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Production of biodiesel from Jatropha Curcas oil with recycling of enzyme

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Abstract— Alternative energy sources are supposed to be the most challenging job of today's world and biodiesel attracted considerable attention as one of the significant alternative energy sources in the future world as an environment friendly, renewable and non toxic fuel. Different edible and non edible vegetable oils are used for biodiesel preparation but the most suitable and cost effective source is non edible vegetable oil of the fruits of Jatropha Curcas plant. Productivity of biodiesel from crude Jatropha Curcas oil through transesterification reaction with methanol depends on many reaction parameters like molar ratio of oil to methanol, reaction temperature, catalyst concentration and stirrer speed or mixing effect of the reaction system. Enzyme as catalyst also play a significant role in transesterification reaction due to eco-friendly and selective nature, purity of the product, minimum purification stage, low temperature requirement and reuse of catalyst. In the present research investigation, effects of reaction parameters on the productivity of biodiesel from crude Jatropha Curcas oil with methanol using non-specific Novozyme 435 (Candida antarctica) have been studied. Moreover, recycling of enzyme is done in our experiment which reduces the cost of the transesterification process for the production of alternative energy sources. The physical properties of Jatropha Curcas methyl ester and diesel fuel have been compared and it shows significant results. So recycling of enzyme for the production of biodiesel from Jatropha Curcas oil can be utilized to mitigate scarcity of non renewable fuel in the future world.

Keywords— Biodiesel, Jatropha Curcas oil, Enzymatic transesterification, Recycling of enzyme.

I. INTRODUCTION

The present depleting condition of conventional fossil based fuels, recent increase in petroleum prices and need for saving our environment by reducing obnoxious gases from conventional fuels have promoted great interest in identifying alternative renewable source of fuel. Among the alternative energy sources, biodiesel (BD) may be a promising option which can be utilized as an environmental friendly, renewable, non toxic and biodegradable fuel. With regard to energy content, BD is the only alternative fuel whose energy content is similar to conventional fuel with no need of altering existing energies [1] and it runs in any conventional, unmodified diesel engine. BD can be used alone or mixed with petroleum based diesel in any proportions. Another major importance of using BD is that its combustion products have reduced levels of particulates, sulphur oxides, carbon oxides, nitrogen oxides and therefore significantly reduces environmental pollution [2, 3]. BD has also shown its ability to meet the energy demand of the world in the transportation, agriculture, commercial and industrial sectors of the economy [4, 5, 6, 7].

BD can be produced from different vegetable oils like Soybean, Sunflower, Rapeseed, Rice bran, Palm, Cottonseed, Jatropha Curcas, Pongamia, Mahua etc., animal fats like Beef, Tallow, Lard etc. and waste oils but the most promising source is the non edible oil of Jatropha Curcas plant. Main advantage of this plant is that it can be grown 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. Use of Jatropha Curcas Oil (JCO) for BD production is significant due to its higher oil content (35-40%) and non edible nature which does not hamper food supply [8, 9].

BD from JCO using chemical catalyst has been studied by several researchers. Hawash et al. [10] transesterified JCO to BD using calcium oxide as a solid base catalyst under supercritical conditions. Berchmans and Hirata [11] investigated production of BD from crude JCO using two stage transesterification process using acid catalyst in the first stage and base catalyst in the second stage. Raja et al. [12] showed that alkaline catalyzed transesterification of JCO with methanol produces higher percentage of BD under definite parametric conditions. But there are several drawbacks associated with the chemical catalyst. It is energy intensive, unwanted side products are generated from the use of alkali catalyst, alkaline wastewater generated from this process requires treatment and the level of free fatty acids and water greatly interfere with the reaction. The risk of free fatty acids or water contamination results in soap formation that makes the separation process more difficult [13, 14]. Hence better and alternative method of production is the use of enzyme as catalyst due to its reusability, specificity, thermo stability, mild reaction conditions in terms of low temperature and its environmental friendliness [4, 15].

Production of BD from JCO using enzyme as catalyst has also been investigated by several researchers. Kumari et al. [16] used immobilized lipase from *Enterobactor aerogenes* using JCO in t-butanol solvent for BD production and they obtained 94% yield. Another study of Aransiola [17] presented the ethanolysis of both crude and pretreated JCO using immobilized lipase enzyme from *Pseudomonas cepacia* and maximum of 72.1wt% fatty acid ethyl ester was obtained at optimized conditions. Veny et al. [18] produced BD from JCO through enzymatic synthesis in a re circulated packed bed reactor and they obtained highest methyl ester yield of 54% from lipase dosage of 10%. Preparation of BD from JCO has

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also been investigated by Zarie et al. [19], Sash and Gupta [20], Shah et al. [21], Wang et al. [22] and they showed that BD can be produced from JCO using different lipases maintaining optimum reaction conditions. So several studies for environment friendly BD production using enzyme as catalyst have been done by different researchers but no studies have been made regarding recycling of enzymatic production of BD from crude JCO using Novozyme 435 (*Candida antarctica*) lipase.

