

PRODUCTION AND CHARACTERIZATION OF BIODIESEL AND GLYCERINE PELLET FROM MACROALGAE STRAIN: *CLADOPHORA GLOMERATA*

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ABSTRACT. Biodiesel was prepared by extracting oil from *Cladophora glomerata* green algae followed by transesterification of the oil using NaOH as a catalyst. The algae Oil extraction was carried out using two different techniques (Soxhlet and refluxing) and similar oil yield was obtained (23-24%). The resulting biodiesel showed desirable physical and chemical properties. Specific gravity, acid value, iodine value, ash content and calorific value of the algae biodiesel were within the specification of American Society for Testing and Materials (ASTM) and European Standards (EN). The analysis of fatty acid methyl ester composition revealed, 63, 27 and 10% for 9-octadecenoic, hexadecenoic and octadecenoic acid methyl ester, respectively. From the production line, two waste streams (glycerol and residual biomass) were combined to form a glycerine pellet. The measured energy content of the glycerine pellet was found to be comparable with firewood. Therefore, *C. glomerata* could potentially be utilized for the production of both biodiesel and glycerine pellet with no net waste in the transesterification process.

KEY WORDS: Algae oil, Biodiesel, Transesterification, Glycerine pellet, Macroalgae, *Cladophora glomerata*

INTRODUCTION

Conventional sources of energy like natural gas, oil and coal have caused immense damage to the environment by increasing the carbon in the atmosphere causing various global climatic events, such as acidic rain and global warming [1]. Currently, bio-fuels are being explored as an alternative source of energy. Among the bio-fuels, biodiesel seems to be at the forefront because of its environmental credentials such as renewability, biodegradability and clean combustion behaviour [2]. The production of biodiesel from oil crops [3, 4], waste oil [5], plant seed oil [6] and fruit and vegetable wastes [7] had been reported in the literature. These feedstocks are mainly known to be the first and second generation sources of biofuel [8].

Algae are the third generation fuel, which are considered as the most promising alternative and renewable feedstock sources for producing biodiesel [9]. Like other vegetable oils or lipids, the algae oils are composed of triglycerides (fatty acid ester of glycerol), which can be converted into methyl esters (biodiesel) and glycerol via transesterification process. Algae have several advantages over conventional crops. Because of their simple cellular structure, algae have higher rates of biomass and oil production, faster growth rate than other conventional crops [10]. The per unit area yield of oil from algae is estimated to be from 20,000 to 80,000 L per acre, per year; this is 7–31 times greater than the next best crop, palm oil [11]. In contrast to the first and second generation, algae can be grown in water bodies (which account two thirds of the earth surface) by avoiding the use of land [12, 13].

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Based on cellular complexity algae could be classified in to two as microalga and macroalga. Microalgae are unicellular, plant like organisms which range from 1–50 μm in size [14] and can be seen only using a microscope. On the other hand, macroalgae are multicellular, macroscopic, and can grow up to 60 m in length [15]. Different research findings reported that both macroalgae and microalgae are important sources of biofuel. Deriving biodiesel from microalgae, however, presents some significant challenges. There are high costs attached to cultivation and harvesting of microalgae, rendering them economically infeasible with current technologies and processes. In addition, the vulnerability of microalgae to contaminants and elements necessitates the use of sophisticated systems such as bioreactors which increase the cost and complexity of operations. Nevertheless, because of their high oil yield, microalgae are widely studied [16-18] as biodiesel feedstock.

Macroalgae present significant advantages in their ease of cultivation and harvesting, and subsequently low costs for both [19]. Besides, macro-algae are effective in nutrients uptake (N, P) from sewage and industrial waste water without being affected [20]. Irrespective of these advantageous of macroalgae over microalgae, there are few studies on macroalgae as biodiesel feedstock [21, 22]. In Ethiopia there are several species of green algae. However, research activities made to utilize them as feedstock for bio-fuel production is scant and at its infancy stage. Therefore, in the present study, one of a macroalgae named *Cladophora glomerata*, collected from lake Hawasa, was studied for its potential of biodiesel production. The oil was extracted, transesterified and characterized using standard methods. Furthermore, the residual biomass of the transesterification process was mixed with glycerol to produce a glycerine pellet which could be used as refuse derived fuels (RDF). The glycerine pellet energy content was estimated and compared with firewood.

