



A Novel Enzymatic Route to Extract Biodiesel from Plant Seeds

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ABSTRACT: Petroleum is a non-renewable energy source that is limited and eventually runs out if it is used continuously. In order to produce bio-diesel, plant seeds are processed and used as a feedstock. This process is made possible by both the storage of resources and the rise in the price of gasoline. In terms of sulphate content, flash point, and biodegradability, biodiesel is superior to petroleum diesel. In this work, an effort has been made to prepare biodiesel manufactured from trans-esterified pongamia pinnata, mahualongifolia, and rice bran. It doesn't include sulphur aromatic compounds. It is biodegradable and harmless. The trans-esterification reaction that produces biodiesel is catalysed by an acid, alkali, or enzymatic catalyst.

Keywords: *Renewable Fuel, Biodiesel, Trans-esterification, enzymatic catalyst, lubricity.*

1. INTRODUCTION

A renewable fuel derived from biomass is biodiesel. Vegetable oils and animal fats are used to manufacture the majority of biodiesels. Diesel fuel derived from petroleum and biodiesel are utilised in the same machinery. The most popular ratios for blending biodiesel with petroleum diesel are 2% (referred to as B2), 5% (referred to as B5), or 20%. (B20). Pure biodiesel can also be produced from biodiesel (B100). Without altering the engines, biodiesel fuel is used in standard diesel engines. Biodiesel blends are sometimes utilised as heating oil. In 2010, global biofuel output increased by 17% from 2009 to reach 105 billion litres (28 billion US gallons), and 2.7% of the world's road fuel came from biofuels which transit, with ethanol and biodiesel playing a significant role. The world's top two producers, the United States and Brazil, together accounted for 90% of the world's 86 billion litres (or 23 billion US gallons) of ethanol fuel produced in 2010. The European Union produced 53% of the world's biodiesel in 2010, making it the top producer in the world.

As of 2011, there were 29 states/provinces and 31 nations with national requirements requiring the mixing of biofuels. The International Energy Agency estimates that by 2050, biofuels might supply more than a quarter of the world's demand for transportation fuels. Simple to use, biodegradable, nontoxic, and practically free of sulphur and aromatics are all attributes of biodiesel. Most diesel engines, especially the more recent ones, use it, and aside from nitrogen oxides, it releases fewer air pollutants and greenhouse gases. It has almost the same energy efficiency as petroleum diesel and is safer to handle. Moreover, it offers advantages for lubricity that fossil fuels do not. It has been discovered that biodiesel blends as low as B2 greatly minimise the quantity of harmful carbon-based pollutants. In comparison to petroleum diesel, biodiesel is becoming an ever more reasonable option as the cost of products derived from oil soars. Using biodiesel lessens reliance on limited fossil fuel supplies. With equipment like the Biofuels, it is relatively simple to process and accessible to all

communities, from metropolitan areas in rich countries to rural areas in developing ones. The surge in U.S. biodiesel consumption since 2001 is attributed to factors including biodiesel's environmental advantages, convenience of use, accessibility to federal and state financial and other incentives, and the federal Renewable Fuels Standard (RFS). The usage of biodiesel is encouraged in several nations. The amount of biodiesel consumed globally in 2001 was almost 0.3 billion gallons. At least 56 nations consumed 9.3 billion gallons of biodiesel in 2016, with five of those countries accounting for 58% of global consumption. The major sources of raw material for making biodiesel in the US and their shares of total biodiesel feedstocks in 2017 are:

- Soybean oil—52%,
- Canola oil—13%
- Corn oil—13%
- Recycled feedstocks, such as used cooking oils and yellow grease—12%
- Animal fats—10%

