Synthesis and Assessment of Physicochemical Attributes of Biodiesel Obtained from Cottonseed (Gossypium arboretum L.) Oil

Bikash Parajuli^{1*}; Dhruba Prasad Poudyal¹; Manoj Kumar Tharu² ¹Department of Chemistry, Birendra Multiple Campus, Tribhuvan University Chitwan, Nepal ²Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal

Corresponding Author:- Bikash Parajuli1*

Abstract:- Biodiesel manufacture is one of the advanced and technical areas for researchers as it is conquering the world because of petroleum shortage and increasing rate together with the environment friendly nature. Biodiesel define as the mono-alkyl esters of fatty acids prepared through trans-esterification reaction.

The use of biodiesel is increasing rapidly worldwide due to its positive impact on diesel engines, combustion processes, and pollution formation. Biodiesel is a wellknown derivative of long-chain fatty acids that can be obtained from renewable sources such as vegetable oils or animal fats. It can be effectively used in diesel engines. The process of preparing biodiesel from raw materials, such as cottonseed oil, involves the trans-esterification of fat, followed by biodiesel characterization testing. Cottonseed oil, non-edible oil, was chosen as the raw material for this experiment, as it does not contribute to food versus fuel conflict. Methanol was used as a catalyst in the trans-esterification process for biodiesel preparation. However, the optimum conditions for biodiesel production are suggested in this paper, a maximum of 77% biodiesel was produced with 20% methanol in presence of 0.5% sodium hydroxide. Furthermore, after the preparation of biodiesel, for its identification, numbers of test was done and the prepared biodiesel was verified.

Keywords:- Biodiesel, Cotton-Seed, Transesterification, Physicochemical Properties of Biodiesel.

I. INTRODUCTION

The scarcity of conventional fossil fuels, growing emissions of combustion generated pollutants, and their increasing costs will make biomass sources more attractive. On the other hand, biomass use, in which many people already have an interest, has the properties of being a biomass source and a carbon-neutral source. Experts suggest that current oil and gas reserves would suffice to last only a few more decades. To meet the rising energy demand and replace reducing petroleum reserves, fuels such as biodiesel and bioethanol are in the forefront of alternative technologies. Accordingly, the viable alternative for compression-ignition engines is biodiesel [1]. World energy demand continues to rise. The most feasible way to meet this growing demand is by using alternative fuels. One such fuel that exhibits great potential is biofuel, in particular biodiesel. The term biofuel can refer to liquid or gaseous fuels for the transport sector that are predominantly produced from biomass. Biofuels include energy security reasons, environmental concerns, foreign exchange savings, and socioeconomic issues related to the rural sector. In developed countries there is a growing trend toward using modern technologies and efficient bioenergy conversion using a range of biofuels, which are becoming cost wise competitive with fossil fuels[2].

Biodiesels are esters of long chain fatty acids synthesized by the process of transesterification by reacting different plant sources like soybean oil [3] or other vegetable oil [4] or even animal fat [5] with an alcohol in order to produce a mono-alkyl ester. The first successful transesterification was performed by Patrick Duffy In 1853 from a vegetable oil after four decades from the invention of first functional diesel engine [6]. Later on in 1937 a Belgian scientist, Chavanne patented a procedure of transforming vegetable oil into fuel by alcoholysis of vegetable oil with ethanol as a result fatty acids from the glycerol were replaced by alcohols [7].

Biodiesel is often blended with conventional diesel and distributed for retail use and 'B' factor is used to state ratio of biodiesel in any other fuel such as B100 for 100% biodiesel, B20 for 20% biodiesel and 80% petrodiesel, B5 for 5% biodiesel mixed in 95% petrodiesel and so on [8] [9]. Only minor or no modification is required in diesel engine to utilize blends up to 20% biodiesel [10] but B100 may require certain modifications to stabilize performance and reduce maintenance [11]. The pure form of biodiesel i.e. B100 can be used or a blend of it with petroleum diesel in any concentration specially in engines with injection pump but most manufacturers set a limit of B5 to B20 [12]. Although few modern diesel engines are not being designed to burn biodiesel as fuel but a remarkable ratio of heavy duty engines are capable of operating with biodiesel blends up to B20 [9].Today, blended form of biodiesel is used in United States for vehicular uses [13] and in United Kingdom for railway usage [14]. Moreover, it is also being used as cleaning oil spills [15] and in generators as fuel [16].