In this context, present research analysis investigated the production of BD from crude JCO using methanol as substrate in hexane media with the help of non-specific enzyme Novozyme 435 (*Candida antarctica*). Here, the effects of different reaction parameters like alcohol to oil molar ratio, reaction temperature, enzyme concentration and stirring speed have been studied on the productivity of BD. Due to higher cost of enzyme, transesterification reaction using enzyme as catalyst is costly compared to chemical catalytic reaction. But the transesterification process cost for BD preparation can be minimized by reusing of enzyme several times. With this view, recycling of enzyme was done in our experiment several times for the production of alternative energy sources. The physical properties of BD produced from our experiment were compared with diesel fuel and the results showed close proximity of properties.

II. Materials

The crude JCO used in this study was provided by M/s. Arora Oils Ltd., Burdwan, West Bengal, India. The enzyme used in the following studies was Novozyme 435 (*Candida antarctica*) immobilized lipase which was a kind gift of Novozyme South Asia Pvt. Ltd. Bangalore, India. The chemicals used in this work such as methanol (99.8% pure) and hexane were purchased from S.D. Fine Chemicals (Mumbai, India). Except otherwise specified all other chemicals used were A.R. Grade.

III. Experimental Methods

For transesterification reaction for BD production, initially 250 ml of crude JCO was taken in an Erlenmeyer flask and heated up to 80^0C to drive off moisture by continuous stirring for about 1 h. After that, transesterification reaction was carried out by adding methanol in an appropriate proportion using solvent hexane fitted with a water condenser and stirred by a magnetic stirrer at different temperatures for 4 hours maintaining other reaction conditions. To the reaction mixture, immobilized enzyme Novozyme 435 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, immediately immersed in boiling water for at least 5 minutes to denature the enzyme and stop the reaction. After that the samples were centrifuged for 15 min at 20^0C 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 and their amounts were determined by thin layer chromatographic method. The enzyme was washed with hexane, dried and reused for the next experiment. Biodiesel characterization was done according to the American Standard Testing Method. Values are reported as mean ± s.d., where n=3 (n=no of observations).

IV. Results and Discussion

Initial characteristics of crude JCO are vital for successful conversion of BD production enzymatically. Table 1 shows the characteristics of crude JCO which was used for the preparation of BD using non specific enzyme Novozyme 435 (*Candida antarctica*). It has been observed from Table 1 that crude JCO contains mainly palmitic, stearic, oleic and linoleic acid with maximum percentage of oleic acid. Effect of reaction parameters for the optimum conversion of BD from crude JCO have been analyzed in the following.

A. Effect of alcohol to oil molar ratio

Effect of alcohol to oil molar ratio is important for optimum conversion of BD. Stepwise addition of alcohol is also significant in enzyme catalyzed transesterification reaction. Our investigation of effect of alcohol-oil molar ratio was done in hexane medium without which the enzyme would be deactivated. Therefore, the transesterification reaction between crude JCO and stepwise addition of methanol in hexane solvent was observed for identifying optimum concentration of methanol to oil molar ratio. Reaction conditions were maintained at temperature 50^0C using 4% (w/w) immobilized enzyme for 4 hours using different molar ratios as shown in Fig. 1. It has been observed from the Figure that maximum BD was obtained with a 4:1 molar ratio of methanol to JCO. Increasing concentration of methanol (5:1 or 6:1) though initially produced higher amount of BD but finally rate of reaction was decreased as evidenced from the Fig. 1. This may be due to the fact that the amount of substrates

Table 1. Characteristics Of Crude Jco

Characteristics	**Value**
Fatty acid composition (%):	
i) Palmitic acid	14.62±0.180
ii) Stearic acid	5.07±0.037
iii) Oleic acid	42.13±0.216
iv) Linoleic acid	35.56±0.194
Calorific value (MJkg-1)	38.93±01.191
Density at 15^0C, kg/m^3	917.2±0.361
Flash point (˚C)	231±3.71
Free fatty acid (as oleic acid), (%)	7.77±0.012
Kinematic viscosity at 40^0C, mm^2/s	34.71±0.191
Triglycerides (%)	89.45±2.03
Water content, (%)	0.9213±0.002

maintaining a ratio of 4:1 (methanol: JCO) actually occupies all the active sites of the enzyme. As active sites are

the only reactive area of enzyme and these are occupied fully with this definite ratio, so further increase in amount of methanol did not enhance product percentage.

