

Biosynthesis and Characterization of Biodiesel from Cottonseed Oil Using *Pseudomonas fluorescences* Lipase and the Performance of its Blend (B20) in diesel Engine

K. Karuppasamy^a, A. Syed Abu Thaheer^b, and C. Ahmed Basha^{c,*}

^aAnna University, Regional Centre,
Tirunelveli- 627 007, Tamil Nadu

^bPET Engineering College, Vallioor- 627 117,
Tirunelveli Dist, Tamil Nadu

^cDepartment of Chemical Engineering, Adhiyamaan College
of Engineering, Hosur-635109, Tamil Nadu, India

Original scientific paper
Received: April 6, 2012
Accepted: January 14, 2013

Lipase-catalyzed alcoholysis of vegetable oils has attracted significant interests in the production of biodiesel. Present work deals with biosynthesis and characterization of biodiesel from cottonseed oil using *Pseudomonas fluorescences* lipase and the performance studies of the blend B20 (contains 20 % biodiesel and 80 % diesel). Response Surface Methodology based Box-Behnken design was used to optimize the transesterification reaction variable – Ethanol/oil molar ratio, catalyst loading and reaction time for production of ethyl esters. The optimized conditions for biodiesel production were found as follows: ethanol to oil molar ratio: 7 mol/mol, catalyst loading: 6 g, and reaction time 68 h. The optimum biodiesel yield was 93.5 %. Properties such as flash point, fire point, density, viscosity and calorific values of biodiesel B20 and diesel were compared. B20 fuel was tested in a single cylinder, four stroke, direct injection, constant speed, compression ignition diesel engine (Kirloskar) to evaluate the performance and emissions.

Key words:

Cottonseed oil, *Pseudomonas fluorescences* lipase, transesterification, Response Surface Methodology, biodiesel, performance and emission parameters

Introduction

Petroleum fuels are mostly used for various purposes such as transportation, irrigation, aviation and power generation all over the world. Hence its reserves have been diminishing increasingly steadily to an alarming level. The use of petroleum fuels in diesel engines produces a high level of NO_x, smoke and particulates, and with the increase in the number of vehicles and spreading industrialization, environmental pollution has been on the rise^{1–5}. The situation has led to the search for an alternative fuel. The substitution of conventional fuels such as diesel and gasoline by renewable biofuel is a potential way to reduce pollution and sustain the development of the country. At the current consumption level of about 85 million barrels per day of oil and 260 billion cubic feet per day of natural gas, the reserves represent 40 years of oil and 64 years of natural gas, and are non-renewable. India is importing 70 % of petroleum-based fuel to meet the excess requirement of 127 million tons per year. At present, India is using approximately 40 million

tons per year of diesel constituting about 40 % of all petro-products.²

The alternative fuel has to be technically feasible, economically competitive, environmentally acceptable and readily available. The oils from plant origin like vegetable oils and tree borne oil seeds are one of the possible alternatives to fossil. Alternative diesel fuel can be obtained from vegetable oils by the transesterification process as mono-alkyl esters can be termed as biodiesel. This alternative diesel fuel is biodegradable and nontoxic, and obtained from renewable biological sources. Usage of biodiesel will allow for the balance to be sought between agriculture, economic development and the environment.⁶

Various properties of vegetable oils^{1,3,8–15} are shown in Table 1. The commonly used methods for biofuel production are blending, micro-emulsification, transesterification and pyrolysis.^{5–8} Among the various methods of producing biodiesel, transesterification is the most commonly preferred process.^{16–21} This process is widely in use as it reduces the viscosity of triglycerides and thereby enhances the physical properties of renewable fuels and improves engine performance.

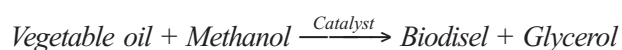
*Corresponding authors: Tel: +91-8870170860;
e-mail: cab_50@rediffmail.com (C Ahmed Basha)

Table 1 – Various properties of vegetable oils

Vegetable Oil	Heating Value (MJ kg ⁻¹)	Density (kg L ⁻¹)	Kinematic Viscosity at 38 °C (mm s ⁻¹)	Cetane Number –	Pour point (°C)	Flash point (°C)
Crambe ¹	40.5	0.948	53.6	44.6	–12.2	274
Safflower ¹	9.5	0.9144	31.3	41.3	–6.7	260
Peanut ¹	39.8	0.926	39.6	41.8	–6.7	260
Palm ¹	–	0.90	39.6	42.0	–	267
Babassu ¹	–	0.946	30.3	38.0	–	150
Corn ⁸	37.1	0.991	–	46.3 ^c	37.6	–
Sunflower ⁸	39.52	0.918	58.5 ^c	37.1	–	–
Deccan hemp ¹¹	38.72	0.93	53 ^b	–	–	255
Jatropha ¹⁴	39.77	0.918	49.9	45.0	–	240
Jojoba ¹⁵	42.76	0.863	24.5 ^d	–	–	292
Linseed ⁸	39.75	–	16.2 ^d	–	–5.0	108
Rubber seed ¹⁰	37.5	0.91	66.2	–	–	198
Soya bean ¹⁰	39.6	0.92	65.0 ^d	–	–	230
Rapeseed ¹²	36.87	0.916	38.0	44–48	15.0	220–280
Turpentine ¹³	44.4	0.860–0.89	2.5 ^b	–	–	38
Mahua ⁹	35.61	0.897	28.58 ^d	–	–	212
Cottonseed ^a	41.8	0.916	50.7 ^d	49.0	2.0	316
Diesel ^a	44.0	0.818	0.301 ^d	52.0	–13.0	53

^a-properties were determined experimentally. (^b, ^c and ^d : viscosity at 30, 27 and 40 °C)

The transesterification process can be done in a number of ways, such as using an alkali catalyst, acid catalyst, biocatalyst, heterogeneous catalyst or using alcohols in their supercritical state. The general reaction is shown below.