The blend ratio, quality and load conditions under which the fuel is combusted regulates the thermal efficiency of biodiesel [17] but in a study it was found that the power output of B40 was superior than traditional hydrocarbonbased diesel at higher compression ratio [18]. The carbon emission is significantly affected by the raw material used to synthesize biodiesel [19] even when it is blended with conventional petrodiesel [20]. For example, in an experiment, the biodiesel manufactured from soy showed the highest carbon emission while that from cooking oil showed least emission [21]. In another study it was found that the blended form particularly B5, B8 and B20 which are regularly used in vehicles resulted in significantly reduced carbon emission than regular diesel [9].

The major factor behind adoption of biofuel program is energy security which is obtained by reducing oildependence or by replacing it with locally available fuel sources [22]. Consequently, many nations worldwide are now involved in accelerating the use of biofuel including biodiesel as an alternate to conventional oil and fossil fuel and legislations and laws have been implemented by governments to boost renewable energy use [23]. Biodiesel has got better lubricant properties than fossil diesel [25]. Its oxygen content improves the combustion process, leading to a decreased level of tailpipe polluting emissions. Biodiesel is non-toxic and quickly biodegrades. The risks of handling, transporting, and storing biodiesel are much lower than those associated with fossil diesel [26]. The competitiveness of biodiesel relies on the prices of biomass feedstock and costs, which are linked to conversion technology [27]. Depending on the feedstock used, byproducts may have more or less relative importance. Biodiesel is not competitive with fossil diesel under current economic conditions, where the positive externalities, such as impacts on the environment, employment, climate changes, and trade balance, are not reflected in the price mechanism [24].

Gossypium arboretum L. is a shrub and commonly known as tree cotton which is native to tropical and subtropical regions of Asia including India, Pakistan and Bangladesh [28].The cottonseed oil contains 70% unsaturated and 26% saturated fatty acids [29] and it has high smoke point (232 °C) similar to other long-chain fatty acids [30]. The high amount of tocopherols in cottonseed oil increases its self-life and contributes to stability [30].

	Table 1	Technical	Properties	of Bio-Diesel
--	---------	-----------	------------	---------------

Common Name	Biodiesel	
Common chemical name	Fatty acid methyl ester	
Chemical formula range	C_{14} - C_{24} methyl ester or $C_{15-25}H_{28-48}O_2$	
Kinematic viscosity range	3.3-5.2	
Density range	860-894(kg/m ³ at 288 K)	
Boiling point range	>475 K	
Flash point range	430-455 K	
Distillation range	470-600 K	
Vapor pressure range	>5 K	
Solubility in water	Insoluble in water	
Physical appearance	Light to dark yellow, clear liquid	
Odor	Light musty/ soapy odor	
Biodegradability	More biodegradable than petroleum diesel	
Reactivity	Stable but avoid strong oxidizing agent	

II. MATERIAL AND METHODS

➤ Materials

To get better yield of bio-diesel fresh cottonseed oil is taken which was obtained from "Kapas Bikash Samiti",Khajura, Banke. Lab grade catalyst (NaOH) which is made by Qualigenes and lab grade Methyl Alcohol made by Qualigenes was taken.

> Methods

• Collection and Purification of Cottonseed Oil

The cottonseeds were collected around Khajura, Banke, 5th state, Nepal. Cotton was dried to obtain the seed, the seed was separated from the cotton and dried, undesired impurities were removed by hand-picking. The seed was prepared for extraction of oil by grinding using a grinding mill. The extracted oil were then filtered by using cotton clothes and collected in air tight container.

