A Comparative Study of Enzyme Efficacy for Biodiesel Production from Jatropha Curcas Oil

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Abstract-

Feasibility *of biodiesel (BD) production from non edible Jatropha Curcas oil (JCO) using different enzymes as catalyst has been studied. BD is considered as non-conventional, alternative, renewable, green energy sources. Optimum production of BD from JCO is directed by the initial characteristics of raw materials along with important reaction parameters like reaction temperature, concentration of enzyme as catalyst, reaction time and molar ratio of initial raw materials. Enzymes play an important role in the transesterification reaction for optimum conversion of BD and in the present research investigation, the effect of variation of enzyme for the production of BD from JCO has been analyzed. Four enzymes namely NS 435, TLIM, RM IM and NS40013 (6%) have been studied in the presence of solvent at 6:1 molar ratio of alcohol to JCO maintaining a temperature of 60^{0}C with 600 rpm of mixing intensity for 8hrs. The research findings show that NS 435 is the most efficient enzyme and contributes highest yield of BD through transesterification reaction between JCO and methanol compared to other enzymes. The properties of Jatropha methyl ester are measured and are in conformity with the ASTM standards.*

Keywords - *Biodiesel, Jatropha Curcas Oil, Enzymes, Methanol, Renewable energy*

I. INTRODUCTION

BD has created a lot of attention as renewable, alternative, green energy sources for the last few decades. Continuous degradation of environment as well as depletion of non renewable energy sources attracted scientists and academicians to explore alternative renewable energy sources. In this regard, BD is most significant due to its easily available raw materials like different vegetable oils / fats and alcohols (1,2). The most suitable approach is the preparation of BD from JCO and alcohol. JCO is obtained from Jatropha Curcas trees which can be cultivated in both non tropical regions and in waste or barren land and grows on almost any terrain, even on gravelly, sandy and saline soils and under adverse climatic condition.

Preparation of BD from different vegetable oils and alcohols in the presence of chemical catalyst has been studied by several researchers (3,4,5). Enzymatic approach for the production of BD has also been done and it has several advantages over chemical catalyst. The main advantages of using enzymes are no by product generation, easy separation of product, mild reaction conditions etc. A comparative study has also been made by the present authors between chemical and biocatalytic transesterification of JCO and alcohol and studies showed that enzymatic method is more effective than base catalytic method with regard to productivity, eco friendliness, selective nature, purity of the product, minimum purification stage, low temperature requirement and reuse of catalyst (6). Du et al (7) studied the perspective for biotechnological production of BD and its impacts. Enzymatic BD production from canola oil was also studied by Dizge et al (8) and the immobilization was done on hydrophobic micro porous styrene – divinylbenzene copolymer (9). They (10) also studied BD production from sunflower, soybean and waste cooking oils using lipase immobilized onto novel micro porous polymer. The transesterification of palm oil with methanol using M. miehei in n-hexane micro aqueous system has been successfully described by Al-Zuhair et al.(11) for determining the optimal conditions for BD production. In another study, Huang et al (12) optimized lipase catalysed transesterification of lard for BD production using response surface methodology. BD from JCO has also been studied by several researchers in the presence of chemical as well as biological catalyst. Kumari et al.(13) used immobilized lipase from *Enterobactor aerogenes* using JCO in t-butanol solvent for BD production and they obtained 94% yield. Another study of Aransiola (14) presented the ethanolysis of both crude and pretreated JCO using immobilized lipase enzyme from *Pseudomonas cepacia* and a maximum of 72.1wt% fatty acid ethyl ester was obtained at optimized conditions. Veny et al.(15) produced BD from JCO through enzymatic synthesis in a recirculated packed bed reactor and they obtained highest methyl ester yield of 54% from lipase dosage of 10%.

In the present research investigation, a comparative study has been made using different enzymes like NS 435, TLIM, RM IM and NS40013 for the production of BD from JCO maintaining optimized reaction parameters. Comparative analysis shows that NS 435 is the most effective enzyme for BD production from JCO as well as any vegetable oils which may be a solution of energy scarcity in future.