In the alkali process, sodium hydroxide or potassium hydroxide is used as a catalyst along with methanol or ethanol. Initially, during the process, alchoxy is formed by the reaction of the catalyst with alcohol, and the alchoxy then reacts with particular vegetable oil used to form biodiesel and glycerol. Glycerol being denser, settles at the bottom and the biodiesel can be decanted. This process is the most efficient and least corrosive of all the processes and the reaction rate is reasonably high even at a low temperature of 60 °C. There may be the risk of free acid or water contamination, and soap formation is also likely to take place which makes the separation process difficult.^{1, 7}

Acid catalyst can be used for producing biodiesel instead of a base catalyst when FFA content is higher in the vegetable oil. The most commonly used acids are sulphuric acid and sulfonic

acid. Although the yield is high, the acids, being corrosive, may cause damage to the equipment and the reaction rate was also observed to be low.⁵ Chemical transesterification has quite a few drawbacks, such as, high energy consumption, difficulty in the recovery of glycerol and high amount of alkaline wastewater from the catalyst. Enzyme catalyzed transesterification of vegetable oil is a good alternative to overcome these drawbacks. There are many reports on biodiesel production using enzyme catalysis by free or immobilized lipases. Immobilized lipase, in particular, is suitable for continuous biodiesel production because of the ease of its recovery from the reaction mixture. In enzymatic transesterification process, the lipase can be immobilized by them in a suitable biomass support particle.

Lipases are widely employed as a catalyst in hydrolysis, alcoholysis, esterification and transesterification of carboxylic esters. Lipases have excellent catalytic activity and stability in non-aqueous media, which facilitates the esterification and transesterification process during biodiesel production. Immobilized enzymes are defined as “enzymes physically confined or localized in a certain

defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously". There are several methods for lipase immobilization, including adsorption, covalent bonding, entrapment, encapsulation, and cross-linking. These immobilization methods have been employed to improve lipase stability for biodiesel production. Entrapment of a lipase entails capturing the lipase within a matrix of polymer. In theory, the entrapped enzyme is not attached to the polymer; its free diffusion is merely restrained. Virtues of the entrapment method for immobilizing lipase are that it is fast, cheap, very easy, and usually involves mild conditions.²² The advantage of immobilization is that the enzyme can be reused without separation. Also, the operating temperature of the process is low (50 °C) compared to other techniques. Disadvantages include inhibition effects which were observed when methanol was used, and the fact that enzymes are expensive.²³ Effective ethanolysis reactions using several extracellular lipases from *Candida* sp.^{24–26}, *Pseudomonas* sp.¹⁷, and *Rhizopus* sp.^{27,28} have been developed by several researchers. With these lipases, methyl ester content in the reaction mixture, which has a yield of more than 90 %, is obtained using either low- or high-water content systems. However, the use of extracellular lipase as a catalyst requires complicated recovery, purification, and immobilization processes for industrial application.⁵

In the present investigation, cottonseed oil, a non-edible type vegetable oil is chosen as a potential alternative for synthesis of biodiesel using *Pseudomonas fluorescences* lipase. RSM has been used to determine the relation between the percentage of biodiesel production in terms of yield (%) and the input parameters such as ethanol to oil molar ratio, catalyst loading and reaction time. The test fuel B20 (consists of 20 % biodiesel and 80 % diesel) was used in single cylinder, direct injection, diesel engine to evaluate the performance and emissions.

Materials and methods

Chemicals such as sodium alginate, ethanol, calcium chloride and hexane were of analytical grade. The non-edible crude cottonseed oil was purchased commercially from the oil mills in Tamilnadu and stored at 4 °C to avoid rancidity of the vegetable oil, and it was used throughout the experimentation. Its quality characteristics were determined according to the standard methods of fats and oils published by the association of oil chemists, having the density of 0.916 g cm⁻³, iodine value of 114 mg I₂/100 g, acid value of 0.6 mg

KOH/g and saponification value of 199 mg KOH/g. The molecular weight of the cottonseed oil was calculated from its saponification and acid values, and was 840. For removing non-hydratable phospholipids, phosphoric acid was added to the oil and heated to 70 °C, which is called the special micelles degumming method. The reaction time for this method is about 5 minutes and it is followed by neutralization through dilute lye.

Pseudomonas fluorescences, MTCC103 was obtained from Microbial type Culture Collection and Gene Bank Chandigarh (India). The culture was maintained in the nutrient agar medium. After 3 days of incubation at 25 °C the agar slants were stored at 4 °C. The liquid medium for the growth of inoculums for bacteria was nutrient agar medium composed of 1.0 g L⁻¹ of beef extract, 2.0 g L⁻¹ of yeast extract, 5.0 g L⁻¹ of peptone and 5.0 g L⁻¹ of sodium chloride.

Inoculums preparation

Inoculums were grown aerobically in 250 mL Erlenmeyer flasks containing the above mentioned medium at 25 °C in an environmental shaker (Remi Scientific) at 200 rpm for 24 h. Active cells were centrifuged in a clinical centrifuge at 1200 rpm, washed with sterile water and used as inoculums.