A precise quantity of cottonseed oil was taken into the beaker. Taken cottonseed oil was heated up to 50° C. by using water bath with the help of thermometer. Specified amount of catalyst (Sodium Hydroxide) was measured with the help of digital balance and dissolved it with required amount of Methanol [31]. The obtained sodium methoxide then poured in cottonseed oil in a required ratio. The oil was placed in a magnetic stirrer with continuous heating in a constant temperature of 50° C. The reaction was allowed to stand for an hour and then stopped. Due to the black color in nature of cottonseed oil, it is allowed to stand for whole night. After complete separation of glycerol and bio fuel, obtained sample was slowly transferred into the clean and dry separating funnel and allowed some time for settlement. Furthermore, glycerol and bio fuel are separated and separated bio fuel was rinsed with about 50 ml of water for their purification. Later on excess water was removed with the help of separating funnel and obtained bio fuel was heated at 100° C for 30 minutes to evaporate excess water from sample. Furthermore obtained biofuel was placed in the desiccators throughout a time to protect against from

moisture. Obtained sample was taken for the further physico-chemical analysis.

III. EXPERIMENTAL

Most of the testing was done in college laboratory where as some of the experiment was done in other than college laboratory. Preparation of biodiesel was done in the laboratory. Mostly all the experimental method was as such as it stated in the upper topic Material and Method.

> Preparation of Bio- Diesel

400 mL of cottonseed oil was taken into the beaker and placed in the heater having magnetic stirrer. It was heated up to 50° C. and regular checkup of temperature was done by the help of thermometer. Meanwhile 3 g of Sodium Hydroxide was taken in another beaker and it was mixed with 100 mL of Methanol and stirred until it get dissolved. Converted Sodium Methoxide was the slowly poured in the beaker having cottonseed oil. While pouring, cottonseed oil

was continuous in its stirring position. After mixing, mixture of Sodium Methoxide and cottonseed oil was placed in continuous stirring and same amount of heat was applied. After mixing, it was allowed to stand for 1 hour for its complete reaction. Later on it was placed in separating funnel for the separation of prepared bio-diesel with glycerin. It was stand for full day and night for complete separation. Due to the black color in nature of cottonseed oil, it took almost a day for complete separation. After it was stand for full night, lower layer of Glycerin was get separated from separating funnel. Remaining bio-diesel was taken in another separating funnel and 50 mL of water was placed in bio-diesel for the purification of bio-diesel. After placing for some time, water was removed from bio-diesel and clean bio-diesel was placed into the conical flask and placed in the desiccators for the drying process.

In the same manner, number of test was done by changing the ratio and their yield was measures. Later on, prepared bio-diesel was taken for other testing purpose.

S. No.	Cottonseed oil (%)	Methanol (%)	NaOH (%)	% yield
1	60	40	0.5	63.33
2	70	30	0.5	66.66
3	80	20	0.5	75.00
4	90	10	0.5	58.33

Table 2 Table Shows the Yield of Biodiesel in Different Ratio

> Physical Appearance

Freshly grinded cottonseed oil was black in color with some odor where as prepared bio diesel was dark orange in color with slight odor.

Determination of Saponification Value

• Preparation of 0.5 M Ethanolic Potassium Hydroxide: 5.6 gm of potassium hydroxide was taken and dissolved in 200 ml of ethanol [**32**].

• Preparation of 0.5 M Hydrochloric acid:

 $8.5\ mL$ of conc. HCl was taken and dissolved in 200 mL of water.

• *Preparation of Phenolphthalein Indicator:*

1 gm of Phenolphthalein was taken and dissolved in 100 mL of Ethanol.

1 g of each sample (oil and biodiesel) was taken and dissolved in 25 ml of 0.5 M ethanolic potassium hydroxide solution, using 250 ml round bottom flask. The flask was heated in a heating mantle fitted with reflux for 30 min with occasional swirling. The resultant solution was titrated with 0.5 M HCl using phenolphthalein indicator. Meanwhile, blank determination was carried out under similar conditions.

➢ Determination of Iodine Value

• Preparation of Wiji's Solution:

0.8 g of iodine trichloride was taken and dissolved in 20 mL of glacial acetic acid. Meanwhile 0.9 g of iodine was

taken and dissolved separately in 30 mL of dichloromethane. Two of the solution was mixed and diluted up to 100 mL with glacial acetic acid [**33**].

• Preparation of 0.1 M Sodium Thiosulphate:

5 g of sodium thiosulphate was taken and mix with 100 mL of water in 200 mL volumetric flask. 400 mg of sodium carbonate was taken and mixed in same flask. Dissolved all component and dilute up to 200 mL with water.

