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1. Introduction

1.1 Background justification for using non edible oil seeds as source of bioenergy

Environmental pollution and diminishing supply of fossil fuels are the key factors leading to search for the alternative sources of energy. Today, 86% of the world energy consumption and almost 100% of the energy needed in the transportation sector is met by fossil fuels (Dorian et al., 2006). Since the world’s accessible oil reservoirs are gradually depleting, it is important to develop suitable long-term strategies based on utilization of renewable fuel that would gradually substitute the declining fossil fuel production. In addition, the production and consumption of fossil fuels have caused the environmental damage by increasing the CO2 concentration in the atmosphere (Westermann et al., 2007).

Currently the most often-used type of biodiesel fuel is vegetable oil fatty acid methyl esters produced by transesterification of high quality vegetable oil by methanol. Biodiesel derived from vegetable oil and animal fats is being used in USA and Europe to reduce air pollution and dependency on fossil fuel. In USA and Europe, their surplus edible oils like soybean oil, sunflower oil and rapeseed oil are being used as feed stock for the production of biodiesel (Ramadhs et al., 2004; Sarin and Sharma, 2007).

Since more than 95% of the biodiesel is synthesized from edible oil, there are many claims that a lot of problems may arise. By converting edible oils into biodiesel, food resources are actually being converted into automotive fuels. It is believed that large-scale production of biodiesel from edible oils may bring global imbalance to the food supply and demand market. Recently, environmentalists have started to debate on the negative impact of biodiesel production from edible oil (Butler, 2006). They claimed that the expansion of oil crop plantations for biodiesel production on a large scale may increase deforestation in countries like Malaysia, Indonesia and Brazil. Furthermore, the line between food and fuel economies is blurred as both of the fields are competing for the same oil resources. In other words, biodiesel is competing limited land availability with food industry for plantation of oil crops. Arable land that would otherwise have been used to grow food would instead be used to grow fuel (Anonymous, 2004). In fact, this trend is already being observed in certain part of this world. There has been significant expansion in the plantation of oil crops for biodiesel in the past few years in order to fulfill the continuous increasing demand of biodiesel. Fig. 1 shows the trend in global vegetable oil ending stocks due to the production of biodiesel in the years 1991–2005 (Anonymous, 2006). Although there is continuous...
increase in the production of vegetable oil; however, the ending stocks of vegetable oils are continuously decreasing due to increasing production of biodiesel. Eventually, with the implementation of biodiesel as a substitute fuel for petroleum-derived diesel oil, this may lead to the depletion of edible-oil supply worldwide.

![Fig. 1. Global vegetable oil ending stock and biodiesel production](image)

1.2 Non edible oil seed crops for biodiesel

In order to overcome this devastating phenomenon, suggestions and research have been made to produce biodiesel by using alternative or greener oil resources like non-edible oils. The non-edible vegetable oils such as *Madhuca indica*, *Jatropha curcas* and *Pongamia pinnata* are found to be suitable for biodiesel production under the experimental conditions (Meher et al, 2006; Sent et al., 2003). Meher et al., (2006) found that the yield of methyl ester from karanja oil under the optimal condition is 97-98%. Oil content in the Castor bean, Hemp and Pongame seed is around 50, 35 and 30-40 % respectively. Neem seed contains 30% oil content. Biodiesel is the pure, or 100%, biodiesel fuel. It is referred to as B100 or “neat” fuel. A biodiesel blend is pure biodiesel blended with petrodiesel. Biodiesel blends are referred as Bxx. The xx indicates the amount of biodiesel blend (i.e., a B80 blend means 80% biodiesel and 20%petrodiesel).

1.3 Objectives

Extensive work has been done on the transesterification of non edible oils; however, no significant work has been done on the optimization, oil characterization and fuel analysis of most of the non edible oil seeds. An optimization study on biodiesel production from castor bean, hemp, neem and pongame was done in detail with one-step alkali transesterification process along with the fuel property analysis of these oils and their blends.