Lipase and its immobilization

Active cells from inoculums were transferred to 250 mL Erlenmeyer flasks containing the production medium maintained at 28 °C in an environmental shaker (Remi scientific) at 250 rpm for 5 days to attain maximum production of lipase. Culture broth was centrifuged at 4000 rpm for 15 minutes and the supernatant was crude lipase solution. The supernatant was brought to 65 % saturation with the addition of solid ammonium sulphate, (NH₄)₂SO₄ followed by centrifugation for 15 minutes at 4000 rpm and 4 °C. The pellet collected as crude enzyme was resuspended in 1mM phosphate buffer (pH 7.0), concentrated using an ultra filtration membrane before storing at 4 °C, and immobilized using sodium alginate entrapment. Alginate solution with a concentration range of 0.5 – 10 % was used for the lipase immobilization and was prepared by dissolving sodium alginate in boiling water and autoclaved at 121 °C for 15 minutes. Both alginate slurry and crude enzyme suspension were mixed and stirred for 10 minutes to get a uniform mixture of alginate/enzyme, which was extruded drop by drop into a cold sterile 0.2 M CaCl₂ solution through a sterile 5 ml pipette from 5 cm height and kept for curing at 4 °C for 1 h. The beads were hardened by re-suspending into a fresh CaCl₂ solution for 24 h at 4 °C with gentle agitation. Finally,

these beads were washed with distilled water to remove excess calcium ions and untrapped enzymes. When the beads were not being used, they were preserved in 0.9 % sodium chloride solution in the refrigerator.

Design of experiments

Response Surface Methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes. The most extensive applications of RSM are in particular situations where several input variables potentially influence some performance measure or quality characteristic of the process. The performance measure or quality characteristic is called the response. The input variables are sometimes called independent variables, and they are subject to the control of the scientist or engineer. The field of response surface methodology consists of the experimental strategy for exploring the space of the process or independent variables, empirical statistical modeling to develop an appropriate approximating relationship between the yield and the process variables, and optimization methods for finding the values of the process variables that produce desirable values of the response.^{29, 30}

The Box–Behnken experimental design of RSM was chosen to find the relationship between the response functions and variables using the statistical software package **Design Expert Software 8.0.4** trial version (**Stat-Ease, Inc., Minneapolis, USA**). The Box–Behnken design can be considered a highly fractionalized three-level factorial design where the treatment combinations are the midpoints of edges of factor levels and the center point. These designs are rotatable (or nearly rotatable) and require three levels of each factor under study. Box–Behnken designs can fit in full quadratic response surface models and offer advantages over other designs. The advantages of the Box–Behnken design over other response surface designs are: (a) it needs fewer experiments than central composite design and similar ones used for Doehlert designs; (b) in contrast to central composite and Doehlert designs, it has only three levels; (c) it is easier to arrange and interpret than other designs; (d) it can be

expanded, contracted or even translated; (e) it avoids combined factor extremes since midpoints of edges of factors are always used. Table 2 gives the parameters and the operating ranges covered. Response surface methodology based Box–Behnken design was used to develop the design matrix. The design matrix consists of 17 experiments.

Statistical analysis

Experimental data obtained from the 17 experiments were fitted in the second-order polynomial equation. This equation gives the relation between the yield of biodiesel produced and the coded independent variables. The polynomial model for the biodiesel yield may be written as follows.

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i < j=1}^3 \sum_{j=1}^3 \beta_{ij} X_i X_j \quad (1)$$

Where β (0=intercept, i=linear, ii=quadratic and ij=interaction) and X_i, X_j ($i=1, 3; j=1, 3; i \neq j$ represent the coded independent variables) are the model coefficients. With the fitted quadratic polynomial equation, 3D model graphs were developed to analyze the interaction between the terms and their effects on biodiesel production yield.

Alcoholysis of cottonseed oil

Transesterification reactions were conducted at different molar ratio of ethanol to substrate. Oil and ethanol were poured into the reaction flask and heated to the reaction temperature with constant shaking using reciprocal shaker (150 oscillations/min; amplitude 70 mm) for 24 h, 48 h, and 72 h. In the subsequent experiments, in which the effect of molar ratio of ethanol to oil was investigated, the volume of oil was kept constant while the volume of ethanol varied. Around 3 mL of hexane was added to the reaction mixture to increase the solubility of the reactants.³¹ The appropriate quantity of immobilized beads based on oil weight was added to the flask. After the reaction was completed, the beads were removed from the reaction mixture by filtration. Biodiesel and glycerol were separated using separating funnel. The parameters affecting transesterification reaction such as ethanol to oil molar ratio, catalyst loading and reaction time were varied as per the design matrix and the yields obtained were recorded for analysis.

Properties of biodiesel

The fuel properties of cottonseed oil ethyl ester, biodiesel blend (B20) and diesel are listed in Table 3. The kinematics viscosity of cottonseed oil is 50.7 mm² s⁻¹ at 40 °C. The high viscosity of the oil is due to its large molecular mass (840), which

Table 2 – Experimental range and levels of independent process variable

Factor	Variable	Unit	Range and levels		
			-1	0	+1
X ₁	Ethanol to oil molar ratio	Mol/Mol	6:1	9:1	12:1
X ₂	Catalyst loading	g	5	10	15
X ₃	Reaction time	h	24	48	72

Table 3 – Properties of biodiesel blend (B20) in comparison with diesel

Property	ASTM Standard	Diesel	Biodiesel/Ethylester	Biodiesel Blend (B20) ^a	Biodiesel of ASTM D6751
Density @15 °C (kg m ⁻³)	ASTM D1298	821.8	856	829.2	870–900
Viscosity @40 °C (mm ² s ⁻¹)	ASTM D445	3.01	5.88	3.48	1.9–6.0
Flash point (°C)	ASTM D93	53	174	82	>130
Fire point (°C)	ASTM D93	61	184	90	–
Cetane Number	ASTM D613	52	51.2	51.8	47 min.
Gross Caloric Value kJ/kg	ASTM D240	44,855	42, 218	43,643	–
Cloud point (°C)	ASTM D2500	8	10	9	–
Pour point (°C)	ASTM D97	–13	–3	–10	–
Sediments (%)	ASTM D2709	NIL	NIL	NIL	0.05

^a-(20% Biodiesel +80% diesel)

is about 20 times higher than that of diesel fuel. The flash point of cottonseed oil ethyl ester is 174 °C, which is much higher than diesel fuel. The heating values of cottonseed oil ethyl esters (42.2 MJ kg⁻¹) are in the same range as compared to diesel fuels (44.8 MJ kg⁻¹). The presence of chemically bound oxygen in vegetable oils lowers their heating values by about 4 %. The cetane number is around 51.2 which is very close to the diesel value.