250 mg of each sample was dissolved in 15 ml of carbon tetrachloride. The solution was mixed with 25 ml Wiji's solution. The flask with the content was stopper and allowed to stand in the dark for thirty minutes at room temperature, to enable oxidation to take place. Then 100 ml of distilled water and 20 ml of 10% potassium iodide solution were added to the content of the flask. The resultant mixture was titrated with 0.1 M Sodium Thiosulphate using 10% starch solution (weight by volume). A blank determination was carried out in the same manner under similar conditions and the reading of both test and blank was noted.

Determination of Acid Value

• Preparation of 0.1 M Potassium Hydroxide:

5.6 g of Potassium Hydroxide was taken and dissolved in 1000 mL of water.

 Preparation of Phenolphthalein Solution: 1% of Phenolphthalein was dissolved in alcohol.

1 g of each sample was dissolved in a 25 mL neutral mixture of solvent (equal volume of diethyl ether and absolute ethanol). The resultant oil solution was titrated with 0.1M Potassium Hydroxide solution after the addition of Phenolphthalein indicator about 3 drops. The titration was continued until the end point was obtained. The reading was recorded after it appears pink color as the end point [**34**].

Determination of Free Fatty Acid

• Preparation of 0.1 M potassium hydroxide:

 $5.6~{\rm g}$ of potassium hydroxide was taken and dissolve in 100 mL of water.

1 g each of oil and biodiesel samples was taken in different conical flask and dissolved in a 25 ml neutral mixture (equal volume of diethyl ether and absolute ethanol). The resultant oil solution was titrated with 0.1 M potassium hydroxide solution with a phenolphthalein indicator added 3 drops. The titration continued until the end point was reached. The end point was recorded as the appearance of a permanent pink color [**35**].

> Determination of Refractive Index

To determine the refractive index, Refractometer of model ZEISS ABBEREF 07 was used. Refractrometer for the determination of refractive index was cleaned with alcohol and clean cotton cloth. After drying the glass slide of refractrometer, a few drops of oil and biodiesel sample were put on the glass slide of the refractometer respectively. Obtained result was observed by eye lens and the centre of the circle was adjusted (taken down) to where it corresponds to the graduated scale pointed to the refractive index. Hence the refractive indexes of the oil and biodiesel sample were noted [**36**].

Determination of Specific Gravity

For the determination of specific gravity, gravity glass was taken and cleaned well and dried well. Firstly, empty gravity glass weight was measured and later on water was poured in gravity glass up to the brim and its weight was noted. Same gravity glass was cleaned and dried and the weight of biodiesel was observed and noted all the weight for the further calculation [**37**].

Determination of Viscosity

For the determination of viscosity, U-shaped viscometer was taken. Firstly it was cleaned and dried. Water was poured in the dried viscometer and its flow of time was measured. In the same manner, oil as well as biodiesel was placed in viscometer and their individual flow of time was measured. All the reading was noted for the further calculation of viscosity.

Determination of Boiling Point

20 ml of biodiesel was poured into a beaker and placed in heating mantle and for the temperature reading, alcohol thermometer was inserted. As the temperature increases, the point at which the sample started boiling was recorded with the help of alcohol thermometer.

> Determination of Cloud Point

For the determination of cloud point, ice bath was prepared with the help of ice and small bucket. Prepared biodiesel was poured in the conical flask and placed in ice bath and alcohol thermometer was place in the conical flask for the reading purpose. After some time a cloudy appearance was observed in biodiesel. During that time, the temperature was noted [**38**].

> Determination of Pour Point

50 mL of sample was taken in conical flask and placed in refrigerator until it get solidify. After it solidify, sample was taken out from the refrigerator and thermometer was placed inside the conical flask. When solid sample just started to melt then the temperature of the sample was noted [**38**].

➢ Moisture Determination

For the determination of moisture of biodiesel, VEEGO automatic Karl Fischer titrator having model number of VEEGO-KF-09/0915 was taken. At first clean and dried beaker was taken and placed in Karl Fischer Titrator for the blank determination. 30 ml of dried methanol was taken and it was neutralized by Karl Fischer reagent. Later on, 1.33 g sample was taken and placed in the neutral methanol beaker for the titration. Titration was carried out by Karl Fischer titrator. After the completion of titration, obtained result was printed with the help of printer.