2. Experimental work

The seeds of castor bean, hemp, neem and pongame were used as raw material for biodiesel production. Seeds of these plants were expelled by using electric oil expeller (KEK P0015-10127), Germany. Methanol 99.9% purity, sodium hydroxide (NaOH) and anhydrous sodium sulphate (Na$_2$SO$_4$) were of analytical grade obtained from Merck (Germany).
2.1 Transesterification

The formation of fatty acid methyl esters (FAME) through transesterification of seed oils requires raw oil, 15% of methanol & 5% of sodium hydroxide on mass basis. However, transesterification is an equilibrium reaction in which excess alcohol is required to drive the reaction very close to completion. The vegetable oil was chemically reacted with an alcohol in the presence of a catalyst to produce FAMEs. Glycerol was separated as a by-product of transesterification reaction (Rao et al., 2008). The transesterification process was carried out using two litres round bottom flask equipped with reflux condenser, magnetic stirrer, thermometer and sampling outlet. One liter crude oil firstly filtered and heated up to 120°C to remove the moisture. The transesterification reaction performed at 6:1 molar ratio of methanol/oil, by using 0.34%, 0.67% and 1.35% (w/w) NaOH as catalyst. The temperature and the reaction time was maintained at 60°C and stirred for 2 hr with stirring velocity of 600 rpm. The resultant mixture was cooled to room temperature for the separation of two phases (Plate 1-4). The upper phase contained biodiesel and lower phase contained glycerin (by-product). Crude biodiesel contains the excess methanol, the remaining catalyst together with the soap formed during the reaction and some entrained methyl esters and partial glycerides.

2.2 Purification

After separation of the two layers, the upper layer of biodiesel was purified by distilling the residual methanol at 60°C. The remaining catalyst was removed by successive rinsing with distilled water by adding 1-2 drops of acetic acid to neutralize the catalyst. The residual can be eliminated by treatment with anhydrous sodium sulphate (Na$_2$SO$_4$) followed by filtration. Transparent blackish liquid was obtained as the final product.

2.3 Acid value

The acid value of the reaction mixture in the first stage was determined by the acid base titration technique (ASTM, 2003).

2.4 GC-MS method

The fatty acid methyl esters (FAMEs) contents were determined by gas chromatography, model GC–6890N coupled with mass spectrometer, model MS–5973 MSD (mass selective detector). Separation was performed on a capillary column DB-5MS (30 m ×0.32 mm, 0.25µm of film thickness). The carrier gas was helium with flow rate of 1.5 mL/min. The column temperature was programmed from 120-300 °C at the rate of 10 °C/min. A sample volume of 0.1µL HOB in chloroform was injected using a split mode, with the split ratio of 1:10. The mass spectrometer was set to scan in the range of m/z 50-550 with electron impact (EI) mode of ionization.

2.5 FT–IR method

Biodiesel samples were characterized by FT–IR, using a Bio-Rad Excalibur Model FTS3000MX in the range 4000 - 400 cm$^{-1}$. The resolution was 1cm$^{-1}$ and 15 scans.

2.6 NMR method

NMR analyses were performed at 7.05 T using Avan CE 300 MHz spectrometer equipped with 5mm BBO probes. Deuterated chloroform and tetramethylsilane were used as solvent and internal standard respectively. $^1$H (300 MHz) spectra were recorded with pulse duration
of 30°, a recycle delay of 1.0 s and 8 scans. The $^{13}$C (75 MHz) spectra were recorded with a pulse duration of 30°, a recycle delay of 1.89 s and 160 scans.

2.7 Fuel properties determination
Various fuel properties of COB, HOB, NOB, POB and their blends B100, B50, B20 and B10 were determined and compared with ASTM.

3. Results and discussion

3.1 Determination of Free Fatty Acid (FFA) content
The FFA has significant effect on the transesterification of glycerides with alcohol using catalyst (Goodrum, 2002). The high FFA content (>1%w/w) will cause soap formation and the separation of products will be exceedingly difficult, and as a result, low yield of biodiesel product would be obtained. It is important to first determine the FFA content of oil. The free fatty acid (FFAs) number of hemp crude oil was 1.76%, neem 2.5% and pongame 2%. According to Anggraini et al., (1999) if the free fatty acid content is more than 3% then the conversion efficiency decreases gradually.