EXPERIMENTAL

Chemicals, apparatus and instruments

Methanol, chloroform, and *n*-hexane (99.7% purity) was purchased from Merck (Darmstadt, Germany), while the sodium hydroxide was from LobaChemie (GmbH, Switzerland). Glycerol standard (99.5% purity) was obtained from VWR International. Trichloethylene (98% purity) was purchased from Thomas Scientific and used after further purification. Apparatus used were: Soxhlet extractor, thermo-regulator heater equipped with stirrer (MGW-LAUDA, D6970, Germany), specific gravity bottle, pH meter (Hanna model No. 02895), rotary evaporator, oven (BTOV 1423), Ferranti portable viscometer model VL, Abbe refractometer, and Hewlett adiabatic bomb calorimeter (model 1242). GC-MS (Little Falls, CA, USA), flame photometry (JENWAY PFP7, Germany) and UV-Visible spectrophotometer (Thermo Scientific, USA) were the instruments used in the biodiesel characterization.

Sample collection and preparation

Samples of *C. glomerata* were collected from Lake Hawassa; Ethiopia from May to July in 2012. The identification of the algae to species level was undertaken on the site by using algae identification field guide. The samples were washed with lake water *in situ* to remove trapped mud, sand, epiphytes and attached particles and then put into clean polyethylene bags. The open air dried algae biomasses were ground with mortar and pestle. The ground algae biomasses were further dried for 20 min at 80 °C to remove moisture.

Oil extraction

Two extraction methods (Soxhlet and refluxing) were employed for the extraction of algae oil. In Soxhlet, 30 g dried algae biomass was placed in a thimble made from filter paper and loaded

to Soxhlet apparatus. Trichloethylene (300 mL) was added in the ratio of 1:10 g/mL and extracted for 18 h and rotator evaporated to remove the solvent. Refluxing was carried for 3 h by mixing 30 g algae biomass and 300 mL Trichloethylene in a distillation flask. The extract was separated by using Buchner funnel under weak vacuum and the solvent was removed using rotator evaporator.

Algae biodiesel production

Transesterification reaction procedure reported by Refaat *et al.* [23] and Muthukumar [24] has been used with modification for the production of biodiesel. In the first step, the amount of the NaOH catalyst required was determined by titration which was found to be 6.3 g NaOH/L oil. Accordingly, 0.63 g of NaOH was taken for 100 mL of algae oil. This amount of NaOH was dissolved in 20 mL of methanol and the mixture was heated to 60 °C before mixing it with the oil. The methanol-NaOH solution was added to algae oil at 60 °C with a volumetric ratio of 1:5. The mixture was then blended by using magnetic stirrer for 1½ h and allowed to settle for 24 h at room temperature to separate glycerol from crude biodiesel. The crude biodiesel was then washed by adding 30% (v/v) of warm water at 50 °C followed by rocking for 5 min. The washing process was repeated four times to make sure that all the soap residuals have been removed. The washed biodiesel was then placed in an oven at 100 °C for 6 h to evaporate any water that might be left during washing. Finally; the volume and weight of the biodiesel were measured, and the sample was stored for characterization.

Determination of physico-chemical properties of algae biodiesel

Both preliminary biodiesel quality tests (soot, 3/27, water solubility and refractive index test) and critical biodiesel tests (density, specific gravity, iodine value, kinematic viscosity, pour point, cloud point, carbon residue, ash content, acid value and calorific value) were determined at petroleum quality test laboratory in accordance with ASTM standard test methods [25].

Fatty acids methyl ester composition of the algae biodiesel was determined using GC-MS. The GC-MS was equipped with an inert XL Mass detector (Agilent-Technologies 5975), auto injector (Agilent-Technologies 7683B series) and HP-5MS 5% phenyl methyl silox capillary column (27 m x 250 µm with film thickness x 0.24 µm). The sample for injection was prepared by diluting 1 µL of the algal biodiesel with 99 µL of *n*-hexane. And from this solution 1.0 µL of the sample was injected into the GC-MS in the split less mode. Helium was used as a carrier gas with a flow rate of 1 mL/min. The temperature of column oven was programmed, it started at 40 °C for 0 min hold and ramped to 100 °C at 4 °C/min and retained for one minute at 100 °C. It was increased from 100 to 310 °C at 10 °C/min where it was finally held for 16 min. The temperature of the injector and MS transfer line was set at 250 and 280 °C, respectively. An electron ionization system (with ionization energy, i.e. 70 eV) was used for GC-MS detection while scanning mass ranged from 33–500 m/z. The fatty acid methyl ester compositions were determined as relative percentage of the total peak area.

