

SMALL SCALE BIODIESEL PRODUCTION FROM *Sargassum* sp AND OPTIMIZATION OF CONDITIONS FOR YIELD ENHANCEMENT

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ABSTRACT

Production of biofuel from excessive natural resources and underutilized plant materials has drawn global attention due to its cost effectiveness, eco friendliness and sustainability compared to the conventional fossil fuels. This work was aimed to select the best marine flora available in the Northern Sri Lankan sea to produce biodiesel and to optimize the conditions to enhance the yield. *Sargassum* sp., *Ulva fasciata*, *Turbinaria ornata*, *Gelidium* sp. and *Thalassia* sp. were collected from the coastal region of the Jaffna peninsula and washed thoroughly sun-dried. The organic solvents used to extract oil from these algal and sea grass species were n-hexane and di-ethyl ether, while alkaline catalysts were used to convert the extracted oil into biodiesel via trans-esterification reaction. When the oil extraction was done separately from the five dried marine plant species using n-Hexane and Di-ethyl ether as solvents, significantly higher quantity of oil was obtained from *Sargassum* sp. than the other tested algal and plant species, hence *Sargassum* sp. was selected for further studies. When the conditions for the extraction of oil from *Sargassum* sp. such as *Sargassum* to solvent(n-Hexane: Di-ethylether) ratio (0:200), amount of algal biomass (100 g), longer contact time (24h), molar ratio (4:1), catalyst amount (0.6 %), temperature (60 °C), reaction time (25min) were optimized before the conversion of oil into biodiesel via trans-esterification reaction, there was a significant increase in the quantity of extracted oil by 11.7 times than the non-optimized conditions. Since the density of the biodiesel (867.3 kg/m³) meets the international standards, the quality of the biodiesel produced from *Sargassum* sp. could be recommended as satisfactory. Large scale multicenter studies need to be done in order to confirm and implement this finding.

Keywords: Biodiesel, *Sargassum* sp, Trans-esterification, Oil extract, Optimization

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1.0 INTRODUCTION

Continued usage of petroleum sourced fuels is now widely recognized as unsustainable, because of depleting supplies. Contribution of the fossil fuels to the accumulation of carbon dioxide in the environment is significantly higher thus they lead to greenhouse effect [1]. The cost of crude oil will continue to rise due to diminishing supply, therefore the production of fuels from alternate sources will be very much needed in the future decades [2]. In this scenario, biodiesel could be considered as one of the best alternative fuels due to its nontoxic nature [3]. Biodiesel has attracted attention during the past few years as a renewable and environmentally friendly fuel because of diminishing petroleum reserves and the deleterious environmental consequences of exhaust gases from petroleum diesel [4, 5]. Biodiesel is the monoalkyl esters of long fatty acids, which is derived from trans-esterification of biological substances [6]. The recent researches have proven that oil production from algae is clearly superior to that of terrestrial plants such as palm, grape seed, soy beans and has potential to completely displace fossil fuel [7, 8, 9]. Marine and freshwater flora can contribute between 20-80 % of the oil production by weight of their dry mass [10]. Marine flora shows much faster growth rates than terrestrial crop plants. The per unit area yield of oil from algae is estimated to be from 20,000 to 80,000 L per acre per year. The use of marine flora as energy crops has potential benefits, due to their easy adaptability to growth conditions, the possibility of growing in adverse environments, either in fresh or marine waters avoiding the use of land [11]. Furthermore, two thirds of earth's surface are covered with water, thus marine algae and plants would truly be renewable option of great potential for global energy needs [12]. Biodiesel offers a major economic advantage over other non- renewable energy sources [13]. It is used as biofuel, power generation [14,15] petrol additive [16, 17] to increase the octane rating and as a solvent [17]. Objectives of the study were to produce biodiesel from different marine flora and to optimize the conditions for biodiesel production from the selected marine flora before and during transesterification reactions in order to enhance the biodiesel yield.

2.0 MATERIALS AND METHODS

Sources of algal species

Marine macro flora such as brown algae *Sargassum* sp., *Turbinaria ornata* (Class: Phaeophyceae), green algae *Ulva fasciata* (Class: Ulvophyceae), red algae *Gelidium* sp., (Class: Florideophyceae) and marine sea grass *Thalassia* sp. were collected from coastal area of the Jaffna peninsula located in the Northern Sri Lanka.

Pretreatment of algal species

The samples were washed thoroughly two to three times using tap water. Then they were directly exposed to sunlight for one week to get a dried biomass. The dried samples were ground separately and the fine powder was passed through a 500 μ sieve [18].