The main feed-stocks for biodiesel generated in other nations include rapeseed oil, sunflower oil, and palm oil. From roughly 10 million gallons in 2001 to nearly 2 billion gallons in 2017, biodiesel consumption increased. Its characteristics and chemical makeup, as well as its potential as a feedstock for biodiesel, are described. Feasibility of edible Oil vs. non-edible oil vs. waste edible oil as biodiesel feedstock energy are described. Biodiesel: Source, production, composition, properties and its benefits. Transesterification of karanja (*Pongamiapinnata*) oil by solid catalysts is experimented. The usage of methanol and jatropha oil in a compression ignition engine is experimentally compared, and the results are described. Study on Acid-Catalyzed Transesterification of Crude Rice Bran Oil for Biodiesel Production is reviewed. The best way to transesterify *pongamia pinnata* oil using an alkali catalyst to produce biodiesel is explained. Diagnose of Biodiesel production from vegetable oils in India is available. Cultivation of *Jatropha curcas* L. Aromatic – Medicinal plants for Biodiesel is discussed. Biodiesel Production from *Mesua ferrea* L. (Nahar) and *Pongamia glabra* Vent (Koroch) Seed Oil are examined.

In this proposed work the materials and process used to extract a biodiesel is discussed in section 2. and the result is discussed in chapter 3. The proposed work is concluded in section 4.

2. MATERIAL AND METHODOLOGY USED FOR EXTRACTING THE BIODIESEL

In this proposed work, *Mahua Longifolia*, *Pongamia Pinnata* and Rice Bran are used to extracted biodiesel. Initially the properties of those materials are studied and then extraction processes are carried out.

Materials Used For Biodiesel Extraction

Mahualongifolia: is a tropical tree that is primarily found in the forests and plains of central and northern India. Mahuwa, Mahua, Mahwa, Mohulo, Iluppai, or Vippachettu are some of its more prevalent names. It is a fast-growing tree with evergreen or semi-evergreen foliage that can reach a height of around 20 metres and is a member of the Sapotaceae family. Being a significant tree in tropical mixed deciduous forests in India, it can survive in arid settings. It is grown for its oleaginous seeds (one tree can produce between 20 and 200 kg of seeds annually, depending on maturity), flowers, and wood in hot, humid climates. The fat, which is solid at

room temperature, is used as a vegetable butter, to make soap or detergents, and to care for the skin. Moreover, it functions as fuel oil. After oil has been extracted, seed cakes are produced, and they make excellent fertiliser. In India's tropical region, an alcoholic beverage is made from the blooms. This beverage is also known to have an impact on animals. The tree's bark and other parts are used for their therapeutic benefits. Due to its use, it is revered by many indigenous tribes. The aboriginal people that live in the forest and regard the tree as a blessing are determined to preserve it. Unfortunately, because it is not favoured by non-native species, the preservation of this tree has been neglected.

Mahualongifolia taxonomy are listed:

Botanical Name : Madhuca longifolia,

Family : Sapotaceae,

Subfamily : Caesalpinioideae,

Tribes : Caesalpinieae,

Genus : Madhuca,

Species : longifolia and Order : Ericaleae

PongamiaPinnata (L.) Pierre: is a fast-growing leguminous tree that may be grown on poor soil and has the potential to produce a lot of oil seeds. These characteristics boost the plant's appropriateness for the large-scale production of vegetable oil needed by the sustainable biodiesel sector. A thorough understanding of the genetics, physiology, and propagation of this legume is necessary for Pongamiapinnata to succeed in the future as a sustainable source of feedstock for the biofuels sector. Research should focus on increasing plant development in relation to oil biosynthesis in particular. The taxonomy of PongamiaPinnata (L. Pierre) is as follows: Fabaceae family, Fabales order, MillettiaPinnata as the scientific name Status of conservation: Least Concern (Population stable) Life Encyclopedia Millets, a higher classification.

Rice Bran: A plant is rice. Bran, the grain's outer shell, and the oil derived from it are both utilised in medicine. In India in particular, rice bran oil is well-known as "good oil" in Asia and Japan. To cure conditions including diabetes, high blood pressure, high cholesterol, alcoholism, and AIDS; to prevent diseases like stomach and colon cancer; to enhance the immune system; to increase energy and boost athletic performance; to improve liver function; and to act as an antioxidant. High cholesterol can also be treated with rice bran oil. Eczema is an allergic skin rash that some people treat by directly applying rice bran to the affected area (ectopic dermatitis). The process of turning brown rice into white rice produces a byproduct called rice bran, which is full of antioxidants that are good for human health. 12%–13% of the oil and 4.3% of the rice bran fraction are highly unsaponifiable. Various plant seeds biodiesel properties are tabulated in table 1.