Determination of Flash Point

For the determination of flash point PMCC Flash Point Tester (GD-261) was used. For flash point determination about 50 mL of biodiesel sample was taken and poured in a sample jar and was placed in a sample compartment. Power of flash point tester was make on and the sample was stirrer by the magnetic stirrer. Thermometer was placed in the thermometer compartment. In some interval of time, spark was given to the sample compartment for the checking purpose. The temperature at which sample get burnt when spark was given was noted [**39**].

Instrumental Characterization (FTIR)

For the characterization of the sample FTIR instrument was taken. The taken instrument was IRTracer- 100, SHIMADZU make. For the characterization, firstly for the base line correction, blank KBR disc was run and its reading was taken. Later on biodiesel sample was placed in the KBr disc pallet without any contamination and scratches. Prepared KBr disc was then placed in the sample compartment with precaution and again it was run for the observation of reading. After biodiesel sample, cottonseed oil in KBr disc was also prepared in the same manner as it was prepared for biodiesel and again its reading was taken. Finally the reading of cottonseed oil and biodiesel was printed for the result purpose.

IV. RESULT AND DISCUSSION

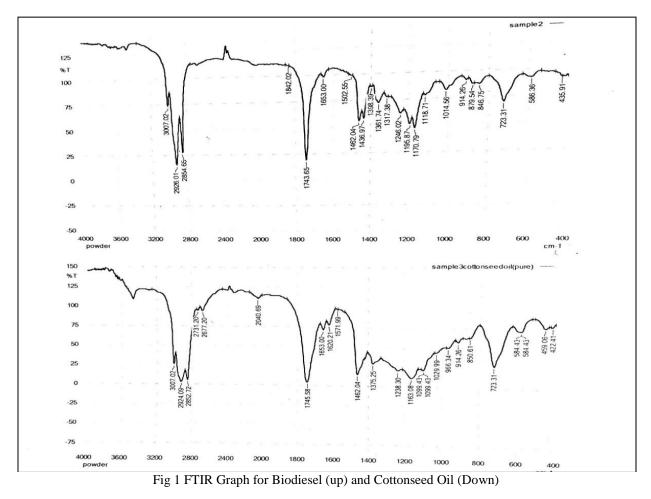
S. No.	Name of Experiment	Biodiesel from (cottonseed oil	Biodiesel from Jatropha (observer value)	Rang(ASTM)
1.	Saponification Value (mg of KOH)	165.52		150-205
2.	Iodine Value	101.68	7.64	100-117
3.	Acid Value	1.50		0.2-1.5
4.	Free Fatty Acid	0.11		< 0.5
5.	Refractive Index	1.45	1.45	1.45-1.46
6.	Specific gravity	0.915	0.87	0.8-0.9
7.	Viscosity	5.20	4.71	1.9-6.0
8.	Boiling Point	210		-
9.	Cloud Point	-5		Location dependent
10.	Pour Point	-10		< 0
11.	Flash Point	130		> 120
12.	Moisture	0.0882		0.05 max

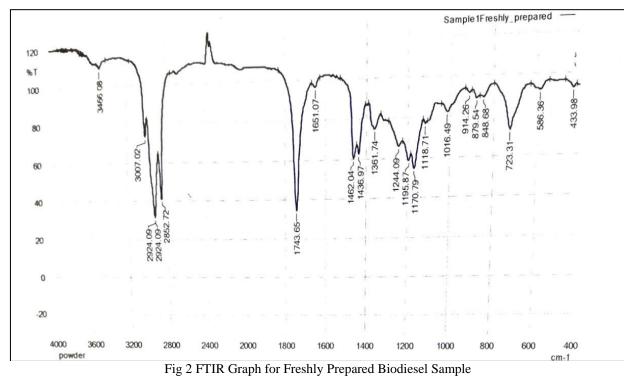
Table 3: Comparison of biodiesel obtained from cottonseed oil and Jatropha [40] with ASTM standard