3.2 Biodiesel yield
To achieve optimum yield of biodiesel from non edible oil seeds, alkali based transesterification was carried out. Alkali-catalyzed transesterification is much faster than acid-catalyzed and is used in commercial production of biodiesel. Even at ambient temperature, the alkali-catalyzed reaction proceeds rapidly usually reaching 95% conversion in 1-2 h (Rachmaniah et al., 2006). As a catalyst in the process of alkaline methanolysis, mostly sodium hydroxide or potassium hydroxide have been used, in concentration from 0.4 to 2% w/w of oil (Meher et al., 2006). Yield of methyl esters of HOB, NOB, POB and COB were investigated by changing catalyst concentrations while molar ratio (6:1) methanol / oil and temperature (60°C) was kept constant. The highest conversion rate was obtained with the catalyst concentration of 0.7 g, under these conditions the biodiesel yield was 74.36%, 70%, 77.16% and 70% for HOB, NOB, POB and COB respectively. When the catalyst concentration was doubled to 1.6 g, it was observed that the ester formation decreased with the increase in sodium hydroxide concentration and soap formation was increased. This is because the higher amount of catalyst may cause soap formation (Attanatho et al., 2004). Soap formation reduces catalyst efficiency, causes an increase in viscosity, leads to gel formation and makes the separation of glycerol difficult (Guo and Leung, 2003). The Conversion efficiency decreased when the catalyst concentration was reduced to half (0.4 g), as low concentration of catalyst suppress biodiesel.

3.3 Fuel properties
The experimental investigation was carried out for different fuel properties and the performance was evaluated according to ASTM D-445, D-1298, D-93, D-1298, D-1500, D-4294 and compared with diesel in Table 2.

3.3.1 Viscosity
High viscosity is the major problem preventing the use of vegetable oils and animal fats directly in diesel engines as it affects the flow of fuel and spray characteristics (Hossain and
Table 1. Biodiesel yields of HOB, NOB, POB and COB by using NaOH as catalyst

Davies, 2010). High Speed Diesel (HSD) has viscosity of 1.3-4.1 @40 °C whereas the viscosities of COB and POB are 5.67 and 5.5 respectively which is slightly higher than the viscosity of HSD. While the viscosities of HOB and NOB are 3.83 and 4.81 which were closer to HSD. It shows that viscosities of these non-edible oil seeds biodiesel were comparable to HSD.

3.3.2 Density

The density of COB, HOB, POB and NOB at 15 °C was found to be 0.88 Kg/L, 0.81 Kg/L and 0.87 Kg/L which are closer to the density of diesel (0.83) 0.86 kg/lit can be used as an alternative fuel.

Table 2. Comparative analysis for fuel properties of NOB, COB, POB and HOB with HSD

3.3.3 Flash point

Flash point is the temperature that indicates the overall flammability hazards in the presence of air; higher flash points make for safe handling and storage of biodiesel (Hossain
and Davis 2010). The Flash Points of biodiesel of NOB, COB and POB were 124°C, 120 °C, 96 °C and 90°C which are higher than that of HSD (60-80 °C) (Fig. 2). For non-edible based seeds oils flash point are higher than fossil diesel (Anonymous, 2007; Pramanik, 2003; Ziejewski et al, 1992).

3.3.4 Sulphur contents
The most valuable result is the reduction and absence of percentage of total sulphur contents in NOB, COB, HOB and POB that will result in reduction of Sox in exhaust gases which is one of the reason of acid rain. Sulfur content of petrodiesel is 20–50 times higher than biodiesels (Shay, 1993).

3.3.5 Cloud point and pour point
Cloud point is the temperature at which a cloud of wax crystals first appear in the oil when it is cooled. The pour point is the lowest temperature at which the oil sample can still be moved. These properties are related to the use of biodiesel in colder region (Arjun et al., 2008). The cloud points of NOB, COB, POB and HOB were 12, -6, 6, -25 °C and the pour point are 9,-9,3 and 6 °C respectively. Pour point and cloud point of all oils were almost within the specified range. Lee et al (1995) argued that the cloud points were affected by the presence of monoglycerides while the pour points were not affected.

