Production of Biodiesel from *Thespesia populnea* seed oil through rapid *in situ* transesterification - an optimization study and assay of fuel properties

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Abstract

Biodiesel production was carried out from *Thespesia populnea* seed oil through rapid *in situ* transesterification. Influence of reaction parameters such as catalyst type and concentration, methanol to biomass ratio, co-solvent volume, temperature and agitation speed on conversion of oil into methyl esters was investigated. The effect of different co-solvents on conversion was evaluated. Optimum methyl ester conversion of 97.80% was achieved at 1.5wt% of KOH catalyst, 5.5:1 (v/w) methanol to biomass ratio, 25vol% tetrahydrofuran-co-solvent, 60°C and 500 rpm within 120min of reaction time. Fuel properties of produced methyl esters were well fitted within the limits of ASTMD 6751 standards. Considering the properties of produced biodiesel, *Thespesia populnea* seed derived biodiesel can be used as potential alternate to fossil diesel fuel.

1. Introduction

Viscosity and high molecular weight are the main barricades that cause incomplete combustion, poor atomization and carbon deposition in the engine when vegetable oil is directly used in the diesel engine. Such impediments are transcended by processing the oils through dilution with diesel, pyrolysis, emulsification, catalytic cracking and transesterification [1]. Chemical conversion (transesterification) methods have been proved efficient and economically viable techniques to produce high quality products despite being very slow [2]. Most widely used and efficient chemical method to reduce the viscosity of oil is transesterification, where triglycerides of fats or oils are converted into esters of alcohols and glycerol. The transesterified form of vegetable oils with alcohol is called as Biodiesel. Most of the world’s biodiesel is produced (United States, South East Asia and Europe) from edible oils, which is economically untenable and exceeds the overall production cost of fossil fuel. At this context, only non-edible, economically viable feed stocks can serve as sustainable resources for biodiesel production [3]. Production of sustainable biodiesel relies on feedstock selectivity and cost effective production processes.
Conventional method of biodiesel production involves two major steps, extraction of oil from biomass (feedstock) followed by transesterification. Hexane is being widely used as extraction solvent. Approximately 3,800L of hexane (for 3,000 tons of seed) is introduced into the atmosphere per day through the hexane extraction in the process of oil recovery. Hexane has been classified under hazardous air pollutants, besides reloading of evaporated solvent add cost to extraction step. Hence, the elimination of hexane usage by alternate methods found advantageous. In addition, extracted oil can be usable only after proper purification and refining, which is a complex process includes, dehydration, degumming, dewaxing, deacidification, dephosphorization [4]. Thus, the reduction in biodiesel production cost is possible by simplifying oil extraction and transesterification processes. On considering it, In Situ transesterification (IST) has gained researchers’ attention. In situ method involves the direct conversion of biomass into biodiesel through the elimination of an expensive lipid extraction process. It is widely acknowledged that IST is the most efficient and economical method of converting triglycerides into biodiesel than conventional transesterification method [5]. *Thespesia populnea*, generally called as Milo (Indian tulip tree), is one of the most widespread, drought resistant species of Pacific Islands. In India, it is found in tropical regions and coastal forests and grown as garden plant [6].

Through meticulous literature survey, it was found that there was no data previously reported on IST process and optimization of non-edible *T. populnea* seed oil (TPO)/*T. populnea* seed hitherto. The main aim of this study was intended to produce biodiesel from enormously available native *T. populnea* seeds by alkali catalyzed IST process and to determine the effect of various reaction parameters such as catalyst type and concentration, methanol to biomass ratio, co-solvent volume, temperature and agitation speed on conversion of TPO into *T. populnea* methyl esters (TPME), which paves the way for economical production of biodiesel from *T. populnea*. The fuel properties of produced TPME (biodiesel) were evaluated with ASTMD6751 and EN14214biodiesel standards.