II. MATERIALS AND METHODS

The JCO used in this study was provided by M/s Arora Oils Ltd., Burdwan, West Bengal, India. The immobilized lipases used, Lipozyme RM-IM (Source: Rhizomucor miehei,) 1,3 specific lipase, TL IM (Source: Thermomyces lanuginosus,) 1,3 specific lipase, NS40013 random lipase, NS 435 (Source: Candida Antarctica), were all donated by M/s. Novozymes South Asian Pvt., Ltd., Bangalore, India, for research purpose. The chemicals used in this work such as methanol and hexane were purchased from S.D. Fine Chemicals (Mumbai, India). Except otherwise specified all other chemicals used were A.R. Grade.

For transesterification reaction of JCO and alcohol in the presence of enzyme, initially 250 mL of crude JCO was taken in an Erlenmeyer flask and heated up to 80^0 C to drive off moisture by continuous stirring for about 1 h. After that, transesterification reaction was carried out by stepwise addition of methanol in an appropriate proportion using solvent hexane fitted with a water condenser and stirred by a magnetic stirrer at a specified temperature for 8 hours maintaining other reaction conditions. To the reaction mixture, immobilized enzyme was added in definite proportion (w/w).

Stepwise addition of methanol was allowed to minimize the deactivation of enzyme. For the analysis of the products, definite amount of samples were withdrawn into a capped vial at suitable intervals. After that the samples were centrifuged for 15 min to remove immobilized lipase. The supernatant part was taken in hexane and no leaching of enzyme was observed in this part. It was then evaporated to dryness and the products were isolated. The synthesis of Jatropha methyl esters (BD) was first monitored by thin layer chromatographic (TLC) method and the typical yield of each reaction product was determined separately by column chromatography. After completion of reaction, the enzyme was washed with hexane, dried and reused for the next experiment. Biodiesel from JCO and methanol using enzyme NS 435 was characterized according to the American Standard Testing Method (ASTM). Values are reported as mean ± s.d., where n=3 (n=no of observation). The formation of BD through transesterification of JCO with methanol was first confirmed by TLC after spotting the lipid mixture on a silica-gel G plate (0.2 mm thick) using hexane-diethyl etheraceticacid (90:10:1) as a developing solvent.The lipid spots were identified by iodine absorption with triacylglycerols, diacylglycerols, monoacylglycerols and biodiesel as standard. Quantitative analysis of Jatropha esters (BD) was determined by column chromatography using silicic acid as an adsorbent and 160 mL of hexane-diethyl ether: 99:1 as eluting solvent.

III.RESULTS AND DISCUSSIONS

The initial characteristics of JCO are important for comparative study with regard to the activity of enzyme for conversion of BD. Table 1and Table II show the fatty acid composition and characteristics of crude JCO. It has been observed from Table 1 that crude JCO contains mainly palmitic, stearic, oleic and linoleic acid with maximum percentage of oleic acid.

Table I : Fatty Acid Composition of Crude Jco

Fatty acids	Amount (%w/w)
Palmitic acid	11.43±0.180
Stearic acid	9.04±0.033
Oleic acid	40.11±0.202
Linoleic acid	37.23±0.182

Table II: Characteristics of Crude Jco

Density at 150 C, kg/m3	Free fatty acid (as oleic acid), (% w/w)	Kinematic viscosity (400C, mm2/s)	Water content, (% w/w)
917.4 ±0.258	2.83 ±0.013	33.15 ±0.201	0.4901 ±0.004

A. Effect of variation of Enzyme w.r.t Molar Ratio of Methanol to JCO

Molar ratio of alcohol to oil is an important factor for maximum production of BD. Fig. 1 shows that nature of enzyme has a significant effect on BD production using different ratios of methanol to JCO. Here, reaction conditions were maintained at temperature 60^0 C using 6% (w/w) immobilized enzymes for 8 hours at 600 rpm using different molar ratios of alcohol to oil. Stepwise addition of alcohol was maintained to avoid deactivation of enzyme.

It has been observed from Fig. 1 that in all ratios of alcohol and JCO, enzyme NS 435 contributed the higher percentage of BD and optimum production of BD was obtained using 6:1 molar ratio of methanol and JCO. Using other enzymes like TL IM and RM IM, enhancement the conversion of BD is insignificant though NS40013 is somewhat more efficient than these two enzymes. Highest efficiency of NS 435 may be due to the fact that the active sites of enzyme Novozyme 435 is more compatible with the substrates like methanol and JCO than other immobilized enzymes. As active sites are the only reactive area of enzyme and these

are more structural friendly and saturated with substrates with a definite molar ratio (6:1), so further change in amount and nature of enzyme did not significantly contribute to the enhancement of product percentage.