Treatment with solvents

Treatment with hexane

Oils were obtained by extracting the algae (10 g) with hexane in a soxhlet extractor for 9 h. The extracted oil was separated by evaporating the solvent in a rotary evaporator at 45 °C for 15 min.

Treatment with di-ethylether

100 g of fine powder of each sample was weighed and added in to 500 ml brown bottle containing 200 ml di-ethylether. The solution was covered and shaken every 30min for 6h and allowed to stand for twenty-four hours in room temperature (27-30 °C). Then it was shaken well and filtered through Buchner funnel suction pump using Whatman (No.1) paper. After filtration, the solvent was removed by evaporation using a rotary evaporator under reduced pressure and at a temperature below 35 °C [19]. About 3.3 g crude extract was obtained and separated through the extraction process from 10 g sample of algal or seagrass. The extracted oil was converted into biodiesel through transesterification reaction in the presence of methanol. In this process triglycerides reacts with alcohols to form the fatty acid ester (biodiesel) and the glycerol [20]. During this reaction the alga and *Thalassia* oil was allowed to react with the methanol in the presence of alkaline NaOH.

OPTIMIZATION OF CONDITIONS FOR BIODIESEL PRODUCTION

Optimized condition for extraction of oil from marine floral biomass

Effect of biodiesel production from different marine floral species

Soxhlet apparatus thimble was filled with 10g of *Sargassum* sp., dissolved in 200 ml n-hexane and allowed 9h for oil extraction. The same oil extraction process was followed for all the tested algal and sea grass samples

Percentage of biodiesel yield = $\frac{\text{Biodiesel yield}}{\text{Oil used}} * 100$ [21, 22]

Effect of solvent ratio

Experimental setup was made by adding different solvent ratio (hexane:di-ethylether 200:0, 100:100,0:200) in a10g of marine floral biomass and the percentage oil yield was determined at the end.

Effect of algal biomass

Oil extraction setup was made by optimized *Sargassum* sp., optimized solvent ratio (hexane:di-ethylether 200:0) with different amount of ground algal biomass (10 g , 20 g, 40 g, 60 g, 80 g, 100 g). Same process was repeated for all the algal species under the optimized conditions.

Effect of contact time

Optimized *Sargassum* biomass 100g was placed on the thimble and experimental setup was allowed for different time periods (6 h ,9 h, 12 h, 18 h, 24 h) with optimized solvent ratio (hexane:di-ethylether 200:0).

Optimization of conditions for transesterification reaction

Effect of molar ratio of methanol to algae

Oil extraction experimental setup was done by *Sargassum* sp., optimized solvent ratio (hexane: diethylether 200:0) optimized algal biomass 100 g, and allowed for optimized time period (24h). Following mol ratio methanol to algal biomass was added to optimized transesterification reaction (2:1, 3:1, 4:1, 5:1, 6:1, 7:1).

Effect of catalyst amount

Oil extraction test arrangement was made with *Sargassum* sp, with the advanced dissolvable proportion (hexane:diethylether; 200:0), improved algal biomass 100 g and took into account streamlined timeframe (24h). Optimized methanol to oil proportion 4:1 was included with the accompanying impetus sum (NaOH) individually 0.3 %, 0.4 %, 0.5 %, 0.6 %, 0.7 % and 0.8 % for transesterification response [23].

Effect of temperature

Oil extraction trial arrangement was made with *Sargassum*, with the improved dissolvable proportion (hexane: diethylether;200:0), optimized algal biomass 100 g and considered upgraded timeframe (24 h) under enhanced transesterification response condition (molar proportion methanol to oil 4:1) with the streamlined impetus sum 0.7 % (NaOH) were blended utilizing attractive stirrer by different response time 5 min to 30 min [24, 25] and that blend was poured in to oil. This item was kept 35 °C to 60 °C diverse temperature in a water bath.

Effect of reaction time

Oil extraction preparation was finished with *Sargassum*, enhanced dissolvable proportion (hexane:diethylether 200:0), upgraded algal biomass 100 g, took into consideration advanced timeframe 24h, streamlined transesterification response condition molar proportion methanol to oil 4:1, optimized impetus sum 0.7 % (NaOH) were through blended utilizing attractive stirrer ,and the blend was poured in to oil. This item was 60 °C with improved temperature and taking into consideration 5min to 30min to response happened in water bath [26].