2. Experimental details

2.1 Materials:

Solvents methanol (99.5%), hexane (99%), tetrahydrofuran (THF) (99.90%), toluene (99.5%), benzene (99%), ethyl acetate (EA) (98%), chloroform (99.7%), KOH and NaOH were purchased from Merck Ltd., Mumbai, India. All the reagents were used of analytical grade. *T. populnea* fruits were collected from the vicinity of Anna University, Chennai. Seeds were separated out and dried in hot air oven at 80°C and the decrease in moisture content was monitored at regular intervals of time until a constant weight of the seeds was measured. The seeds were ground, sieved through 120mesh to ensure the homogeneous particle size and stored in an air tight container until use.

2.2 Selection of co-solvent:

The oil content of seeds was determined by Soxhlet extraction with n-hexane. 100g of the seed biomass was extracted with 250ml of n-Hexane using Soxhlet apparatus for 3h at 65°C. 20.70% (w/w) of oil has been extracted and the extraction rate was considered as 100%. Five organic solvents chloroform, toluene, benzene, THF and EA were chosen to evaluate the co-solvent for IST. Solvent extraction was carried out for 100g of biomass with each solvent of 250ml at their respective boiling temperatures for 3h. Extraction rate of each solvent was calculated by using the formula [7],

\[
\text{Extraction rate} = \left( \frac{\text{Weight of oil extracted with test solvent in 3h (g)}}{\text{Weight of oil extracted with n-hexane (g)}} \right) \times 100(\%) \quad (1)
\]
2.3 Conduct and optimization of IST:

IST was conducted for the whole seed according to the procedure as follows. Schott bottles of 250ml capacity each were added with prefixed quantity of alkaline methanol (NaOH or KOH dissolved in methanol) and measured quantity of THF. A quantity of 25g of seed biomass was added per bottle, when the reaction mixture attains the set temperature. The entire process was carried out in an incubator shaker with adjustable agitation speed, temperature and time. When the reaction was complete, the flasks were taken out, kept undisturbed to settle down seed particles as well as to cool down the reaction mixture at room temperature. The contents of the flasks were vacuum filtered to separate seed biomass with liquid portion. Methanol and co-solvent (THF) were distilled off at 65°C for reuse. The resultant liquid portion was transferred into separating funnel for gravity separation of methyl ester and glycerol phases. Fatty acid composition of ester phase was analyzed by Gas Chromatography (GC).

Experimental trials for optimization of IST process were conducted over various parameters to evaluate their influence on methyl esters production from *T. populnea*. Parameters investigated includes, catalyst type and concentration (wt%, on biomass weight basis), methanol to biomass ratio (v/w), volume of co-solvent (vol%, on volume of methanol basis), reaction temperature, agitation speed and reaction time was fixed to 120min. Alkaline catalysts KOH and NaOH were employed and the concentration of efficient catalyst (KOH) was varied between 0.5-2.5wt%. Methanol: biomass ratio (v/w) was changed over 4.0:1-6.0:1 and co-solvent volume was changed between 0-30%. Reaction temperature was varied from 30°C-70°C and agitation speed was employed over 400-600 rpm. After the reaction, mass of each crude methyl esters sample was recorded and conversion of *T. populnea* oil into methyl esters (%) was determined through GC analysis. All the experiments were done in triplicates.

2.4 Analytical methods:

After each reaction, the purity of each crude methyl ester sample was analyzed by GC (GC-2010, Shimadzu), equipped with a flame ionization detector (FID) and a capillary column of 30m length, 0.25mm diameter and 0.25μm film thickness. Initial temperature of the oven was maintained at 140°C for 5min, raised to 280°C at the rate of 10°C/min. Detector temperature was maintained at 300°C. Helium was used as a carrier gas. The injection split ratio was 10:1 with flow rate of 1.2mL/min. Samples for analysis were prepared in n-heptane solvent. 1µl of the sample solution was injected into GC per each time. Methyl heptadecanoate was used as an internal standard. The purity of methyl esters was determined based on the peak area of methyl ester over reference by using the following equation [8],

\[
Purity(\%) = \left( \frac{\text{area of FAME}}{\text{area of reference}} \times \frac{\text{weight of reference}}{\text{weight of crude biodiesel}} \right) \times 100
\]

Purity refers to the conversion of TPO into its methyl esters or formation of TPME. The fatty acid composition of TPME was analyzed by GC. TPME produced in the due course of optimization was characterized through proton NMR (Nuclear Magnetic Resonance) spectroscopy (Bruker 500 MHz, USA).