3.4 Biodiesel blends
Blending oils with diesel fuel was found to be a method to reduce chocking and extend engine life. Zhang and Gerpen (2006) investigated the use of blends of methyl esters of soybean oil and diesel in a turbo-charged, four cylinder, direct injection diesel engine modified with bowl in piston and medium swirl type. They found that the blends gave a shorter ignition delay and similar combustion characteristics as diesel (Orchidea et al., 2007). Table 3 presents data pertinent to the mixture of petroleum diesel and biodiesel in the following fashion:

- Petroleum diesel (90%)- biodiesel (10%) : B10
- Petroleum diesel (80%)- biodiesel (20%) : B20
- Petroleum diesel (50%)- biodiesel (50%) : B50

The fuel properties of biodiesel blends NOB, COB, POB and HOB were compared with HSD. It was found that viscosity was slightly higher as the proportion of biodiesel in the mixtures increased. However, this event does not affect the atomization characteristics. Viscosity of B20 is very close to the viscosity of diesel. So that the biodiesel of B10, and B20 blends can be used without any heating arrangement or engine modification.

The density of different blends of methyl esters were increased with increase in blend percentage. The blends of B10, and B20 of NOB and HOB were closer to the density of diesel. The flash points of different blends of methyl esters are increased with increase in methyl ester percentage. It is also observed that the flash points of pure biodiesel were in comparison with HSD. Thus, it can be used as a fuel without any fire accidents.

3.5 Biodiesel analysis
The chemical composition and characterization of COB, HOB, NOB & POB based on GC-MS, NMR & FTIR analysis were shown in Fig 3-6.
### Table 3. Fuel properties of Biodiesel Blends and HSD

| Fuel Properties | PLANTS | B 100 % | B 50 % | B 20 % | B 10% | HSD |
|-----------------|--------|---------|--------|--------|-------|-----|
| Kinematic viscosity @ 40°C cSt | NOB | 4.81 | 3.80 | 3.30 | 3.10 | 1.3-4.1 |
| | COB | 5.67 | 4.43 | 4.48 | 3.52 | 1.3-4.1 |
| | POB | 5.532 | 6.23 | 4.1849 | 3.7959 | 1.3-4.1 |
| | HOB | 3.83 | 6.331 | 5.113 | 4.223 | 1.3-4.1 |
| Density @15°C Kg/L | NOB | 0.8785 | 0.8578 | 0.8476 | 0.8450 | 0.8295 |
| | COB | 0.8873 | 0.88722 | 0.88720 | 0.88111 | 0.8343 |
| | POB | 4.086 | 0.92 | 0.5072 | 0.1639 | 0.8295 |
| | HOB | 0.8195 | 0.8145 | 0.8224 | 0.8116 | 0.8343 |
| Flash Point (°C) | NOB | 124 | 72 | 64 | 65 | 60-80 |
| | COB | 96 | 86 | 83 | 77 | 60-80 |
| | POB | 90 | 83 | 72 | 70 | 60-80 |
| | HOB | 120 | 108 | 91 | 75 | 60-80 |

### 3.5.1 Gas Chromatography and Mass Spectrometry (GC-MS)

The composition of FA were analyzed by gas chromatography after converted into their corresponding methyl esters (FAME) (Knothe, 2001). The use of mass spectrometer would eliminate any ambiguities about the nature of eluting materials since mass spectra unique to individual compounds would be obtained. Fatty acids (FA) components of biodiesel produced from COB, NOB, POB and HOB oil obtained by GC is presented in Table 4.

| FA | Carbon number | COB | NOB | POB | HOB |
|----|---------------|-----|-----|-----|-----|
| Myristic Acid | C14:0 | 0.7 | - | - | - |
| Palmitic Acid | C16:0 | 0.9 | 14.9 | 10.6 | 15 |
| Stearic Acid | C18:0 | 2.8 | 14.4 | 6.8 | 65 |
| Oleic acid | C18:1 | 90.2 | 61.9 | 49.4 | 15 |
| Linoleic Acid | C18:2 | 4.4 | 7.5 | 19.0 | 5 |
| Linolenic Acid | C18:3 | 0.2 | - | - | - |
| Arachidic Acid | C20:0 | - | 1.3 | 4.1 | - |
| Eicosenic acid | C20:1 | - | - | 2.4 | - |
| Docosanoic acid | C22:0 | - | - | 5.3 | - |
| Tetracosanoic acid | C24:0 | - | - | 2.4 | - |