The amount of free glycerol in the biodiesel was measured with a UV-Visible spectrophotometer based on the method developed by Keppy *et al.* [26]. The sample was first treated with sodium periodate which reacts with free glycerol in the sample to generate formaldehyde. Then reaction between this formaldehyde and acetyl acetone produced the yellow complex, 3,5-diacetyl-1,4-dihydrolutidine. This yellow compound exhibited a maximum absorbance peak at 410 nm, where its concentration in the sample was measured. The concentration of the complex was proportional to the amount of free glycerol in the sample. For the calibration graph, stock standard solution of glycerol (1000 mg/kg) was prepared by diluting 1 g of glycerol solution into a 1 L solvent (1:1 ratio of deionized water and ethanol) and series of six glycerol reference standards were prepared in the range of 0 (blank) to 18.75 mg/kg from

the stock solution by serial dilution. Working standards and the samples were treated with 1.2 mL of a 10 mM sodium periodate solution and shaken for 0.5 min. Each solution was then treated with 1.2 mL of a 0.2 M acetylacetone solution, placed in a water bath at 70 °C for 1 min and stirred manually. The solutions were immediately placed in cold water to stop the reaction. Finally, the absorbance of the complex formed was plotted against the concentration of the glycerol standard solutions. The calibration curve showed very good correlation coefficient value (0.997). Based on this calibration curve the free glycerol content in algal biodiesel sample was determined.

Flash point was calculated based on Eq. (1), which relates calorific value (heat of combustion), viscosity, density and flash point [27]. Except flash point all other variables can be obtained experimentally.

$$\text{Heat of combustion} = 0.4527v - 0.0008\rho - 0.0003FP + 40.3667 \quad (1)$$

where, heat of combustion (MJ/kg), v is viscosity (mm^2/s), ρ is density (g/L), and FP is flash point ($^{\circ}\text{C}$).

The metals (sodium, potassium, magnesium and calcium) content were determined using a flame photometer. The sample (5 g) was taken in a silica crucible and heated on a hot plate and the residue was further burned to ash in a muffle furnace at 525 °C. After dissolving the ash in a concentrated sulphuric and nitric acids, the metal content was analyzed using a flame photometer. The flame photometer was first calibrated by aspirating series of standard solutions and then the sample solutions were aspirated and quantified.

Glycerine pellet preparation

Glycerine pellet formation is fairly straight forward. The raw materials (waste glycerine and waste biomass) were mixed by weight ratio and blended by hand in a large mixing bowl. The ratios of glycerine (30 g) to waste biomass (50 g) were then mixed to produce a crude unfinished material. This raw pellet is transferred into the mold, a short length of PVC with one end sealed. The diameter of the PVC pipe is 12.5 mm, and its length is 101.6 mm. The mold helps the pellet retain its cylindrical shape. Then a short PVC rod, slightly smaller than the PVC internal diameter, was inserted into the open end of the mold to compress the pellet. Pressure was applied by hand for 2 min. This pressure not only reduced the pellet size, but also facilitates the glycerine to permeate the materials and form a single firm unit as shown in Figure 1. Finally, the calorific value or energy content of the sun-dried glycerine pellet was determined by bomb calorimeter.



Figure 1. Glycerine pellet produced from waste glycerine and biomass.

RESULTS AND DISCUSSION

Algae oil yield

The amounts of algae oil extracted by Soxhlet and refluxing were 24.2 and 23.4%, respectively. Statistical analysis (t-test at probability 5%) showed no significant difference in the amount of oil obtained using the two methods. Nevertheless, in terms of time, refluxing is much better than Soxhlet. Because 7.26 g of oil was extracted after 18 h of Soxhlet extraction while 7.01 g is obtained within 3 h of refluxing time.

The amount of oil obtained from *C. glomerate* was found to be higher than the oil content of other macroalgae species reported before. For example, Suganya *et al.* [21] reported 11.14% oil yield from *E. Compressa* macroalgae by Soxhlet extraction. Similarly, El Maghraby and Fakhry [28] obtained 1.56-4.14% oil yield from three different macroalgae species (*Rhodophyceae*, *Chlorophyceae* and *Phaeophyceae*).