After IST process, the residual seed meal was re-extracted with n-hexane by Soxhlet extraction method to obtain oil fraction remainsin the meal. The difference in total oil to the fractional oil gives the amount of oil extracted in IST or the amount of oil dissolved in methanol.

2.5 Fuel properties analysis of TPME:
Methyl esters of *T. populnea* subjected to some important fuel properties by following ASTM and EN standard methods. Density was measured by digital density meter (Anton paar, DMA 35) according to ASTM D5002. Kinematic viscosity by calibrated viscometer (ASTM D445), acid value by titration method (ASTM D664), copper strip corrosion (ASTM D130), cloud point (ASTM D2500), pour point (ASTM D97), cold filter plugging point (ASTM D6371), cetane number (ASTM D613), flash point by closed cup testing method (ASTM D93) and oxidation stability by Rancimat method (EN 14112).

3. Results and Discussion

3.1 Influence of catalyst type and concentration on TPME formation:
Alkaline catalysts are very efficient in converting oil into biodiesel compared to acid catalysts, on the other hand, the presence of even trace amounts of acid catalyst causes engine corrosion [9]. In the present study, two alkaline catalysts KOH and NaOH have been tested for their efficiency towards IST of *T. populnea*. Figure 1, shows the effect of alkaline catalyst type on conversion of TPO into TPME at pre-fixed conditions of 2wt% of catalysts, 6.0:1 (v/w) methanol to biomass ratio, 30 vol% of co-solvent, 60°C of reaction temperature and agitation speed of 600rpm. Methyl ester formation was monitored for 120min. From the figure, it is apparent that KOH has shown better conversion than NaOH. KOH was capable of converting 85.93% TPO into TPME whereas it was only 80.13% with NaOH. Even though NaOH has shown initially higher conversion rate (KOH-51.03% and NaOH-53.90% at 15min), on time prolongation the yield was declined. It was noticed that, during the downstream processing, both ester phase and glycerol phases separated easily with KOH catalyst. This may be due to the soft nature of potassium soaps formed than sodium salts. This observation complies with that of Leung and Guo [10]. Due to higher conversion ability at given conditions, KOH was selected as an efficient catalyst for IST of TPO.

![Figure 1. Effect of alkaline catalyst type on the conversion of TPO into TPME.](image)

Figure 2, represents the influence of catalyst concentration on conversion of TPO into TPME with time. Conversion has shown linear relationship with catalyst concentration up to 1.5wt%. Equilibrium conversion of 88.90% was achieved at 120min with 1.5wt% catalyst concentration and the reaction was almost complete even at 90min. Conversion of TPO into TPME was drastically reduced from 88.90% to 73.70% with increase in catalyst concentration from 1.5wt% to 2.5wt%. At higher catalyst concentrations,
formation of large amounts of soaps was noticed. This could be due to that addition of excess amount of alkaline catalyst causes participation of more triglycerides in saponification reaction. Hence the reduction in conversion was observed beyond 1.5wt% catalyst concentrations. Similar findings were reported by Leung and Guo [10] in producing biodiesel from Canola oil. It was reported that biodiesel yield was declined from 85wt% to 65wt% with increase in NaOH catalyst concentration from 1wt% to 1.6wt%.