> Instrumental Characterization

Infrared is a common spectroscopic technique used for quantitative and qualitative analysis. In the biodiesel measurement, the fatty acid methyl ester (FAME) has a characteristic absorption at 1745 cm⁻¹ due to the carbonyl group. ASTM methods specify this wavelength. From a study, there are units that use an integration of the side $(1750 - 1760 \text{ cm}^{-1})$ of the carbonyl peak (~ 1743 cm⁻¹). The peak typical of the methyl ester (OCH₃) at 1436 cm⁻¹ is very narrow and moves along the raw oil peak. The ester carbonyl group stretching vibration at 1740 cm⁻¹ is shown by

strong bands, esteric –COC vibration at 1171 and 1195 cm⁻¹ reveals medium intensity bands, and the presence of the $(CH_2)_n$ group vibration band is seen at 723 cm⁻¹. The absence of broad band at 2500-3300 cm⁻¹ region which confirms the low moisture and free fatty acid content of the sample. Presence of CH bond is confirmed by the sharp starching band at the range of 2800-3000 cm⁻¹ (2926 cm⁻¹). Similarly, IR bands in the region 1370–1400 cm for O-CH₂ groups in glycerol (moiety of triglycerides, diglycerides, and monoglycerides) were present in the IR spectra of crude oils [**41**].





V. CONCLUSION

This study has shown that the production of biodiesel can be possible from cottonseed oil which is not used in vegetable or eating purpose. During the preparation of biodiesel, both the concentration of methanol and catalyst (Sodium Hydroxide) is equally important most of the properties evaluated for the biodiesel conform to the ASTM standard values. It could be concluded from this study that the biodiesel produced from Cottonseed oil is a potential replacement for fossil diesel while the production and effective usage of biodiesel will help to reduce the cost of protecting the atmosphere from the hazards in using fossil diesel and hence will boost the economy of the country.

VI. ACKNOWLEDGEMENT

Authors are pleased to dedicate this work to Asst. Prof. Bodh Babu Bhattarai, T.U., Resham Raj Acharya, technical assistant of Kapas Vikash Samiti, Banke and Santa Prasad Dhungana, Birendra Multiple Campus.

REFERENCES

- Dominic O., Lovet N.E., Callistus N.U., Sandra O.A. Optimization of biodiesel production from refined cotton seed oil and its characterization, 26, 103–110, 2017
- [2]. Martini N., Schell S. Present State of Science and Future Developments, Proceedings of the Symposium, 20, 343–378, 1997.
- [3]. "Monthly US Raw Material Usage for US Biodiesel Production 2007-2009" (PDF). assets.nationalrenderers.org. 2010. Archived (PDF) from the original on November 02, 2023. Retrieved March 23, 2012

- [4]. Costa, Gustavo GL; Cardoso, Kiara C.; Del Bem, Luiz EV; Lima, Aline C.; Cunha, Muciana AS; de Campos-Leite, Luciana; Vicentini, Renato; Papes, Fábio; Moreira, Raquel C.; Yunes, José A.; Campos, Francisco AP (2010-08-06). "Transcriptome analysis of the oil-rich seed of the bioenergy crop Jatropha curcas L". *BMC Genomics.* **11** (1): 462. doi:10.1186/1471-2164-11-462. ISSN 1471-2164. PMC 3091658. PMID 20691070.
- [5]. O'Connell, Deborah (2008). "Biofuels in Australia: Issues and Prospects. A report for the Rural Industries Research and Development Corporation" (PDF). *bioenergy.org.nz.* Archived from the original (PDF) *November* 02, 2023. Retrieved 23 March 2012.
- [6]. Duffy, Patrick (1853). "XXV. On the constitution of stearine". Quarterly Journal of the Chemical Society of London. 5 (4): 303. doi:10.1039/QJ8530500303. Archived from the original on November 02, 2023. Retrieved 2019-07-05.
- [7]. Knothe, G. "Historical Perspectives on Vegetable Oil-Based Diesel Fuels" (PDF). INFORM, Vol. 12(11), p. 1103-1107 (2001). Archived (PDF) from the original on November 02, 2023. Retrieved 2007-07-11.
- [8]. "Biodiesel Basics Biodiesel.org". biodiesel.org. 2012. Archived from the original on November 02, 2023. Retrieved May 5, 2012.
- [9]. Omidvarborna; et al. (December 2014).
 "Characterization of particulate matter emitted from transit buses fueled with B20 in idle modes". *Journal of Environmental Chemical Engineering*. 2 (4): 2335–2342. doi:10.1016/j.jece.2014.09.020.