The physical properties, including the cetane number, were evaluated experimentally for all diesel/biodiesel blends used in this work.

Performance and emission test

The performances of cottonseed oil ethyl ester blend (B20) were studied in comparison with diesel fuel. The compression ignition engine used for study was KIRLOSKAR TV-1, single cylinder, constant speed, vertical, water cooled and direction

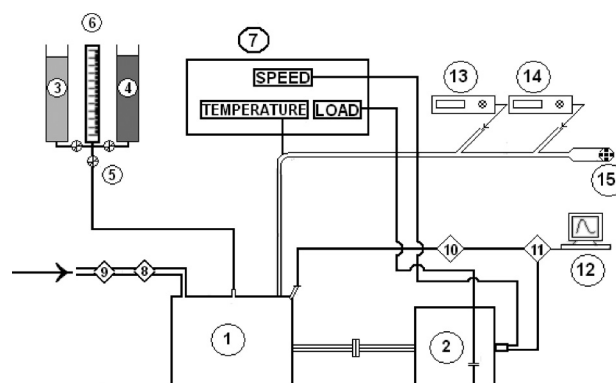


Fig. 1 – Schematic diagram of experimental setup: 1. Kirloskar TV-1 Diesel Engine, 2. Eddy current Dynamometer, 3. Diesel tank, 4. Biodiesel blend tank, 5. Control valve, 6. Fuel measuring burette, 7. Control panel, 8. Air stabilizing tank, 9. Air filter, 10. Charge amplifier, 11. Indimeter, 12. Data Acquisition System, 13. AVL DI gas analyzer, 14. AVL smoke meter, 15. Silencer.

injection diesel engine and the specification details are given in Table 4. The experimental set-up is shown in Fig. 1. The engine was coupled with an eddy current dynamometer for applying different load conditions and it was controlled by a control system provided with a control panel (consisting of a speed, temperature and a load indicator). The engine was always run at its rated speed. The fuel injection system consists of three-hole type injector with a MICO plunger pump of 8 mm diameter operated by the camshaft. The injection timing recommended by the manufacturer is 23° before TDC (static). The operating pressure of the nozzle was set at the rated value of 220 bar. Provision was made in the cylinder head surface to mount a piezoelectric transducer for measuring the cylinder pressure. The fuel flow was measured by the use of a 50 cc graduated burette and a stopwatch. Chromel Alumel (k-type) thermocouple was installed to measure the exhaust gas temperature of the engine.

Table 4 – Engine specification details

Make	Kirloskar
Model	TV-1
Type	Vertical, single cylinder, four stroke, water cooled, DI diesel engine.
Number of cylinder	1
Bore x stroke	87.5x 110 mm
Compression ratio	17:1
Rated power	5.2 kW
Rated speed	1500 rpm
Injection pressure	220 kgf/cm ²
Start of injection	23° before TDC
Dynamometer	Eddy current

The smoke intensity was measured by an AVL 413 smoke meter and Nitrous oxide (NO_x), Carbon monoxide (CO), Hydrocarbon (HC) were measured by an AVL 444 Di gas analyser. Performance parameter and emission characteristics of engine were taken for diesel, biodiesel blend (B20 %) from lower load to full load condition. The tests were repeated three times and each test was done for 3 h. Finally the average value of the three readings was taken for the calculation.

Results and discussion

Optimization of different parameters for biodiesel production by RSM

The experimental readings for synthesis of biodiesel were fitted in the second-order polynomial equation using **design expert software trial version 8.0.4**. The final empirical model in terms of coded factor (Y) is shown in Eq. (2)

$$Y = 70 - 9.4X_1 - 0.4X_2 + 13.53X_3 - 5X_1X_2 - 10.95X_1X_3 - 12.2X_2X_3 - 8.7X_1^2 + 0.6X_2^2 - 3.4X_3^2 \quad (2)$$

where the values of X_1 , X_2 , and X_3 are in terms of coded factors and represent ethanol to oil molar ratio, catalyst loading and reaction time, respectively. The positive sign in front of the terms indicates a synergistic effect, whereas the negative sign indicates an antagonistic effect. The quality of the model developed was evaluated based on the correlation coefficient value. The R^2 value for Eq. (2) was 0.9387. This indicated that 93.87 % of the total variation in the biodiesel yield was attributed to the experimental variables studied. Analysis of variance (ANOVA) was further carried out to justify the

adequacy of the model.^{29,30} The ANOVA for the quadratic model for biodiesel yield is listed in Table 5. From the ANOVA for response surface quadratic model for the yield of biodiesel, the Model F-value of 11.91 and Prob > F value of 0.0018 implied that the model was significant. For the model terms, values of Prob > F, which was less than 0.05, indicated that the model terms were significant. In this case, ethanol/oil molar ratio (X_1), X_3 , X_1X_3 , X_2X_3 , and X_1^2 were significant model terms whereas catalyst loading (X_2), X_1X_2 , X_2^2 , and X_3^2 were all insignificant to the response.