Table 4. Fatty acid content of the methyl esters from non edible oil seeds

Oleic acid was the most common FA found in Castor oil, Pongame oil and Neem oil. COB was found to have 90.2 % oleic acid (18:1), while NOB have 61.9 % oleic acid (18:1). Myristic acid was present only in COB, Eicosenic acid, Docosanoic acid and Tetracosanoic acid were absent in all oils except for pongame oil. HOB was found to contain 65% stearic acid (18:0), 15% oleic acid (18:1), 5% linoleic acid (18:2),15% palmitic acid (16:0), Stearic and Palmitic acids are the major saturated FA found in HOB. It contains approximately 20% unsaturated fatty acids. COB was found to contain 90.2 % oleic acid (18:1),4.4% linoleic acid (18:2), 0.9%
palmitic acid (16:0) and 2.8% stearic acid (18:0). Oleic acid is the major unsaturated fatty acid found in COB. It contains approximately 94% unsaturated fatty acids. POB was found to contain 49.4% oleic acid (18:1), 19.0% linoleic acid (18:2), 10.6% palmitic acid (16:0) and 6.8% stearic acid (18:0). It contains approximately 70% unsaturated fatty acids. NOB was found to contain 61.9% oleic acid (18:1), 7.5% linoleic acid (18:2), 14.9% palmitic acid (16:0) and 14.4% stearic acid (18:0). Oleic acid is the major unsaturated fatty acids found in NOB. It contains approximately 69% unsaturated fatty acids. The amount and type of fatty acids in the biodiesel determines the viscosity, one of the most important characteristics of biodiesel. Due to the presence of high amount of long chain fatty acids, POB may have a slightly higher viscosity compared to HOB, COB and NOB. The FAMEs of these species also meet the specification of 90/95% boiling point limit of 360°C specified in ASTM-D6751 and in other biodiesel standards. Generally, the FAMEs, which are mainly comprised of carbon chain lengths from 16 to 18, have boiling points in the range of 330–357°C; thus the specification value of 360°C is easily achieved. Besides, the concentration of linolenic acid and acid containing four double bonds in FAMEs should not exceed the limit of 12% and 1%, respectively (Pasto et al., 1992). FAMEs of all species are within the specified limit, so they are suitable for the production of biodiesel.

3.5.2 FT-IR analysis for fatty acid methyl esters
The FT-IR spectra in the mid-infrared region have been used to identify functional groups and the bands corresponding to various stretching and bending vibrations in the samples of oil and biodiesel. The position of carbonyl group in FT-IR is sensitive to substituent effects and to the structure of the molecule (Safar et al., 1994). The methoxy ester carbonyl group in HOB, NOB, COB and POB was appeared at 1743 cm\(^{-1}\), 1741 cm\(^{-1}\), 1742 cm\(^{-1}\) and 1743 cm\(^{-1}\) respectively. The band appeared at 3465 cm\(^{-1}\) showed the overtone of ester functional group (Gelbard et al., 1995). The C-O stretching vibration in HOB and COB showed two asymmetric coupled vibrations at 1119 cm\(^{-1}\) and 1171 cm\(^{-1}\) due to C-C(=O)-O and 1017 cm\(^{-1}\) due to O-C-C (Table 5).

| Sample type | Catalyst | C=O (cm\(^{-1}\)) Ester Carbonyl | C-O(cm\(^{-1}\)) |
|-------------|----------|----------------------------------|-----------------|
| NOB         | NaOH     | 1741.1                           | 1014.2          |
| COB         | NaOH     | 1742                             | 1017            |
| POB         | NaOH     | 1740                             | 1014.4          |
| HOB         | NaOH     | 1743                             | 1017            |

Table 5. Chemical Composition of non edible seed biodiesel based on FT-IR analysis

3.5.3 \(^1\)H NMR
Biodiesel of HOB, COB, POB and NOB were characterized by \(^1\)HNMR spectroscopy as summarized in Table 6. Gelbard et al., (1995) reported that the spectroscopic determination of yield of transesterification reaction utilizing HNMR depicting its progressing spectrum (Gerlbard et al., 1995). The characteristic peak of methoxy protons was observed as a singlet at 3.669 ppm, 3.64 ppm, 3.5 ppm and 3.67 ppm for HOB, POB, COB and NOB and a triplet of \(\alpha\)-CH\(_2\) protons at 2.31 ppm, 2.8 ppm, 2.24 ppm and 2.28 ppm for HOB, POB, COB and NOB. These two peaks are the distinct peaks for the confirmation of methyl esters present in biodiesel.
Table 6. Chemical Composition of non edible oil seed biodiesel based on $^1$HNMR analysis

| Sample type | CH$_3$-Methoxy proton (ppm) | $\alpha$ CH$_2$ Proton (ppm) | CH=CH Unsaturation (ppm) |
|-------------|-----------------------------|-----------------------------|--------------------------|
| NOB         | 3.64                        | 2.28                        | 5.32                     |
| COB         | 3.5                         | 2.247                       | 5.247-5.487              |
| POB         | 3.674                       | 2.816                       | 1.263-1.310              |
| HOB         | 3.65                        | 2.31                        | 5.31-5.39                |