Figure 2. Influence of catalyst concentration (KOH) on conversion of TPO into TPME with time

3.2 Influence of methanol to biomass ratio on conversion of TPO into TPME:
Figure 3 presents the influence of methanol to biomass ratio on conversion of TPO into TPME. Methanol to biomass ratio was varied from 4:1 to 6:1(v/w). Significant hike in conversion was observed with the increase in the methanol to biomass ratio from 4:1 to 5.5:1(v/w) under the conditions of 1.5wt% KOH catalyst, 30vol% of co-solvent, 60°C of reaction temperature, agitation speed of 600rpm at 120min. However, excessive addition of methanol (>5.5:1) led to reduction in TPME formation. This could be due to that, higher concentrations of methanol accelerate the dissolution of produced FAME into the glycerol phase, which would intricate the FAME recovery in downstream process and leads to the loss of FAME with glycerol phase [11].

Figure 3. Influence of methanol to biomass ratio on conversion of TPO into TPME
3.3 Influence of co-solvent volume on TPME formation:
Though methanol can act as extraction solvent, its polarity is a crucial barrier for the extraction of total lipids from the biomass. However, the addition of co-solvent to the reaction could hasten up the reaction rate by accelerating the mass transfer of lipids into methanol [7]. Among five organic solvents tested for their extraction rate (%), THF has shown better results with 83.57% extraction rate and hence it has been taken as an efficient co-solvent for this study. Table 1 shows the extraction rate of the solvents employed.

Table 1. Extraction rate (%) of selected organic solvents at their respective boiling points.

| Solvent            | Boiling temperature(°C) | Amount of oil extracted (g) | Extraction Rate (%) |
|--------------------|-------------------------|-----------------------------|---------------------|
| n-hexane (Soxhlet Extraction) | 68°C                    | 20.70±0.1                   | 100                 |
| Toluene            | 110.6°C                 | 13.8±0.2                    | 66.67±0.45          |
| Chloroform         | 61.2°C                  | 14.2±0.3                    | 68.60±0.67          |
| THF                | 67°C                    | 17.3±0.1                    | 83.57±0.33          |
| Benzene            | 80.1°C                  | 11.0±0.2                    | 53.14±0.83          |

It is widely acknowledged that due to its moderate polarity, THF could extract both polar and neutral lipids. The relative mass difference between the polar ester groups and non-polar alkyl chains present in the oil determines the volume of co-solvent required[12]. The effect of THF volume on TPO conversion was carried out under the optimal conditions derived from previous section. Variation in TPME formation with time on the addition of different volumes of THF is presented in figure 4. With increase in THF volume from 0% to 25vol%, the conversion of TPO into TPME was increased from 61.57% to 96.10%. This could be due to enhanced inter phase miscibility between oil (triglycerides) and methanol through eliminating mass transfer problems [13]. Further increase in THF quantity to 30vol% resulted in reduced conversion. The decrease in TPME formation beyond optimal value of 25% co-solvent volume could be due to the dilution of triglycerides by excessive co-solvent and thus slowing down the reaction to occur. Qian et al [14] have observed similar trend with petroleum ether as co-solvent for the conversion of cotton seed oil.

Figure 4. Effect of co-solvent volume on conversion of TPO into TPME.
From figure 4, the graph obtained at 0% of co-solvent did not reach its plateau state even at 120min reaction time, indicating that in the absence of co-solvent, IST of TPO requires elevated conditions including time, which makes the process expensive. On the other hand, in the absence of co-solvent, only 65% of the total oil was dissolved into methanol. The rapidity of the process could be revealed by comparing the % conversions in the presence and absence of co-solvent. Figure 5 represents the proton NMR spectrogram of TPME formed in the presence of THF (co-solvent) at 25vol% and 15min of reaction time. A singlet at around 3.6ppm corresponds to –OCH₃ protons of the ester, indicating the existence of biodiesel in the sample. Experiments of Caglar [15] on conversion of Colza oil has shown that due to the presence of THF, the reaction was complete with in a short time of 10min with 99.89wt% yield and concluded that THF has mitigated the process cost through the formation of homogenous system.