- [10]. "Biodiesel Handling and Use Guide, Fourth Edition" (PDF). National Renewable Energy Laboratory. Archived from the original (PDF) on November 02, 2023. Retrieved 2011-02-13.
- [11]. "Biodiesel Handling and Use Guide" (PDF). *nrel.gov.* 2009. Archived (PDF) from the original on *November* 02, 2023. Retrieved December 21, 2011.
- [12]. "OEM Statement Summary Chart Archived 2016-04-07 at the Library of Congress Web Archives." Biodiesel.org. National Biodiesel Board, 1 Dec. 2014. Web. November 02, 2023.
- [13]. "National Biodiesel Board, 2007. Chrysler Supports Biodiesel Industry; Encourages Farmers, Refiners, Retailers and Customers to Drive New Diesels Running on Renewable Fuel". Nbb.grassroots.com. 2007-09-24. Archived from the original on *November* 02, 2023. Retrieved 2010-03-15.
- [14]. Virgin launches trials with Britain's first biofuel train *Rail* issue 568 20 June 2007 page 6
- [15]. French McCay, D.; Rowe, J. J.; Whittier, N.; Sankaranarayanan, S.; Schmidt Etkin, D. (2004). "Estimation of potential impacts and natural resource damages of oil". *J. Hazard. Mater.* **107** (1–2): 11– 25. doi:10.1016/j.jhazmat.2003.11.013. PMID 15036 639.
- [16]. Monyem, A.; Van Gerpen, J. (2001). "The effect of biodiesel oxidation on engine performance and emissions". *Biomass Bioenergy*. 20 (4): 317–325. doi:10.1016/s0961-9534(00)00095-7. Archived from the original on *November 02, 2023*. Retrieved 2018-11-22.
- [17]. ASTM Standard D6751-12, 2003, "Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels," ASTM International, West Conshohocken, PA, 2003, doi:10.1520/C0033-03, astm.org.
- [18]. Muralidharan, K. K.; Vasudevan, D. D. (2011). "Performance, emission and combustion characteristics of a variable compression ratio engine using methyl esters of waste cooking oil and diesel blends". *Applied Energy*. 88 (11): 3959– 3968. doi:10.1016/j.apenergy.2011.04.014.
- [19]. Landwehr, K.R.; Hillas, J.; Mead-Hunter, R.; Brooks, P.; King, A.; O'Leary, R.A. (2021). "Fuel feedstock determines biodiesel exhaust toxicity in a human airway epithelial cell exposure model". J. Hazard. Mater. 420: 126637. doi:10.1016/j.jhazmat.2021.126637. PMID 34329109.
- [20]. Landwehr, K.R.; Hillas, J.; Mead-Hunter, R.; King, A.; O'Leary, R.A.; Kicic, A. (2023). "Biodiesel feedstock determines exhaust toxicity in 20% biodiesel: 80% mineral diesel blends". J. Chemosphere. 310: 136873. Bibcode:2023Chmsp.310m6873L. doi:10.10