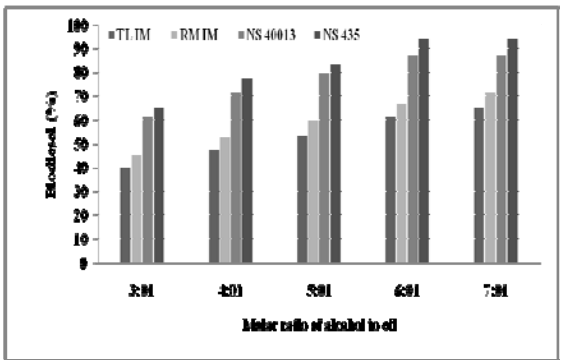

Fig. 1 Effect of Variation of Enzyme W.R.T Molar Ratio of Methanol to JCO For BD Production

B. Effect of Variation of Enzyme w.r.t Reaction Temperature

Activity and efficacy of enzyme depends on reaction temperature which is one of the important parameters for maximum conversion of product because activation energy plays a significant role for a reaction. Here experiments were performed over the temperature range of 30 to 70^0 C at 6:1 methanol: JCO molar ratio by using different enzymes (6% w/w) for 8 hrs (Fig. 2).

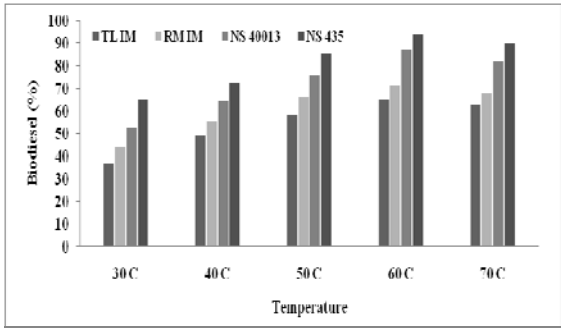

Fig. 2 Effect of Variation of Enzyme W.R.T Temperature for BD Production

It is revealed from Fig. 2 that increasing temperature increases the conversion of BD production for all enzymes but a maximum production was obtained when the reaction temperature was maintained at 60^0 C using NS 435 immobilized enzyme. After that, increasing temperature decreases conversion of BD for all the cases. This is due to the inactivation of enzyme at higher temperature as each enzyme is active up to a certain temperature beyond which it becomes deactivated. Higher temperature also volatilize methanol which hampers the proper ratio of methanol: JCO.

C. Effect of Variation of Enzyme w.r.t Mixing Intensity

Stirring of the reaction mixture helps to enhance the contact between substrates as well as with enzymes which has an important role to get optimum conversion of product. Proper mixing by optimum stirring also helps to transfer the reactants from the bulk of the liquid to the external surface of enzyme and finally in to the pores of active sites. This ultimately results accomplishment of complete conversion of product.

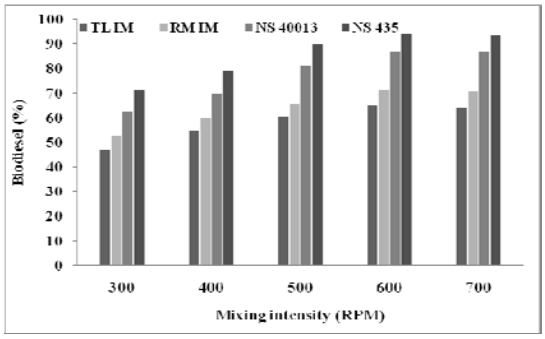

Fig. 3 Effect of Variation of Enzyme W.R.T Mixing Intensity for BD Production

From Fig. 3**,** we can observe that the enzyme NS 435 contributed maximum conversion of BD among the four enzymes at any mixing intensity used for the present investigation and 600 rpm was the optimum mixing intensity for maximum conversion. Beyond that, no further enhancement of conversion was observed by increasing agitation. So, NS 435 is the suitable enzyme for BD production from JCO at 600 rpm maintaining other proper reaction conditions.

D. Effect of Variation of Enzyme w.r.t Reaction Time

Duration of transesterification reaction is one of the determining factors for optimum conversion of BD. It has been observed from Fig. 4 that for any duration of time, enzyme NS 435 contributed best conversion and it was achieved after 8 hrs of reaction between methanol and JCO (6:1) at temperature 60^0C with a mixing intensity of 600 rpm. There is no significant change of production by increasing time of reaction beyond 8 hrs.