3.4 Effect of reaction temperature on TPO conversion:

Figure 6, is the graphical representation of effect of temperature on TPO conversion with time. At lower temperature of 30°C, the conversion was only 64.87%. The conversion has increased to 96.10% with increase in temperature from 30°C to 60°C. At 60°C, the asymptotic curve represents that almost total lipids in the biomass are converted into methyl esters, further increase in reaction time beyond 120min will not give any significant change in conversion. Thus, 60°C was chosen as an optimum temperature. At 30°C, the resultant curve did not reach its saturation state, indicating that the maximum yield can be achieved only at prolonged reaction times. Increase in temperature to 70°C caused the reduction in conversion to 88.93%. In this case, the reduction in conversion at higher temperature (70°C) could be due to the partial vaporization and bubble formation of both methanol and co-solvent, causing unavailability of methanol for the conversion [16].
3.5 Effect of agitation speed on conversion of TPO:

The effect of agitation speed on ester formation is shown in figure 7. Agitation speed was varied from 400-600rpm under the optimal conditions discussed before: 1.5wt% KOH catalyst, 5.5:1(v/w) methanol to biomass ratio, 25vol% of THF co-solvent and 60°C temperature. From the figure, it can be understood that there was no significant difference in the conversion at different agitation speeds. It was only 1.70% (96.10% at 600rpm to 97.80% at 500rpm). It implies that in this case, agitation speed has no considerable effect on TPO conversion. This is probably due to the presence of co-solvent which hinders the mass transfer limitation barrier between oil and methanol. These reports coincide with that of Zenget al. [7].

3.6 Characterization and fuel properties:

Major fatty acids (≥ 5%) present in TPME produced through alkali catalyzed IST of TPO are presented in table 2a. In the present discussion, TPME contains 39.8% of linoleic and 29.7% of oleic acids as majorly contributed unsaturated fatty acids.
Table 2 a. Major fatty acids (≥ 0.5%) present in TPME.

| Fatty acid       | Component (%) |
|------------------|---------------|
| Linoleic (18:2)  | 39.8          |
| Oleic (18:1)     | 29.7          |
| Palmitic (16:0)  | 25.2          |
| Stearic (18:0)   | 2.6           |
| Myristic (14:0)  | 1.9           |

Fuel properties of TPME determined through ASTM D6751 and EN14214 standard methods are presented in Table 2b. Fatty acid composition of methyl esters is the crucial factor, which influences the fuel properties to a greater extent.

Table 2 b. Fuel properties of TPME compared with ASTM and EN standards and petroleum derived diesel fuel.

| Fuel Property                      | TPME   | ASTM D6751 | EN14214 | Petroleum Diesel |
|------------------------------------|--------|------------|---------|------------------|
| Density (g/cm³)                    | 0.871  | --         | 0.86-0.90 | 0.82-0.845       |
| Kinematic viscosity (at 40°C, mm²/s) | 4.12   | 1.9-6.0    | 3.5-5.0  | 1.3-2.4          |
| Acid value (KOH mg/g)              | 0.19   | 0.50 max   | 0.50 max | --               |
| Cloud point (°C)                   | 7.8    | Report     | --       | -15 to 5        |
| Pour point (°C)                    | 6.3    | --         | --       | -15 to -35      |
| Cold filter plugging point (°C)    | 6.0    | --         | --       | --               |
| Flash point (°C)                   | 163    | 93 min     | 120 min  | 60-80            |
| Copper strip corrosion (3h at 50°C) | 1a     | No. 3max   | No. 1max | No. 3max        |
| Oxidation stability at 110°C(h)    | 2.86   | 3 min      | 6 min    | --               |
| Cetane number                      | 55.7   | 47 min     | 51 min   | 40 min           |