3.5.4 $^{13}$C NMR

The spectrum of $^{13}$C NMR of the HOB, POB, COB and NOB were shown in (Table 7) which shows the characteristic peaks of ester carbonyl (−COO−) and C−O at 174.2 and 51.4 ppm for HOB, 173.28 ppm and 51.45 for POB, 174.19 and 51.32 for COB and 174.23 and 51.37 for NOB respectively.

| Sample type | -COO- (ppm) | C-O (ppm) |
|-------------|-------------|-----------|
| NOB         | 174.23      | 51.37     |
| COB         | 174.19      | 51.32     |
| POB         | 173.28      | 51.45     |
| HOB         | 174.21      | 51.35     |

Table 7. Chemical Composition of Biodiesel in $^{13}$CNMR

Fig. 2. Flash Point of Biodiesel blends
Fig. 5. COB chemical profile: A- FT-IR spectrum, B- 1H NMR spectrum, C- Gas chromatogram & D- 13C NMR spectrum
Fig. 6. HOB chemical profile: A- FT-IR spectrum, B- 1H NMR spectrum, C- Gas chromatogram & D- 13C NMR spectrum
Fig. 7. NOB chemical profile: A- FT-IR spectrum, B- 1H NMR spectrum, C- Gas chromatogram & D- 13C NMR spectrum
Fig. 8. POB chemical profile: A- FT-IR spectrum, B- 1H NMR spectrum, C- Gas chromatogram & D- 13C NMR spectrum
Plate 1. Extraction of oil by electric oil expeller

Plate 2. Filtration of crude oil
Plate 3. Mixing of sodium methoxide catalyst

Plate 4. Separation of glycerin as by product
4. Conclusion

In this study an optimized protocol for biodiesel production from non edible seeds of castor bean (*Ricinus communis* L.), hemp (*Cannabis sativa* L.), neem (*Azadirachta indica* A. Juss.) and pongame (*Pongamia pinnata* (L.) Pierre.) converted into fatty acid methyl esters (FAME) through base catalyzed transesterification using an optimum ratio of 1:6 (Oil : Methanol) at 60 ºC. The fuel properties of biodiesel blends i.e. B100, B50, B20, B10 were compared with ASTM standards. Biodiesel from these sources was analyzed for qualitative and quantitative characterization by using H1NMR, C13NMR, GC-MS and FT-IR techniques. Based on qualitative and quantitative analysis of Biodiesel and their byproducts, it is concluded that the bioenergy from these species can be feasible, cost effective, environment friendly, if mass plantation of such resources may initiated in suitable places at global perspective.

5. List of abbreviations

| Abbreviation | Description |
|--------------|-------------|
| ASTM         | American Society for Testing and Materials |
| B100         | 100 % biodiesel |
| B20          | 20 % biodiesel + 80 % high speed diesel |
| B10          | 10 % biodiesel + 90 % high speed diesel |
| B5           | 5 % biodiesel + 95 % high speed diesel |
| B50          | 50 % biodiesel + 50 % high speed diesel |
| C13NMR       | Carbon - Nuclear Magnetic Resonance |
| COB          | Castor Bean Oil Biodiesel |
| FA           | Fatty Acid |
| FAME         | Fatty Acid Methyl Esters |
| FFA          | Free Fatty Acid |
| FT-IR        | Fourier Transfer – Infra Red |
| GC-MS        | Gas Chromatography – Mass Spectrometer |
| H1NMR        | Hydrogen – Nuclear Magnetic Resonance |
| HOB          | Hemp Oil Biodiesel |
| HSD          | High Speed Diesel |
| MSD          | Mass Selective Detector |
| NOB          | Neem Oil Biodiesel |
| POB          | Pongame Oil Biodiesel |

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