Statistical analysis

All the experiments were made in triplicates and the average values were used to plot the graphical representation. Statistical analyses were performed using Minitab 17.0 version. The data were

analyzed using one- way ANOVA. Tukey's multiple comparison test was used to determine significant difference at $p < 0.05$.



Figure 1: Stepwise process of extraction (a) Ground algal biomass, (b) Oil extraction by soxhlet apparatus, (c) Oil and solvent mixture, (d) Solvent evaporation by rotary evaporator, (e) Extracted oil, methanol and NaOH, (f) Unpurified biodiesel.

3.0 RESULTS AND DISCUSSION

Extraction of oil from algal biomass

Effect of biodiesel production from different marine flora

From these marine algae and seagrass species (*Sargassum*, *Ulva*, *Turbinaria*, *Thalassia*, *Gelidium*) higher amount of oil producing marine flora was selected by, based on following properties. Amount of oil produced by marine floral, amount of crude extract, pH of oil from marine floral species. The amount of oil yield from *Sargassum*, *Ulva*, *Turbinaria*, *Thalassia*, *Gelidium* were 11.6 ml, 5.2 ml, 4.8 ml, 2.3 ml, 0.9 ml (Figure 2) respectively and the amount of crude extract (oil + residue) were 3.3gram, 2.7 g, 1.9 g, 1.5 g, 0.7 g (Figure 3) respectively. Significantly the highest amount of oil yield (11.6 ml) and the crude extract (3.3 g) were obtained from *Sargassum* than the other species. Amount of oil produced by marine algae and seagrass species, and the amount of crude extract were directly proportional to the quantity of the oil produced and the final biodiesel yield. There was no significant difference in the pH values of the biodiesels produced among the different algal and plant species tested (Figure 4). Researches done in the past were also reconfirmed by this finding [15, 16].

Effect of solvent ratio

When different solvent ratio hexane:di-ethylether was used in the extraction process, significantly higher amount of fraction of oil yield (0.0751) was obtained with *Sargassum* sp. The effect of solvent ratio on hexane:di-ethylether percentage of oil yield extracted is shown in Table 1. Hexane has great ability to extract oil when compare to di-ethyl ether with a boiling point of 69 °C it is able to retain its liquid stage at all atmospheric conditions than for extreme climates. Contrast to hexane, di-ethyl ether is high volatile solvent boiling point (34 °C) approximately equal to room temperature. Because of this reason, the oil extraction of hexane: di-ethylether,0:200 ratio was not possible with soxhlet apparatus method. Therefore, ratio hexane: di-ethyl ether, 0:200 was tried using Buchner funnel suction pump method. [27, 28].

Effect of algal amount

When the amount of algal biomass initially used was 100g, oil yield was significantly increased by 68 % with *Sargassum* sp. than the other tested marine species. Oil yield was increased with the increasing amount of biomass (Figure 5). Therefore, in addition to the quantity of the algal sample, it could be concluded that the size of the algal particles also influenced in the final oil yield. [29, 30].

Table 1: Amount of oil extracted using different solvent combination

S.no	n-Hexane (ml)	di-ethyl ether (ml)	Fraction of oil extracted
1	200	0	0.0751*
2	0	200	0.0321
3	100	100	0.0542

Effect of contact time

When the contact time was varied from 5 to 24 h, significantly higher oil yield was obtained (10.18%) with *Sargassum* species at 24 h. Percentage yield at larger contact time could be explained by the fact that enhanced interaction between the solvent and biomass occurred after long contact time, which lead to homogenous mixing [31]. It was found that the biodiesel with higher oxidation stability is preferable as low oxidation stability can affect the quality of biodiesel. Biodiesels with poor oxidation stability can be easily remedied by adding antioxidants during this reaction [32]. Therefore, 24 h (Figure 6) would be sufficient enough to facilitate the oxidation process and oil yield from the plant substrate [8, 33, 34].

Transesterification reaction

Effect of molar ratio of methanol to oil

The stoichiometric ratio for transesterification requires 3 moles of alcohol and 1 mole of triglyceride to yield three moles of fatty acid alkyl esters and one mole of glycerol. However, higher quantity of alcohol is preferred to shift the equilibrium to form esters [20]. In this study, different range of 6 molar ratios of methanol to algal oil (2:1, 3:1, 4:1, 5:1, 6:1, 7:1) were examined. Significantly higher amount of biodiesel was obtained at 4:1 methanol to oil ratio than the other ratios used (Figure7). When higher molar ratio of methanol to oil is increased, the excess amount of oil promotes the forward reaction [35]. When the range of methanol to oil ratio was 2:1 to 7:1, maximum yield was obtained (0.945%).