Fig. 2 shows the effect of ethanol to oil molar ratio and catalyst loading on the yield of biodiesel synthesis at constant reaction time (48 h). The low ethanol to oil molar ratio and the increase in catalyst loading caused high fatty acid ethyl ester contents due to abundant active sites of immobilized enzyme beads and sufficient mass contact. At the lowest catalyst loading, an increase in the ethanol to oil molar ratio decreased the ethyl esters content.

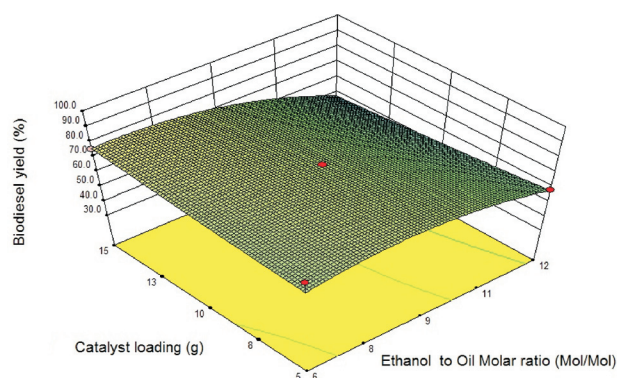


Fig. 2 – Response surface plot showing the effect of ethanol/oil molar ratio and catalyst loading on biodiesel yield

Table 5 – ANOVA for response surface quadratic model for biodiesel yield

Source	Sum of square	DF	Mean	F	p-value
Model	3728.093	9	414.2325	11.91277	<0.0018
X_1 :Ethanol to Oil Molar ratio (Mol/Mol)	706.88	1	706.88	20.32892	<0.0028
X_2 :Catalyst loading (g)	1.28	1	1.28	0.036811	0.8533
X_3 :Reaction time (h)	1463.405	1	1463.405	42.08556	<0.0003
X_1X_2	100	1	100	2.875865	0.1337
X_1X_3	479.61	1	479.61	13.79294	<0.0075
X_2X_3	595.36	1	595.36	17.12175	<0.0044
X_1^2	318.6947	1	318.6947	9.165231	<0.0192
X_2^2	1.515789	1	1.515789	0.043592	0.8406
X_3^2	48.67368	1	48.67368	1.39979	0.2754
Residual	243.405	7	34.77214	–	–

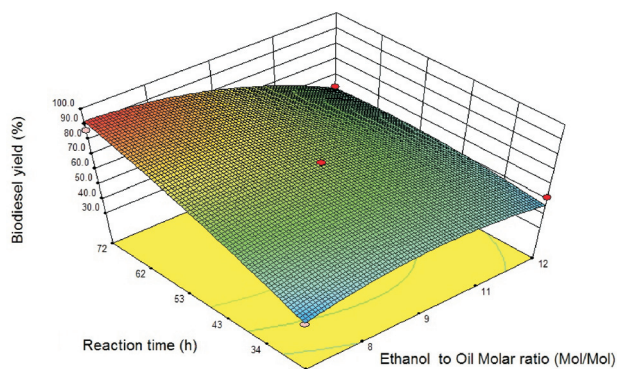


Fig. 3 – Response surface plot showing the effect of ethanol/oil molar ratio and reaction time on biodiesel yield

This is due to deactivation of catalyst at excess ethanol content. Fig. 3 shows the 3D response surface plot of interaction between varying concentration of ethanol to oil and reaction time on biodiesel yield at a catalyst quantity of 10 g of immobilized enzyme beads. It can be ascertained from the surface plot that the biodiesel yield increases with increasing reaction time. The immobilized enzyme beads in the reaction mixture speed up the first two steps of the transesterification reaction involving conversion of triglycerides to monoglycerides and slow down the third step, conversion of monoglycerides to methyl esters. Fig. 4 shows the effect of catalyst loading and reaction time on the biodiesel yield (ethanol/oil molar ratio was fixed at 9 Mol/Mol and reaction temperature was fixed at 37 °C). The biodiesel yield was found to increase with increasing catalyst loading, ethanol/oil molar ratio and reaction time. The highest yield was obtained when all the three variables were at the optimum point within the range studied.

The optimum conditions for the three variables, ethanol to oil molar ratio, catalyst loading, and reaction time were obtained using numerical optimization feature of Design Expert Software

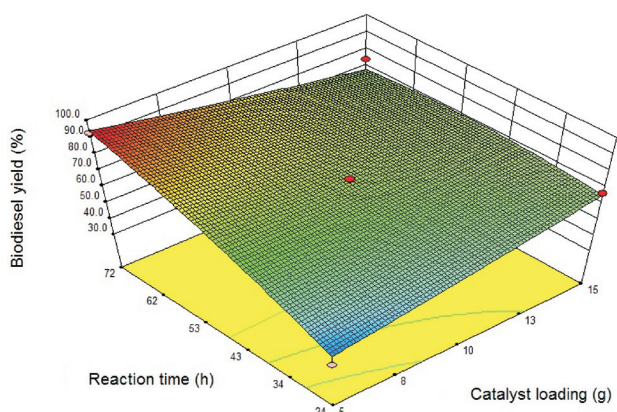


Fig. 4 – Response surface plot showing the effect of catalyst loading and reaction time on biodiesel yield

8.0.4. The optimized conditions found by Design of Experiments were as follows: $X_1 = 7$ Mol/Mol, $X_2 = 6$ g and $X_3 = 68$ h. The theoretical fatty acid ethyl ester content predicted under the above conditions was $Y = 93.5\%$. In order to verify the prediction of the model, the optimal reaction conditions were applied to three independent variables for biodiesel synthesis. The average conversion yield was $93.5 \pm 0.7\%$. This demonstrated that response surface methodology with appropriate experimental design can be effectively applied to the optimization of the process of factors in a chemical reaction. This study focused on the application of response surface methodology to the optimization of biodiesel synthesis conditions using lipase as catalyst.