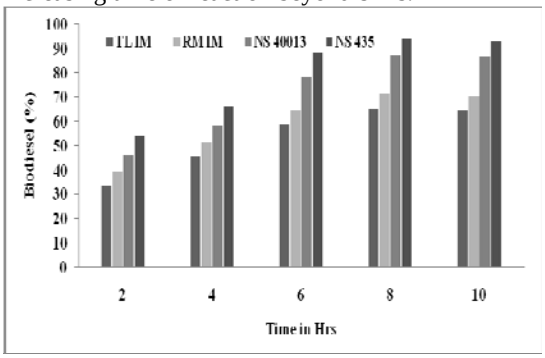

Fig. 4 Effect of Variation of Enzyme W.R.T Reaction Time for BD Production

E. Comparative Study of BD Production

It has been observed from the study that maintaining the reaction conditions of 6:1 molar ratio of alcohol to JCO at temperature 60^0C with 600 rpm of mixing intensity for 8hrs, the immobilized enzyme NS 435 contributed maximum production of BD (94%) than any other enzymes used in this experiment which is revealed in Fig. 5 below.

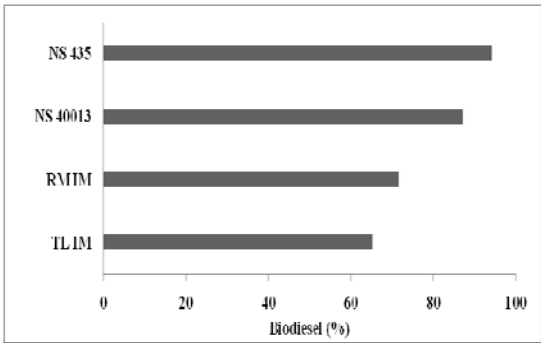

Fig. 5 Comparative BD Production Using Different Enzymes

F. Biodiesel Characterization

Properties of BD is important for applying in diesel engines because the performance and emission characteristics of the diesel engine can only be obtained by using good quality of BD. Characteristics of BD produced in our experiment using enzyme NS 435 has been analyzed and compared with the biodiesel standards. TABLE 2 shows that the characteristics of BD produced in our study are quite comparable with the BD standards and diesel fuel. It has been observed from TABLE 2 that though calorific value of Jatropha BD is somewhat less than conventional diesel fuel but with regard to other characteristics Jatropha BD is analogous to diesel fuel.

Table III : Characteriscics of BD

Properties	Jatropha BD	BD standard	Diesel fuel	Test method
Specific gravity (150C)	0.87 ±0.001	0.86 to 0.90	0.82-0.95	AST M D 6751-02
Kinematic Viscosity (mm2/s)	4.94 ±0.007	1.96 to 6.0	1.3-4.1	AST MD-445
Density at 150C, (kg/m3)	879.1 ±0.312	865-900	820-860	AST MD-4052-96
Calorific value (MJ/kg)	39.27 ±0.212	33 to 40	45	AST M-6751
Cloud point (0C)	5.1 ±0.003	5	-----	AST M D-2500
Flash point (0C)	±0.232			MD-93
Cetane number	54 ±0.169	40 min	50	AST MD-6751
Acid number	0.29	0.5 max	------	AST MD-664-01

IV. CONCLUSIONS

Effect of nonspecific immobilized enzymes namely NS40013, NS 435 (Candida Antarctica), TLIM (Thermomyces lanuginosus) and RM IM (Rhizomucor miehei) for the production of biodiesel from Jatropha Curcas oil and methanol in solvent hexane has been studied in the present research investigation. It has been observed from the experimental findings that immobilized enzyme NS 435 is the most effective enzyme for the production of biodiesel from JCO and methanol maintaining temperature at 60 C and mixing intensity 600 rpm using 6% (w/w) concentration of enzyme Novozyme 435 for 8 hrs. Stepwise addition of methanol helps to avoid inactivation of enzyme.

Characteristics of biodiesel produced are also comparable with standard biodiesel and diesel fuel. So biodiesel produced in this way can be effectively utilized in commercial scale also. In conclusion, this process of biodiesel production utilizing NS 435 as catalyst may be an alternative solution for the scarcity of conventional fossil fuels in future.

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