Table1. Various plant seeds biodiesel properties

LEAVES	DENSITY(g/cc)	VISCOSITY (cSt)	FLASH POINT(°c)
Pongamia pinnata	924	40.2	225
Jatropha curcas	914	31.2	163
Azadiratchta indica	919	49.7	227
Simarouba glauca	865	4.7	151
Mahua longifolia	960	24.6	232

Oryza sativa	935	28.7	200
Hevea brasiliensis	960	60	250
Simmondsia chinensis	863	35	295
Carthamus tinctorius	914	31.3	260
Camelina sativa	926	40	220
Calophyllum inophyllum	910	38.17	224
Citrullus colocynthis	927	40.2	225
Helianthus tuberosus	916	33.9	274
Moringa oleifera	883	40.17	180
Glycine max	913	32.6	254
Gossypium hirsutum	914	33.5	234
Zea mays	909	34.9	277
Linum usitatissimum	923	27.2	241
Latin crambe	904	53.6	274
Brassica napus	911	37.0	246

Methodology Used To Extract Biodiesel

The process of extraction of biodiesel from the plant seeds is done through the methods like transesterification and post treatment process

Trans-esterification Process

Trans-esterification method is employed in this process. This involves acid trans-esterification and alkali trans-esterification. Methanol or Ethanol or acetone or n-hexane is used as solvent that separates water, oil, etc from the sample.

To Extract the biodiesel 250 grams of mahualongifolia seeds, 250 grams of pongamiapinnata seeds and 250 grams of rice bran are taken. The total yeild of 750 grams of seeds is 250 ml of biodiesel. For transesterification of triglyceride to ester, it take 90-120 minutes to complete the conversion. The longer the reaction time the more the hydrolysis of ester would occur. The longer the storage time of biodiesel, the higher the viscosity. The process steps are explained in the following sessions.

Acid Transesterification: The seeds of Pongamiapinnata, seeds of Mahualongifolia and Rice bran are collected together. The seeds are selected according to their conditions, where the damaged seeds are discarded before seeds in good conditions and then cleaned, deshelled and dried at high temperature at 100-105 ° c for 30 minutes. Seeds where then taken for oil extraction. Mechanical press extraction method-(Single chamber and double chamber oil Expeller) is used for the extraction of all types of oil. This process requires extra time and recovers oil in fewer amounts as compared to other methods. Seeds are grained into paste using matron pestle, by methanol consequently. After graining it into paste, the samples is soaked in methanol or any other solvents. The samples are in solvents to extract crude oil.

Known amount of methanol is added to the sample and is heated in the water bath at 50°C to 60°C to extract oil efficiently. It is allowed to settle and the oil is extracted by using saucillate apparatus. The extracted oil is measured. Oil is taken in a washed air dried beaker and equal amount of methanol is added to it. In another words oil and methanol is taken at 1:1 ratio respectively. 1% of H₂SO₄ is prepared by adding 1 ml of concentrated H₂SO₄ to 99ml of distilled water. 1 ml of 1% H₂SO₄ is added to the mixture. The mixture setup in the laboratory shown in figure.1.



Fig. 1 Acid transesterification process lab setup

The mixture is heated at 50°C to 60°C for 90 minutes. The mixture is cooled and settled and transferred to a separating funnel. A layer of solution containing excess methanol and other impurities formed over the surface of the oil. So the top layer is discarded. The bottom layer is taken as a sample of alkali transesterification.

Alkali Transesterification : The measured output of acid transesterification is taken as input. 1% NaOH is prepared by adding 1 ml of NaOH to 99 ml of distilled water. The solution is added to methanol to produce sodium methoxide and then the mixture is added to the oil and is heated at a temperature of 50°C to 60°C for about 60 minutes. The solution is constantly stirred.