Performance parameters

The variations of brake thermal efficiency with biodiesel blend (B20) and diesel at different brake power are shown in Fig. 5. It has been observed that the brake thermal efficiency increases with the increase in brake power of both fuels. The maximum brake thermal efficiency of diesel is about 32.4 % whereas that of B20 fuel is 32.1 % at full load. The slight reduction of brake thermal efficiency with biodiesel Blend (B20) was attributed to poor spray characteristics, poor air fuel mixing, higher viscosity, and lower calorific value.⁴

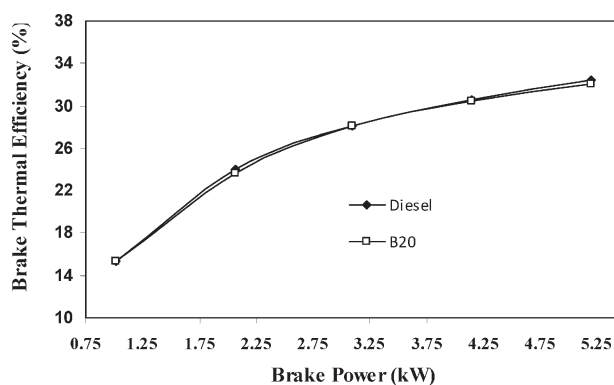


Fig. 5 – Brake thermal efficiency of B20 and diesel with respect to brake power

The variation of specific energy consumption (SEC) with the brake power of diesel fuel and B20 is shown in Fig. 6. It is observed that B20 has higher SEC than diesel fuel. The higher specific energy consumption is due to the lower energy content of the biodiesel blend. Already there are reports that biodiesels prepared from vegetable oils have high SEC.^{3,32,33} The esters converting the chemical energy to mechanical work are comparable with diesel fuel. This specific energy consumption (Fuel Consumption x Heating Value / Brake Power) measures the amount of input energy required to de-

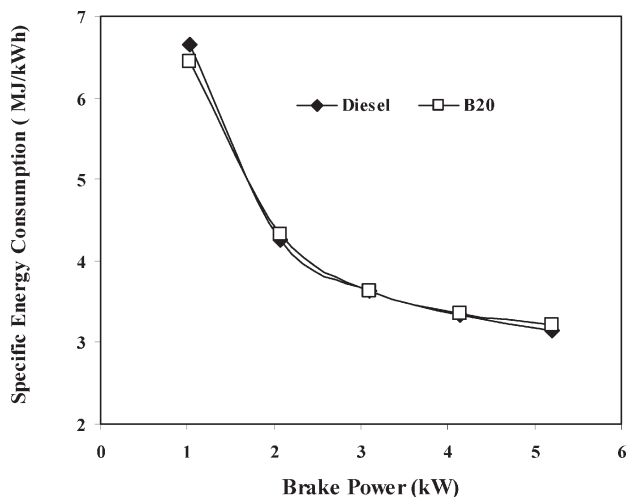


Fig. 6 – Specific energy consumption of B20 and diesel with respect to brake power

velop one kilowatt power. The mass flow rate and heating value are different for B20 and diesel, and hence a simple comparison is made in terms of specific energy consumption.

Emission parameters

The emission characteristics of biodiesel are of special interest in relation to meeting the environmental norms. Fig. 7 shows the variation of smoke density with brake power for diesel and B20. A smoke emission is formed due to incomplete combustion of fuel. It is observed that B20 has 8 % lesser smoke density than the diesel fuel at maximum load condition. This may be due to the presence of oxygen molecules present in the ethyl esters, which results in better combustion of the fuel.⁹

The variation of exhaust gas temperature with brake power output is shown in Fig. 8. It is seen from the figure that the exhaust gas temperature of the biodiesel blend (B20) increases with the increase in brake power. Exhaust gas temperature of

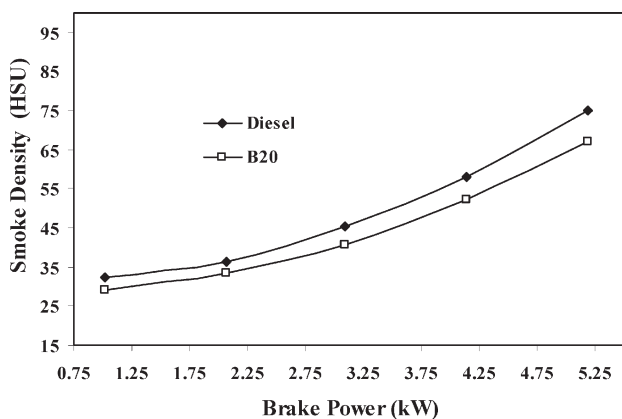


Fig. 7 – Smoke density of B20 and diesel with respect to brake power

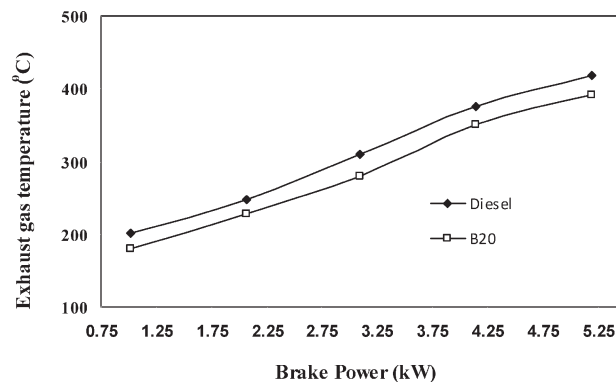


Fig. 8 – Exhaust gas temperature of B20 and diesel with respect to brake power

neat diesel fuel is higher than that of B20. This is due to the oxygen present in the ester molecule that enhances the combustion process thus increasing the temperature. At maximum load conditions, the exhaust gas temperature of B20 is less than that of diesel.¹⁰ The maximum temperature of exhaust gas at peak load is 392 °C with the ethyl esters of cottonseed oil and 418 °C with the diesel.