Biodiesel yield

The biodiesel prepared using 100 g algae oil, 20 g methanol, 0.7 g NaOH at 60 °C and 1½ h reaction time yielded 96.8 g biodiesel and 22 g glycerol as shown in Figure 2. The loss of 1.2 g might be attributed to some un-reacted alcohol, residual catalyst and emulsion removed during the washing stage of the transesterification process. Conversion yield is set as one biodiesel production test parameter by EN (EN14103), which ranges between 96-100%. So, the conversion yield obtained in this work ($96.8 \pm 0.4\%$) falls within the EN specification. This indicates that the oil from *C. Glomerata* is a potential candidate for biodiesel feedstock.

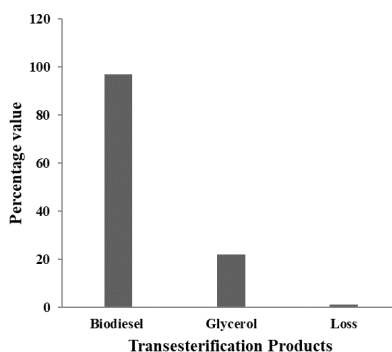


Figure 2. Biodiesel and glycerol percentage yield from algae oil.

The biodiesel yield obtained from this study is much higher than those reported by other researchers; Muthukumar *et al.* [24] and Mondal *et al.* [29] reported 60 and 80% biodiesel yield, respectively, from two different algae strain.

Preliminary quality test

Methanol and biodiesel were mixed in the ratio of 27:3, agitated and settled for 30 min. As a result, no formation of layer, turbidity and cloud was observed which indicates a positive result for 3/27 test. Similarly, in water solubility test clear layers were formed implying that the biodiesel also passes this preliminary test.

The amount of CO₂ released from combustion is an indication of the efficiency of the combustion reaction. Table 1 shows that the time required for color change in gasoline and biodiesel is less compared to kerosene; the more CO₂ is available the faster the color change

takes place. When gasoline and biodiesel is burning more CO₂ is released than it is in kerosene burning. Hence less time is required to cause color change in the universal indicator. This indicates that the combustion efficiency of biodiesel is comparable with gasoline but better than kerosene. Soot occurs when a combustion reaction is not complete. The more soot, the less efficient the combustion process. As it can be seen in Table 1, the biodiesel forms less soot compared to gasoline and kerosene. This is because algal biodiesel contains oxygen which increases efficiency of combustion. In addition, it contains less of heavy oil residues that are normally found in diesel fuel.

Table 1. Soot testing efficiency of biodiesel.

Fuel type	Time of color change (min)	Initial color of universal indicator	Soot observations at glass funnel	Final color of universal indicator
No Fuel (control)	-	-	-	-
Diesel (gasoline)	5.7	Violet	Medium soot	Red
Biodiesel	6.0	Violet	Less soot	Red
Kerosene	7.2	Violet	More soot	Red

Refractive index is the other preliminary parameter that can be quickly evaluated. As can be seen in Table 2, the biodiesel derived from *C. glomerata* has a refractive index of 1.437 which is within the acceptable range. This implies that heavier molecules are converted into lighter one's during transesterification process. In general, the preliminary biodiesel quality test results suggest that there is high probability that the biodiesel prepared would also meet the critical quality test specifications.

Critical parameters quality test

From Table 2 it can be seen that most of the critical chemical and physical quality control parameters are in good agreement with ASTM and EN test specifications.

Table 2. Biodiesel quality test result.

Properties	ASTM and EN standard values			Result of the present study
	Method	Limits	Units	
Methyl ester	EN14103	96.5	% W	96.8
free glycerol	D6584	0.02 Maximum	Mass %	0.000247
Flash point	Calculated	92 Minimum	°C	103.0667
Refractive index (30°C)	-	1.38 -1.49		1.437
Acid number	D 974	0.50 Maximum	mg KOH/g	0.197
Iodine value	EN1411	120 Maximum	g I/100g	101.4973
Kinematic viscosity (40 °C)	D445	1.9 - 6.5	mm ² /s	6.794
Cloud point	D2500	Not given	°C	-3
Pour point	D97	Not given	°C	-7
Density (15 °C)	EN14214	860-900	Kg/m ³	889
Specific gravity (15.56 °C)	D1298	870 -900	Kg/m ³	891
Calorific value	-	-	MJ/kg	39.37
Na + K content	EN 14538	5 Maximum	ppm	0.67
Ca + Mg content	EN 14538	1 Maximum	ppm	0.62