150875. Bibcode:2023Chmsp.310m6873L. doi:10.10 16/j.chemosphere.2022.136873. PMID 36252896. S2 CID 252938667.

- [21]. Robinson, Jessica (September 28, 2015). "Nation's strictest regulatory board affirms biodiesel as lowest-carbon fuel". National Biodiesel Board. Archived from the original on *November 02, 2023*.
- [22]. Sheehan, John; Dunahay, Terri; Benemann, John; Roessler, Paul (July 1998). "A look back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae" (PDF (3.7 Mb)). Close-out Report. United States Department of Energy. Archived (PDF) from the original on November 02, 2023. Retrieved 2007-01-02.
- [23]. Sorda, G.; Banse, M.; Kemfert, C. (2010). "An Overview of Biofuel Policies Across the World". *Energy Policy*. **38** (11): 6977– 6988. doi:10.1016/j.enpol.2010.06.066.
- [24]. Sensoz S., Angin D., Yorgun S. Influence of particle size on the pyrolysis of rapeseed, 19, 271–279, 2000.
- [25]. Ganesh Lamichhane, Sujan Khadka, Sanjib Adhikari, Niranjan Koirala, Dhruba Prasad Poudyal, Biofuel Production from Waste Cooking Oils and its Physicochemical Properties in Comparison to Petrodiesel Nepal J Biotechnol. (3): 87-94, 2020
- [26]. Sippy K. Chauhan and Anuradha Shukla. Environmental Impacts of Production of Biodiesel and Its Use in Transportation Sector Submitted: November 10th 2010Reviewed: May 9th 2011Published: September 6th 2011 DOI: 10.5772/20923
- [27]. Renewable Fuel Standard: Potential Economic and Environmental Effects of U.S. Biofuel Policy (2011)**Chapter:** 4 The Economics and Economic Effects of Biofuel Production https://www.nap.edu/catalog/13105/renewable-fuelstandard-potential-economic-and-environmentaleffects-of-us
- [28]. Abedin, Sultanul (1979), "Gossypium arboreum", in Ali, S.I.; Qaiser, M. (ed.), Flora of Pakistan, vol. 130, St. Louis: University of Karachi & Missouri Botanical Garden, p. 30
- [29]. "Basic Report: 04502, Oil, cottonseed, salad or cooking". United States Department of Agriculture. Archived from the original on *November 02, 2023*.
- [30]. Jones, Lynn A.; King, C. Clay (1996). "Cottonseed oil". In Y. H. Hui (ed.). Bailey's Industrial Oil and Fat Products, Edible Oil and Fat Products: Oils and Oilseeds. New York: Wiley. ISBN 978-0-471-59426-0.
- [31]. G. Antolin,F.V.Tinaut, Y. Brice~no, V. Castano, C. P_erez, A.I. Ramirez. Optimisation of biodiesel production by sunflower oil transesterification, pp.111–114, 83 (2002)
- [32]. B. H. Knight. Determination of Saponification Value of Natural Waxes. Vol.19, no. 5, pp. 359, 1947. https://pubs.acs.org/doi/pdf/10.1021/ac60005a029
- [33]. W. D. Pocklington, DETERMINATION OF THE IODINE VALUE OF OILS AND FATS Pure 84 Appl. Chem., Vol. 62, No. 12, pp. 2339-2343,1990

- [34]. Atabani A. E., Silitonga A. S., Badruddin I. A., Mahlia T. M. I., Masjuki H. H., & Mekhilef, S. A comprehensive review on biodiesel as an alternative energy resource and its characteristics. Renewable and Sustainable Energy Reviews, 16(4), 2070– 2093,2012, (online), (Url: http://www.biodieselmagazine.com/articles/1917/acomprehensive-analysis-of-biodiesel/)
- [35]. Bouaid A., Vázquez R., Martinez M., & Aracil J. Effect of free fatty acids contents on biodiesel quality. Pilot plant studies. Fuel, 174, 54–62, 2016
- [36]. Benjumea P., Agudelo J. & Agudelo A. Basic properties of palm oil biodiesel-diesel blends. Fuel, 87(10-11), 2069-2075, 2008
- [37]. Bala B.K. Studies on biodiesel from transformation of vegetable oil for diesel engine, 1-43, 2005.
- [38]. Akhil A G, Mohammed P. K. Akhilesh S., Muhammad Ashif C A,Salman Khan,Dr.Rajesh Kanna, Determination of Cloud And Pour Point of Various Petroleum Products., 6, 1-4, 2017
- [39]. Petro Industry News," Modified Continuously Closed Cup Flash Point Standard Accepted as a Safe Alternative Method in Various Fuel Specs", **2014**.
- [40]. Ahana S., Asmita B., Peter K. & Sushil L. A-Case-Study-on-Jatropha-curcas. Powershift Nepal, 2014 (http://tude.org.np/wp-content/uploads/2016/08/pdf (accessed Oct 28, 2018)).
- [41]. Sabrina N. Rabelo, Vany P. Ferraz, Leandro S. Oliveira, and Adriana S. Franca FTIR Analysis for Quantification of Fatty Acid Methyl Esters in Biodiesel Produced by Microwave-Assisted Transesterification International Journal of Environmental Science and Development, Vol. 6, No. 12, 2015