3.6.1 Density and kinematic viscosity. Density and Kinematic viscosity of TPME were measured as 0.871g/cm³ and 4.12mm²/s, respectively. The values are in good agreement with the standards. Density affects the atomization of fuel when burn in air free ignition systems. Viscosity depends on fatty acid
structure, increases with increase in saturation of fatty acid chain and decreases with unsaturation [17]. High viscosity causes improper combustion of fuel and leads to enhanced black smoke emission. The low viscosity of TPME could be attributed to the presence of high proportion of linoleic acid. The density and kinematic viscosity measured in this investigation are almost same with that of biodiesel produced from sesame oil [18], a widely cultivated potential oil feedstock of tropical and subtropical regions of the world.

3.6.2 Cold flow properties and Flash point (FP). The temperature at which visible solid cloudy wax crystals formed on decreasing the temperature of biodiesel is called Cloud Point (CP). In the present study, crystal formation was observed at 7.8°C, which is relatively lower than palm oil (17°C), an extensively used feedstock for biodiesel production [19]. The lower CP of TPME could be due to high proportion of unsaturated fatty acids than saturated fatty acids in the mixture. The temperature below which severe crystal formation obstructs the free flow of fuel is Pour Point (PP). The PP of TPME has been measured as 6.3°C. FP is the lowest temperature at which enough vapor of combustible mixture formed over the fuel, will be ignited by the ignition source. FP of biodiesel produced in this study was determined as 163°C. The higher FP than the petro-diesel (60-80) indicates its flame safety to store as well as to have around. Moreover, CP and PP of TPME is strongly affected by its fatty acid composition and made the produced biodiesel more suitable for hot climatic conditions.

3.6.3 Copper strip corrosion. Storage and stability of a fuel rely on its corrosive nature. Copper strip corrosion test is a measure of corrosion behavior of a fuel. Corroding nature of TPME was determined through ASTM D130 standard method. Copper rods placed in TPME for 3h at 50°C has shown only a slight change in the color. These results coincide with the previous reports on biodiesel produced from T. populnea [20].

3.6.4 Cetane number (CN). Cetane number, a primary indicator of fuel quality is the measure of ignition capacity of the fuel. Higher the CN, higher is the efficiency of the fuel to ignite in the engine and lesser will be the time lag. TPME has met the requirements of ASTM, EN standards with 55.7 CN, CN of TPME obtained in the present study is slightly lower than the value 59.8, reported earlier [20]. This dissimilarity could be attributed to the difference in the saturated fatty acid composition.

3.6.5 Oxidation Stability (OS). Oxidation stability of a biodiesel largely depends on unsaturated fatty esters. Oxidation stability of TPME was determined as 2.86 (induction period in hours) by Rancimat method at 110°C according to EN 14112 standard. The susceptibility of TPME to oxidation could be due to high unsaturated fatty acid composition (~70%) specifically, linoleic acid- 39.8% and oleic acid-29.7%. Eventhough T. populnea methyl esters failed to meet the standards’ requirement, it has oxidation stability higher than that of biodiesel fuels derived from used frying oil (1.2), tallow (1.2) and sunflower oil (2.0) [21, 22].

4. Conclusion
In the current exploration, IST of TPO with alkaline catalyst (KOH) was reported. Among the variables studied, catalyst type and concentration, methanol to biomass ratio, co-solvent volume and temperature have shown significant effect on conversion of TPO into TPME. Experimental data showed that 98.30% of the total oil was dissolved into methanol and the conversion was 97.80% under the conditions of 1.5wt% KOH, 5:5:1 (v/w) methanol to biomass ratio, 25 vol% tetrahydrofuran (THF) co-solvent, 60°C temperature and 500 rpm agitation speed. Introduction of co-solvent THF in to the reaction has enhanced the dissolution of oil into the reaction mixture and conversion of TPME. Produced TPME has good fuel
properties, suitable to current diesel engines. From the reports of investigation, it can be inferred that production cost of biodiesel from *T. populnea* could be reduced by using IST process.

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