The solvent vapour ascends and pours into the chamber holding the thimble of solid. The condenser makes sure that any solvent vapours cool and fall back down into the chamber where the solid material is located. Warm solvent is gradually poured into the chamber containing the solid substance. In the heated solvent, some of the desirable chemicals dissolve. A syphon side arm automatically empties the Soxhlet chamber when it is almost full, with the liquid then flowing back down to the distillation flask.

Within eight hours following extraction, this cycle is permitted to recur numerous times. The target component is concentrated in the distillation flask after numerous cycles. After extraction, the solvent is eliminated, usually using a rotary evaporator at 40 to 50 degrees Celsius. The extracted solid's insoluble component is separated out and is left in the thimble. Lastly, the solution is poured into the separating funnel and allowed to sit there for roughly 50 minutes without being touched. A two-layer solution is created, with the top layer holding the biodiesel components being kept and the bottom layer being thrown away. The entire process of alkali transesterification is shown in figure2.



Fig.2 Alkali transesterification lab setup

The top layer of oil is collected as the end product of alkali transesterification process. The quantity of oil containing biodiesel contents is measured and it is taken as an input for post treatment process.

2.2 Post Treatment Process

The oil containing biodiesel is washed with distilled water against and again to remove the macro impurities. The oil is heated at 60°C for 60 minutes. The mixture is allowed to settle and is observed. A solution containing excess methanol/ solvent, glycerol and other impurities is drained out.

The water content present in the oil is removed by adding known amount of anhydrous sodium sulphate. The added anhydrous sodium sulphate salt is removed by filtering the oil through the masculine cloth. The oil can also be filtered by using wattmann paper. Impurities present in the oil are also removed during filtration. Filtering process is shown in figure 3.



Fig.3 Filtering process in the lab

The filtered oil is the end product biodiesel which is golden yellow in color. The biodiesel is heated at 110°C for 10 minutes. It is done to remove the moisture content in the biodiesel. Then the golden yellow colored biodiesel is collected and the quantity is measured and finally good quality biodiesel was subjected for chemical analysis.

3. RESULT AND DISCUSSION

Process variable optimization is required for maximal biodiesel synthesis from vegetable oils. Experiments are carried out to investigate the influence of process variable variation on biodiesel yield. In each experiment, one of the response parameters is changed while the others remain constant. The four factors of interest are the amount of methanol, the catalyst, the reaction time, and the reaction temperature. It is founded experimentally that the optimum conditions for effective biodiesel production of Pongamiapinnata oil is 20% w/w methanol, 1% w/w NaOH, 120 min and 60 degree Celsius

Finally the biodiesel is extracted from 750 gram mixture of Pongamiapinnata, Mahualongifolia and Rice bran by transesterification process using methanol in duration of 1 week with the cost of Rs.8000.



Fig.3.1.Biodiesel

3.1 Calculation of Oil Content

The percentage of oil is calculated by using equation 1

$$\% \text{ of oil} = [(w_2 - w_1) \times 100] / w \quad \text{-----} \quad (1)$$

Where, w = weight of sample, w₁ = weight of the beaker with glass, w₂ = weight of the beaker with glass and oil weight is w₂ – w₁.

4. CONCLUSION

The three different types of seeds obtained from indigenous trees such as pongamiapinnata, mahualongifolia and Rice bran are used for the process of extracting biodiesel oil. Oil content of these seeds is also determined to check their suitability for commercial extraction. Information on the physical properties such as weight, length, width, bulk density and moisture content, and mechanical properties like hardness, crushing strength of the seeds are studied and recorded. Physical and mechanical properties of the seeds is also help to modify the existing mechanical oil expeller in order to extract oil. In the current investigation, it has confirmed that the mixture of oil is used as air resource of biodiesel. The experimental result shows that alkaline catalyzed transesterification is a promising area of research for the production of biodiesel in large scale. The viscosity of

Jatropha oil reduces substantially after transesterification is comparable to diesel. Biodiesel characteristics like density, viscosity, flash point, cloud point and pour point are comparable to diesel. The above process of extraction of bio-diesel from the plant seed is economically not beneficial because the cost and time of doing this process is high. Hence our future work is to extract an bio-diesel in an economically beneficial manner and the yield of the biodiesel should be in an increased amount.

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