The properties of the fatty acid component and their structural features determine the fuel properties of biodiesel. Important fuel properties influenced by the fatty acid profile include density, specific gravity, acid value, pour and cloud point, viscosity and heat of combustion [30, 31]. The major fatty acid methyl ester composition of *C. Glomerata* was found to be hexadecenoic acid methyl ester (C16:0) (27%), 9-octadecenoic acid methyl ester (C18:1) (63%) and octadecenoic acid methyl ester (C18:0) (10%). The mass spectra of these three fatty acid methyl esters showed 99% spectral similarity with NIST-05 mass spectral library.

The total content of unsaturated fatty acid methyl ester (63%) was found to be higher than the total saturated fatty acid methyl esters (37%) in the biodiesel. This is further evidenced by the higher iodine value (103.1 g I/100 g) which indicates the presence of more unsaturated fatty acid methyl ester. The iodine value was also within the limit of EN standard (≤ 120 g I/100 g). Free glycerol is an undesirable by product in the transesterification reactions. It is known to cause clogging in fuel filters, injectors and deposit in the bottom of fuel storage tanks [25]. To prevent these operational problems, ASTM and EN limits free glycerol to 0.02 weight percent maximum. In the present work, free glycerol content of the biodiesel was determined to be 2.47 mg/kg or 0.000247 weight percent, which is well below the ASTM and EN limit set.

Specific gravity has been described as one of the most basic and most important properties of fuel because some important performance indicators such as cetane number and heating values are associated with it [31]. From 10 to 60 °C, specific gravity values of palm oil biodiesel have been reported to be 1.033413 to 1.035419 times that of fossil diesel [32]. Similarly, the specific gravity of the biodiesel from this study was found to be 1.0433255 times that of petroleum diesel (at the reference temperature 15.56 °C). The specific gravity obtained (891 kg/m³) fall well within the limit specified for biodiesel fuels (870–900 kg/m³) by the ASTM Standard. This value is also comparable with previous reports on palm kernel biodiesel (883 kg/m³), and soy bean biodiesel (880 kg/m³) [31, 32]. Density of the biodiesel (889 kg/m³) also falls within EN (EN14214: 860-900) tolerable limit. It is also comparable with previous studies; *Eruca sativa* (881 kg/m³), rapeseed (875 kg/m³), and spent frying oil (873 kg/m³) and frying oil biodiesel (873 kg/m³) [33, 34].

The acid value indicates the degree of fuel ageing during storage, as it gradually increases due to hydrolytic cleavage of ester bonds. High fuel acidity has been discussed in the context of corrosion and the formation of deposits within the engine [30]. To mitigate these negative effects, the EN and ASTM Standards allows a maximum acid value of 0.5 mg of KOH/g. The acid value of the algae biodiesel gave 0.197 KOH/g which is in agreement with the specified limit, implying that the algae biodiesel will not pose problem on the long-term performance of the engine.

Cold flow properties of biodiesel are expressed in terms of cloud point (CP) and pour point (PP). CP is the temperature at which a liquid fatty material becomes cloudy due to formation of crystals while PP is the lowest temperature at which it will still flow [30]. The cloud and Pour point of algae biodiesel was -3 and -7 °C, respectively. The CP and PP values of the algae biodiesel in the present study, is much better than the CP and PP values of biodiesels derived from other feed stocks (olive oil: -2 and -3 °C; rapeseed oil: -2 and -9 °C; soybean oil: 0 and -2 °C; sunflower oil: 2 and -1 °C; tallow: 17 and 15 °C) [30]. The saturated fatty acids have significantly higher melting point than unsaturated fatty acids. For example, due to its high content of saturated compounds, tallow and palm oil methyl esters have CP of 17 and 13 °C, respectively. In contrast, feed stocks with relatively low concentrations of saturated long-chain fatty acids generally yield biodiesel with much lower CP and PP [30, 35]. Thus, the lower CP and PP value observed in the present study is attributable to the higher proportion of the unsaturated methyl ester (C18:1) and less proportion of the saturated methyl esters (C18:0 and C16:0) found in the algal biodiesel.