The variation in NO_x concentration with brake power for B20 and neat diesel fuel is shown in Fig. 9. NO_x formation in the cylinder depends on the engine in-cylinder temperature and the rate of combustion. The two most important factors in determining the NO_x formation by the combustion process are stoichiometric air fuel ratio and the peak combustion temperature.³³ It can be observed that the formation of NO_x for B20 and diesel at full load condition are 1102 ppm, and 1174 ppm. B20 has 6 % lower NO_x emission when compared to that of diesel operation at full load condition. This is due to the decrease in in-cylinder temperature, which is reflected in lesser exhaust gas temperature.

Fig. 10 shows the plot of carbon monoxide emission of B20 and diesel fuel operation at the rated engine speed of 1500 rpm at various brake

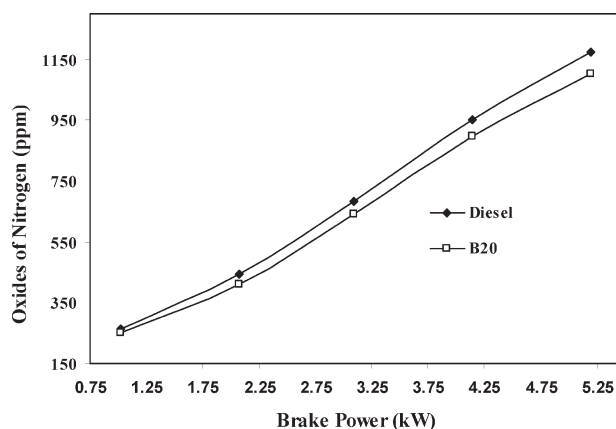


Fig. 9 – Oxides of nitrogen of B20 and diesel with respect to brake power

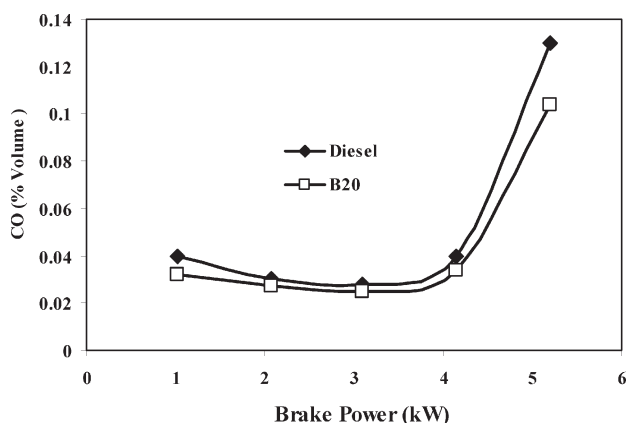


Fig. 10 – Carbon monoxide emission of B20 and diesel with respect to brake power

power. The fuels produce a low amount of carbon monoxide emission at lighter load levels and give out more emissions at higher loading conditions. The carbon monoxide emissions are found to be increasing with the increase in load. This is typical with all internal combustion engines since the air–fuel ratio decreases with the increase in load. CO emission is the ideal emission product assessor. CO concentration in the exhaust emission is negligibly small when a homogeneous mixture is burned at stoichiometric air–fuel ratio mixture or on the lean side stoichiometric. It is observed that, the engine emits more CO while using diesel compared to B20 under all loading conditions. CO emissions for diesel and B20 are 0.13 % and 0.104 % volume at full load. The reduction in CO emissions of the B20 was due to the availability of 11 % of oxygen content in biodiesel which helps complete combustion of biodiesel blend B20.¹⁰

Fig. 11 shows the HC emissions of diesel and B20 with respect to brake power. At smaller loads,

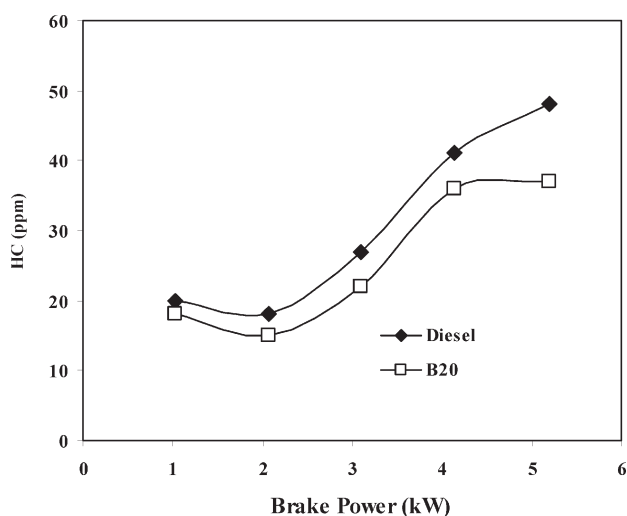


Fig. 11 – Hydrocarbon emission of B20 and diesel with respect to brake power.

oxidation reactions were very slow due to lower temperature and lean mixture. Unburnt HC particles were formed in the core of the spray and the regions just outside the flame zone. They were also formed at the point where the fuel spray touches the wall and thereby got quenched. HC emissions for diesel and B20 were 48 ppm and 37 ppm. It is observed that B20 has 23 % less HC emission than diesel. As the load was increased, heat released by the fuel also increased, which resulted in improved combustion and consequently the unburnt HC level started decreasing.

Conclusions

Experiments were conducted on a single cylinder, four strokes, water cooled, direct injection, diesel engine using cottonseed oil ethyl ester blend (B20) and diesel as fuels. Based on the findings the following conclusions were drawn.