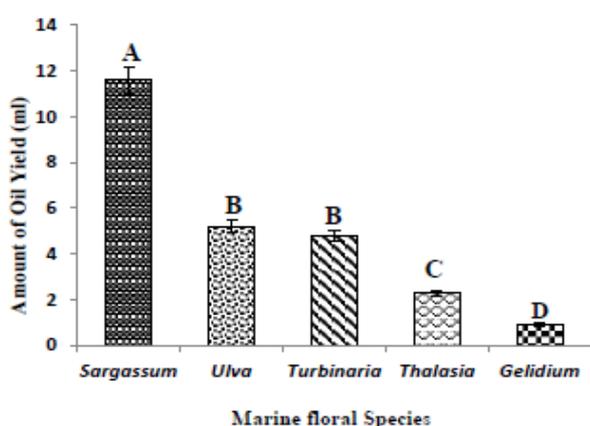


Figure2: Effect of oil yield from selected marine floral species

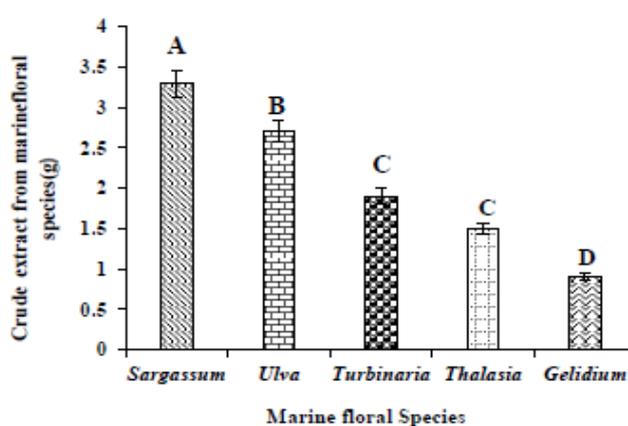


Figure3: Effect of crude extract from selected marine floral species

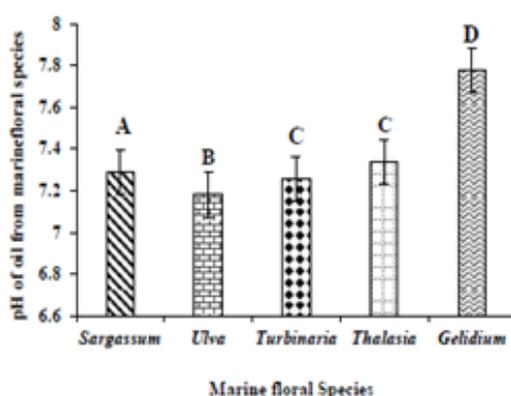


Figure4: Effect of Ph of oil from selected marine floral species

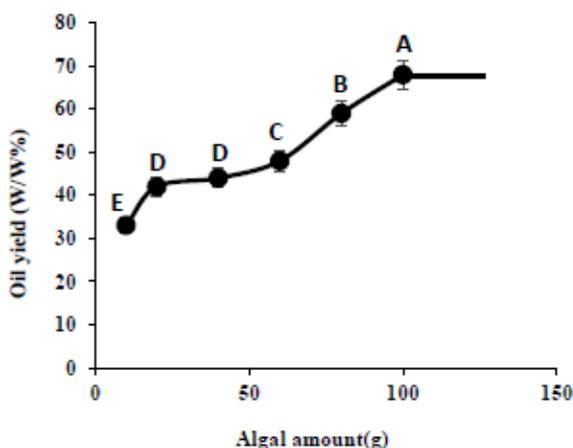


Figure 5: Effect of different algal amount of oil yield from *Sargassum* species

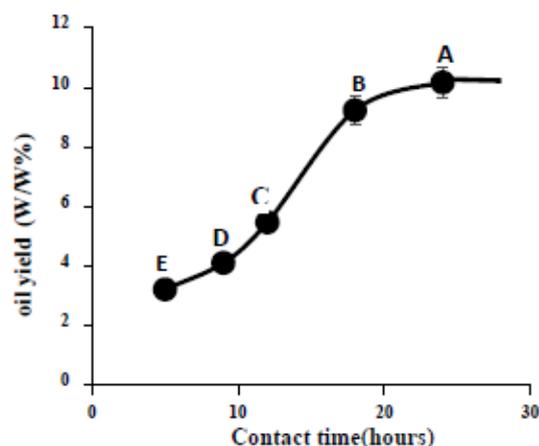


Figure 6: Effect of different contact time of oil yield from *Sargassum* species

Effect of catalyst amount

When different amounts of catalyst (0.2, 0.4, 0.6, 0.8, 1.0) were used, significantly higher amount of biodiesel production (0.93 % - Figure 8) was obtained when 0.6 %, and 0.7 % were used. The role of catalyst in transesterification reaction is very important. This reaction can be carried out with both, acid or alkali catalyst. However, using acidic catalyst has the disadvantages due to elevated rate of transesterification reaction and the reaction conditions are mild, consumption of methanol is significantly less, catalyst is less corrosive, and the acid catalyst process requires a high methanol to oil molar ratio and high acid catalyst concentration [14]. Commonly used alkaline catalysts include NaOH and KOH. In this study NaOH was used because of its cheap and effective [31, 36, 37].

Effect of temperature

The biodiesel production was obtained after the addition of methanol and alkali catalyst mixture to oil, these were kept under different temperature range 35 °C to 70 °C. Maximum amount of biodiesel was produced in between 45 °C to 60 °C temperature range. Therefore, the temperature has a considerable influence in the biodiesel yield produced. In this study, significantly