The kinematic viscosity (6.71 mm²/s) of the algae biodiesel was slightly higher than ASTM (1.9-6.0 mm²/s) standard value. The nature of dominant fatty acids, carbon chain length, and degree of saturation are important factors that determine viscosity [30, 35, 36]. For instance, viscosity increases with the number of CH₂ moieties in the fatty ester chain and decreases with an increasing unsaturation [37]. This implies that presence of multiple bonds imparted low viscosity to biodiesel. Therefore, in the present study, the dominant monounsaturated oleic acid (63%) is responsible for the slight increment of kinematic viscosity of the produced algae biodiesel. Compared with the literature value, the viscosity of the algae biodiesel in the present work (6.71 mm²/s) is much lower than castor oil biodiesel (10.43 mm²/s) [38].

Heating of combustion (calorific value) of a fuel is the thermal energy released per unit quantity of fuel when the fuel is burned completely, and the products of combustion are cooled back to the initial temperature of the combustible mixtures. It measures the energy content in the fuel. The calorific value of algae biodiesel reported in this study (39.37 MJ/kg) was found to be comparable with previous studies on soybean (39.48 MJ/kg), rapeseed (39.46 MJ/kg), and jatropha (39.45 MJ/kg) [39]. The calculated value of flash point for algae biodiesel was found to be 103.0667 °C which is within ASTM (≥ 92 °C) recommended limit. Other parameters such as alkaline (Na and K) and alkaline earth metal (Ca and Mg) content also meet the requirement specified by ASTM (Table 2). It is well known that the content of these metals (Na, K, Ca and Mg) in biodiesel is mainly determined by the efficiency of the washing step. The results indicate that the washing procedure used was efficient in removal of these metals from the produced algae biodiesel.

Glycerine pellet evaluation

A key element of this part of the study is energy estimation of glycerine pellet which is produced in our laboratory. The bomb calorimeter result revealed that the glycerine pellet has 13.65 MJ/kg of energy. As it can be seen in Table 3, the energy content of glycerine pellet from this study is comparable with other competing energy sources implying that it could be used as an additional refuse derived fuel (RDF). The glycerine pellet burns cleaner than the coal currently used as fuel in many industries. Additionally, since two pre-existing waste streams are used to make the pellets, it doesn't require new raw materials. Also, the energy required for making it is substantially less than the energy required to produce coal. Most importantly, the glycerine pellet is nearly carbon-neutral fuel.

Table 3. Energy content of different RDF and glycerine pellet (Brady *et al.* [40]).

Fuel source	Energy (MJ/kg)
Coal	11-24
Dry wood	14.7-17.4
Waste plastic	29-40
Household waste (RDF)	12-16
Household (RDF)	13-16
Paper sludge (RDF)	12.5-22
Waste wood	15-17
Glycerin pellet (from this study)	13.65

FAO [41] report indicates that 1 kg of fuel wood gives 13.8 MJ and one cubic meter of fuel wood equals to 750 kg [42]. Based on this data, one cubic meter of fuel wood is equal to 758 kg of glycerine pellet. Consequently, 1 kg glycerine pellet replaces 0.0013 cubic meter of fuel wood. In general, the cost of raw material is often the biggest obstacle to large scale commercialization of biodiesel. It is estimated to cost 70-85% of biodiesel production cost [43]. Therefore, utilization of biodiesel residuals to generate commercially important by products (i.e. glycerine pellet) is a recommended approach to decrease the cost of biodiesel.

CONCLUSION

The green algae *Cladophora glomerata* oil was extracted and chemically converted via alkaline transesterification reaction to biodiesel (fatty acid methyl ester). The oil yield obtained from *C. glomerata* in this study is much better than most of the macroalgae strain reported from previous studies. The biodiesel produced mainly composed of monounsaturated (63%) and saturated (37%) fattyacids. All the determined parameters of algae biodiesel were found to comply with ASTM and EN standard values implying its suitability as diesel fuel substitute. Furthermore, the

glycerine pellet prepared, could also be used as an important source of fuel, replacing firewood particularly in the developing nations like Ethiopia. However, large scale production for commercial purposes requires optimization of different parameters such as glycerol to biomass ratio, pellet size, a pilot scale operation and holistic economic evaluation of the production process.

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