- Biosynthesis of biodiesel using *Pseudomonas fluorescences* lipase catalyzed ethanolysis of cottonseed oil could be improved by response surface methodology and an ethyl ester yield of 93.5 % was achieved after optimization.

- The brake thermal efficiency of B20 was 0.9 % lower than that of diesel fuel at full load conditions.

- Specific energy consumption of B20 was 0.07 MJ kWh⁻¹ higher than the diesel fuel at maximum load conditions.

- Ethyl esters blend have less emissions when compared with diesel.

Cottonseed oil is a renewable fuel and its performance and emissions have slight variation from diesel fuel. It can be considered for use as a blended fuel for existing diesel engine without modification, as well as more environmentally friendly.

References

1. Barnwal, B. K., Sharma, M. P., *Renewable Sustainable Energy Rev.* **9** (2005) 363
2. Sonare, N. R., Rathod, V. K., *J. Mol. Catal. B: Enzym.* **66** (2010) 142
3. Agarwal, D., Kumar, L., Agarwal, A. K., *Renewable Energy* **33** (2008) 1147
4. Nabi, M. N., Rahman, M. M., Akhter, M. S., *Appl. Therm. Eng.* **29** (2009) 2265
5. Srivathsan, V. R., Narasimhan, S. L., Muthukumar, K., *Bioresour. Technol.* **99** (2008) 397
6. Meher, L. C., Vidya S. D., Naik, S. N., *Renewable Sustainable Energy Rev.* **10** (2006) 248
7. Fangrui, M., Hanna, M. A., *Bioresour. Technol.* **70** (1999) 1
8. Ramadhas, A. S., Jayaraj, S., Muraleedharan, C., *Renewable Energy* **29** (2004) 727

9. *Kapilan, N., Reddy, R. P.*, *J. Am. Oil Chem. Soc.* **85** (2008) 185
10. *Ramadhas, A. S., Muraleedharan, C., Jayaraj, S.*, *Renewable Energy* **30** (2005) 1789
11. *Hebbal, O. D., Vijayakumar Reddy, K., Rajagopal, K.*, *Fuel* **85** (2006) 2187
12. *Labeckas, G., Slavinskas, S.*, *Energy Convers. Manage.* **50** (2009) 802
13. *Karthikeyan, R., Mahalakshmi N. V.*, *Energy* **32** (2007) 1202
14. *Narayana Reddy, J., Ramesh, A.*, *Renewable Energy* **31** (2006) 1994
15. *Huzayyin A. S., Bawady A. H., Rady, M. A., Dawood A.*, *Energy Convers. Manage.* **45** (2004) 2093
16. *Dahai, Y., Wang, Z., Chen, P., Jin, L., Cheng, Y., Zhou, J., Cao, S.*, *J. Mol. Catal. B: Enzym.* **48** (2007) 51
17. *Shweta, S., Gupta, M. N.*, *Process Biochem.* **42** (2007) 409
18. *Sriappareddy, T., Shinji, H., Takanori, T., Talukder, M. R., Akihiko, K., Hideki, F.*, *J. Mol. Catal. B: Enzym.* **48** (2007) 33
19. *Talukder, M. M. R., Wu, J. C., Nguyen, T. B. V., Fen, N. M., Melissa, Y. L. S.*, *J. Mol. Catal. B: Enzym.* **60** (2009) 106
20. *Zheng, Y., Quan, J., Ning, X., Zhu, L.-M., Jiang, B., He, Z.-Y.*, *World J. Microbiol. Biotechnol.* **25** (2009) 41
21. *Jegannathan, K. R., Leong, J.-Y., Chan, E.-S., Ravindra, P.*, *Fuel* **89** (2010) 2272
22. *Tan, T., Lu, J., Nie, K., Deng, L., Wang, F.*, *Biotechnol. Adv.* **28** (2010) 628
23. *Shimada, Y., Watanabe, Y., Sugihara, A., Tominaga, Y.*, *J. Mol. Catal. B: Enzym.* **17** (2002) 133
24. *Zheng, L. I., Li, D., Jike, L., Xiaolei, G., Zixin, Y., Tianwei, T.*, *Chin. J. Chem. Eng.* **18** (2010) 870
25. *Rodrigues, A. R., Paiva, A., Silva, M. G., Simoes, P., Barreiros, S.*, *J. Supercrit. Fluids* **56** (2011) 259
26. *Lu, J., Nie, K., Xie, F., Wang, F., Tan, T.*, *Process Biochem.* **42** (2007) 1367
27. *Lee, J. H., Kim, S. B., Kang, S. W., Song, Y. S., Park, C., Han, S. O., Kim, S. W.*, *Bioresour. Technol.* **102** (2011) 2105
28. *Kazuhiro, B., Shinji, H., Keiko, N., Masaru, K., Takeshi, M., Akihiko, K., Hideo, N., Hideki, F.*, *J. Mol. Catal. B: Enzym.* **17** (2002) 157
29. *Hameed, B. H., Lai, L. F., Chin, L. H.*, *Bioresour. Technol.* **90** (2009) 606
30. *Wang, Y., Wu, H., Zong, M. H.*, *Bioresour. Technol.* **99** (2008) 7232
31. *Mohamed M. S. and Uwe B.*, *Enzyme Microb. Technol.* **33** (2003) 97
32. *Sukumar, P., Vedaraman, N., Boppana, Ram, V. B., Sankar-narayanan, G., Jeychandran, K.*, *Biomass Bioenergy* **28** (2005) 87
33. *Saravanan, N., Nagarajan, G., Puhon, S.*, *Biomass Bioenergy* **34** (2010) 838