higher biodiesel production was obtained at 60 °C, which is in the agreement with the available literature [38, 39]. When the temperature was optimized as 60 °C, biodiesel production was increased by 9.4 times (from 0.1 % -0.942 %) than the non-optimized condition. At 60 °C, biodiesel yield was 0.942 % and this was the maximum quantity for the production of biodiesel because there is a limitation in the boiling point of methanol. Biodiesel would reduce the amount of methanol used in the reaction mix, because of the forward reaction in transesterification reaction, so that the methanol becomes insufficient for further reactions. At 70 °C, biodiesel yield was 1.81 % (Figure 9) and the

reduction might be due to the higher temperature that could accelerate the saponification of triglycerides and had a negative effect on the product yield. Moderately higher temperatures in addition to the carbon source induce saponification of triglycerides in the formation of soap [17, 26].

Effect of reaction time

When the reaction time was kept at 25 minutes, biodiesel yield significantly increased (1.1 times - Figure 10) than the non-optimized reaction time. The reaction time required for the optimization of the alkaline catalyst transesterification reaction depends not only in the reaction temperature, but also on the degree of mixing in the process [40]. The reaction rate of the transesterification increased with the increasing temperature and the rate of mixing and this confirms the previous findings [41].

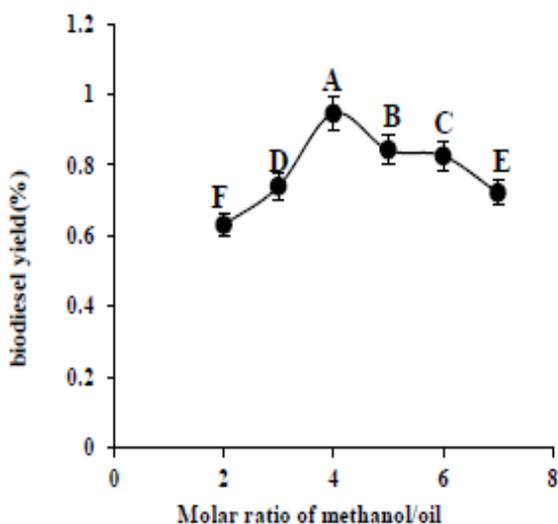


Figure 7: Effect of different molar ratio of methanol/oil of biodiesel yield from *Sargassum* species

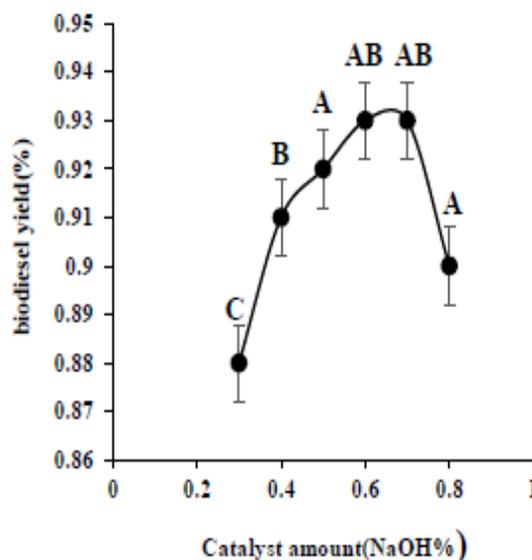


Figure 8: Effect of different catalyst amount of biodiesel yield from *Sargassum* species