B. Effect of reaction temperature

Activation energy plays a vital role for maximum conversion of product formation in any reaction and it has been mainly controlled by reaction temperature. In our study, for identifying suitable temperature, experiments were performed over the temperature range of 30 to 60^0C at 4:1 methanol: JCO molar ratio with 4% (w/w) enzyme for 4 hrs (Fig. 2).

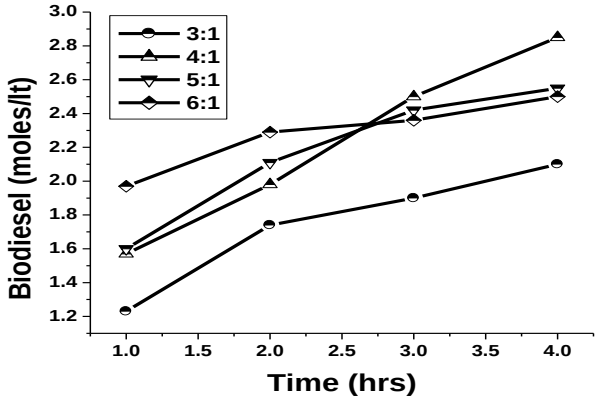

Fig.1Effect of molar ratio of methanol to JCO on biodiesel production

It is revealed from the figure that increasing temperature increases the activation energy of the reaction up to certain limit and the maximum conversion of BD production was obtained when the reaction temperature was maintained at 50^0C. Moreover, at 50^0C, there was a smooth increasing of rate of reaction as evidenced from Fig. 2. After that increasing temperature did not enhance the conversion of BD rather it was decreased. This is due to the fact that enzymes are active up to a certain temperature and beyond that particular temperature it becomes deactivated. In our study, immobilized enzyme Novozyme 435 becomes deactivated near 60^0C temperature. So, initially though higher temperature enhances the transesterification reaction but after 2 hrs, increasing rate of reaction was very slow and finally, the production was hampered as evidenced from Fig.2. Higher temperature may also volatilize methanol which hampers the proper ratio of methanol: JCO.

C. Effect of enzyme concentration

Enzyme catalyst plays a big role for smooth conversion of product without conducting side reactions as well as easy separation of desired product. Effect of concentration of enzyme for BD production from transesterification of JCO and methanol in solvent hexane was investigated here. For this purpose, the enzyme concentration was varied from 3-5% at 4:1 methanol: JCO molar ratio maintaining a temperature of 50^0C for 4 hrs. Fig. 3 shows that increasing concentration of enzyme enhances BD production and we get maximum BD at 4% enzyme concentration. It has been observed from the figure that increasing enzyme concentration from 4 to 5% though initially enhances the rate of BD production but later it could not significantly increase the biodiesel production. This may be due to the fact that higher amount of enzyme contributes higher amount of active sites initially but later due to enzyme aggregation all active sites cannot be exposed to the substrates for further enhancement of reaction.

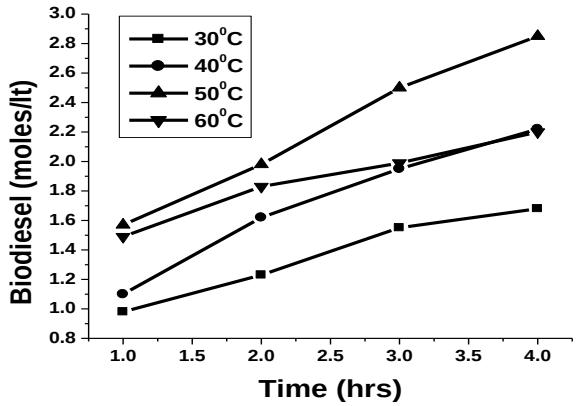

Fig.2 Effect of temperature on biodiesel production

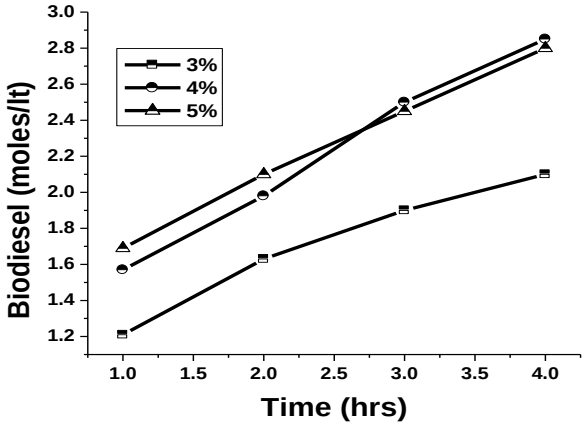

Fig.3. Effect of enzyme concentration (percentage) on biodiesel production

D.Effect of mixing intensity (Stirrer speed)

