of the engine which intakes the fuel and burns to produce rotational power. The figure indicates that as the load increases the BSFC values decreases and in particular, for n-hexanol solvent based biodiesel and commercial biodiesel it saturates thereafter. Further, the result also shows that for the lower load (0-8 Nm), n-hexanol solvent based biodiesel resulted in 0.7% BSFC than diesel and n-hexane based bio-fuel which gave 0.76% and 0.72% BSFC respectively. This shows that at lower loads hexanol solvent
based biodiesel consumed less fuel that the commercial and blended n-hexane based bio-fuel. Relatively, low-load operation provided a good performance for biodiesels than commercial diesel. This is because the overall combustion time of the biodiesel was longer than diesel fuel.

![Comparison of Load (Nm) Vs Hydro carbon %](image1)

**Figure 10.** Comparison of Load (Nm) Vs Hydro carbon %.

### 7.1.1.6. Engine characterization for Load (Nm) Vs Brake Thermal Efficiency (%).

The figure 12 shows that at lower loads and higher loads, n-hexanol based bio diesel exhibits a Brake Thermal efficiency of around 17% and 22% respectively. Results of the thermal efficiency tests state that *Spirulina platensis* based bio-diesel in the ratio 20/80 blends produced reasonable and better efficiency compared to the commercial diesel.

### 7.2. Gas Chromatographic Analysis report of hexane based algae oil extract.

To confirm the chemical compounds present in the algal extract, the gas chromatographic analysis was done. The results of the analysis of algae oil extract (hexane and hexanol) tested in Amrita Research Centre Coimbatore, is shown in table 3 and 4. This shows that different chemical compound and nutrients are present in the algal oil. The data given in table 3 for hexane based algae extract shows the maximum percentage of glycopeptides, lipids, and trimethylsilyl.

![Comparison of Load (Nm) Vs BSFC %](image2)

**Figure 11.** Comparison of Load (Nm) Vs BSFC %.
In general, the lipid content is the significant factor responsible for increasing the quality and efficiency of the biodiesel. Higher the lipid content better will be the efficiency of biodiesel. This analysis helps to choose a suitable solvent for algae extract preparation. However, lipids content are found to be very less (5%) for hexanol based oil extract. The results also show that hexanol based bio-oil has lipid content lesser than hexane based extract oil. Moreover, it is found that even after removing the excess solvents in the oil, 75% of hexanol is still present in the algal oil. The Gas Chromatographic Analysis for hexanol based oil extract is shown in table 4.

### Table 3. Gas Chromatographic Analysis for hexane based oil extract

| Sl no | Compound name         | Quantity in percentage |
|-------|-----------------------|------------------------|
| 1     | Maytansine            | 10.83%                 |
| 2     | succinamide           | 0.53%                  |
| 3     | Phenazine             | 14.83%                 |
| 4     | Beta carotene         | 10.35%                 |
| 5     | dibenzafuanoctanol    | 0.68%                  |
| 6     | trimethylsily         | 25.8%                  |

8. Conclusions
The present work concludes that the hexane based solvent proposed in the present study is found to be suitable for extracting the lipid content present in the *Spirulina platensis* species. It is significant to
note that this solvent does not alter the nutrients of the algae during the extraction process. The BG11 medium is found to be highly efficient as well for algal growth. The gas chromatographic analysis reveals that the hexane and hexanol based algae extracts contains 35% and 5% lipids respectively. Further, the emission test proves that the quality of bio-fuel meets the desirable fuel standards stipulated by ASTM. The results of the study thus prove that BD20 generates lesser emission of noxious pollutants than commercial diesel. Hence it is proven that Spirulina platensis species serves as an efficient source for biodiesel extraction with hexane as the solvent. Furthermore, it is concluded that this algal species is found to be an effective alternate to the conventional energy sources.

9. References

[1] Rincon J, Canizares P and Garcia MT 2005 Ind. Enng. Chem. Res. 44 4373-9
[2] Diphare M and Muzenda E 2013 Influence of Solvents on the Extraction of Oil from Waste Lubricating Grease: A Comparative Study Int. Conf. Agricultural, Environment and Biological Sciences ( Pattaya, Thailand) P 76-78
[3] Topare N S, Raut S J, Renge V C, Khedkar S V, Chavanand Y P and Bhagat S L 2011 Int. J. Chem. Sci. 9 1746-50
[4] Nithya K, Sathish A, Gangadharan D, Vinaykumar A, Tharakan D and Sruthi B 2015 Int. J. Chem. Tech Res. 8 1947-56
[5] Demirbas A, Demirbas M F 2010 Algae energy: algae as a new source of biodiesel (London Dordrecht Heidelberg New York :Springer)
[6] Schenk P M, Thomas-Hall S R, Stephens E, Marx U C, Mussagn J H, Posten C, Kruse O and Hankamer B 2008 Bioenergy Res. 1 20-43
[7] Spolaore P, Joannis-Cassan C, Duran E and Isambert A 2006 J. Biosci. Bioeng. 101 87-96
[8] Ragauskas A J, Williams C K, Davison B H, Britovsek G, Cairney J, Eckert C A, Frederick W J, Hallett J P, Leak D J, Liotta C L and Mielenz J R 2006 Sci. 311 484-9
[9] Cuellar-Bermudez S P, Romero-Ogawa M A, Rittmann B E and Parra-Saldivar R 2014 J. Pet. Environ. Biotechnol. 5 184
[10] Hossain A S, Salleh A, Boyce A N, Chowdhury P and Naqiuddin M 2008 Am. J. Biochem. Biotechnol. 4 250-4
[11] Qiang H and Richmond A 1996 J. Appl. Phycol. 8 139-45
[12] Pirt S J, Lee Y K, Richmond A and Pirt M W 1980 J. Chem. Technol Biotechnol. 30 25-34
[13] Chisti Y 2008 Trends Biotechnol. 26 126-31
[14] Klass D L 1998 Biomass for renewable energy, fuels, and chemicals (Barrington, Illinois, United States : Elsevier)
[15] Batista A P, Gouveia L, Bandarra N M, Franco J M and Raymundo A 2013 Algal Res. 2 164-73
[16] Mendes R L, Nobre B P, Cardoso M T, Pereira A P and Palavra A F 2003 Inorganica Chim. Acta. 356 328-34
[17] Mahesh S, Desalegn T and Alemayehu M 2013 Indian. J. Energy. 2 116-20
[18] Salehzadeh A and Naeemi A S 2015 Int. J. Biosci. 6 250-4
[19] Vonschak A and Richmond A 1988 Elsevier. 15 233-47
[20] Pandey J P, Pathak N and Tiwari A 2010 JABU. 1 93-102
[21] Kannan G R, Karvembo R and Anand R 2011 Appl. Energy. 88 3694-703.
[22] Ribeiro N M, Pinto A C, Quintella C M, da Rocha G O, Teixeira L S, Guarieiro L L, do Carmo Rangel M, Veloso MC, Rezende M J, Serpa da Cruz R and de Oliveira A M. 2007 Energy Fuels. 21 2433-45
[23] Venkatesan S P and Kadireesh P N 2016 Int. J. Ambient Energy. 37 64-7
[24] Gürü M, Koc A, Can Ö, Çinar C and Şahin F 2010 Renew. Energy. 35 637-43
[25] Kumar P, Sharma M P and Dwivedi G 2016 Egypt. J. Pet. 25 255-61
[26] Raslavčiūsis L and Bazaras Ž 2010 Fuel. Process. Technol. 91 1049-54
[27] Dwivedi G, Jain S and Sharma M 2011 Int. J. Energy Sci. 1 105-109