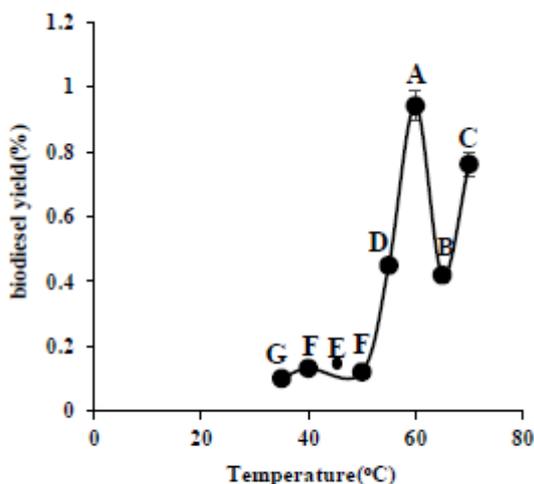


Figure 9: Effect of different temperature of biodiesel yield from *Sargassum* species

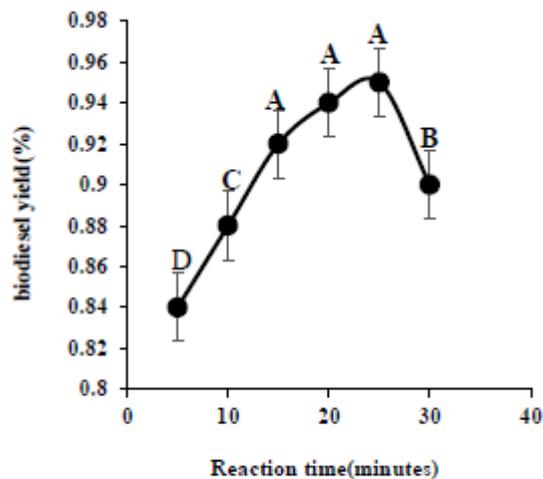


Figure 10: Effect of different reaction time of biodiesel yield from *Sargassum* species

Biodiesel analysis

The quality of biodiesel was assessed by measuring its properties such as flash point, fire point, cloud point and density as shown in Table 2. There was no significant difference between, petrodiesel and the produced biodiesel in the major biodiesel properties. Properties of the produced biodiesel fulfilled the international standards set by the USA ASTM D6751. The physical and chemical properties of the produced biodiesel, along with results from other researchers’ work on similar biodiesels, are compared with other non-edible biodiesels and petrodiesel and it was found that all the properties and qualities of the biodiesel fulfilled the international standards (USA ASTM D6751 and European Union EN14214) [42, 43]. Since the density of the biodiesel (867.3 Kg m^{-3}) falls between the internationally accepted standards, the quality of the biodiesel produced from *Sargassum* sp. could be recommended as satisfactory. Many researchers showed that densities of biodiesels do not vary considerably, as the density of methanol and oil are close to the density of produced biodiesel, which usually varies between 850 and 900 Kg m^{-3} [44, 45, 46].

Table 2: Comparison of the biodiesel properties with the petrol diesel

Properties	Petro diesel	Biodiesel
Flash point (°C)	134	142
Fire point (°C)	154	159
Cloud point (°C)	4	3
Density (Kg m^{-3})	865.2	867.3

4.0 CONCLUSION

Among the marine flora (algae and *Thalassia* sp.) tested, brown macroalgae, *Sargassum* sp was an efficient raw material for the biodiesel production, where oil extraction and transesterification were the two prime steps. Oil yield from *Sargassum* sp. was significantly increased by 3 times after optimization of pre-transesterification conditions such as solvent ratio, algal amount, and contact time. Biodiesel yield from *Sargassum* sp. was significantly increased by 11.7 times after transesterification conditions such as molar ratio methanol/oil, catalyst amount, reaction temperature, reaction time. Optimization of conditions before and after transesterification, significantly increased the biodiesel yield from the dry mass of *Sargassum* sp. Since the density of the biodiesel (867.3 Kg^m⁻³) meets the international standards, the quality of the biodiesel produced from *Sargassum* sp. could be recommended as satisfactory. Therefore, widely spread excessively available marine *Sargassum* sp. could be a good natural source for the production of biodiesel. Large scale multicenter studies and efficient purification procedures need to be carried out in order to proceed this towards commercial applications.

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