Proper mixing intensity in a reaction system can only be obtained by suitable stirring and it has an important role to get optimum conversion of product. Proper mixing by optimum stirring actually helps to transfer the reactants from the bulk liquid to the external surface of enzyme and finally in to the pores of active sites. By this way, the proper contact between active sites of enzyme and substrates is necessary for complete conversion of product and it can only be obtained by controlling effective stirring. In this study, the effect of speed of agitation was investigated in the range of 200 to 800 rpm for 4 h (Fig. 4). It was found that the percentage conversion was increased with increasing stirring speed and reached maximum

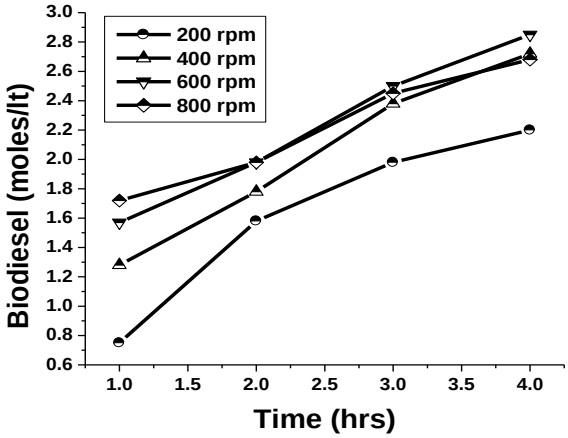

Fig.4. Effect of stirrer speed on biodiesel production

at 600 rpm maintaining other optimum parameters. Beyond that, no further enhancement of conversion was observed with increasing agitation. This may due to the fact that increasing mixing intensity hampers the proper contact of reactants with enzymes and / or decreases the time of contact between reactants and enzyme. So conversion decreases.

E. Recycling of enzyme

Process cost is one of the determining factors for commercialization of any product. As enzymes are costly, so cost of enzymatic transesterification process can only be reduced by recycling of enzyme several times. In our study, recycling of enzyme has been successfully done twenty times after separation of enzyme from each batch. It has been observed from Fig. 5 that after reusing of 20 times of Novozyme 435, the percent conversion of BD production is more than 85% and the enzyme is still active for further transesterification reaction. In the very first batch, BD conversion was 95.63% but the conversion was 86.33% in the 20^{th} batch. So, recycling of enzyme has been successfully done in our experiment and recycling procedure contributes good conversion. It has been observed from Fig. 5 that after each recycle, conversion of BD production decreases to some extent. This may be due to the fact that separation of enzyme after each batch probably includes very little handling loss. It may also be the reason that the moisture content of enzyme varies after each batch which plays significant role in enzyme efficiency.

F. Biodiesel characterization

The good quality of BD is very important for the performance and emission characteristics of the diesel engine. So BD from JCO in our study has been critically analyzed and compared with the biodiesel standards. Table 2 shows the methyl ester composition Jatropha biodiesel.

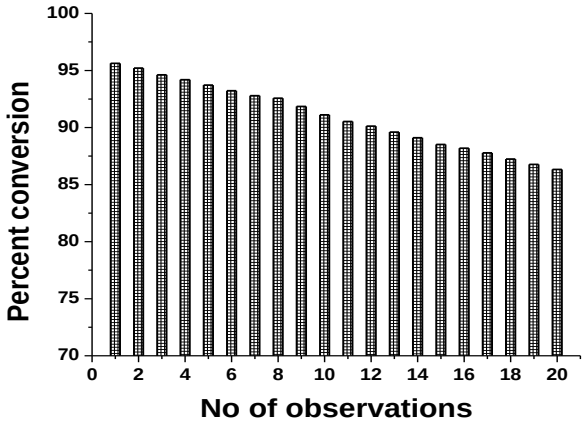

Fig. 5 Recycling of enzyme for biodiesel production

Table 2 Methyl Ester Composition Of Jatropha Biodiesel

Methyl ester (% w/w)	**Jatropha biodiesel**
Methyl myristate	0.1±0.001
Methyl palmitate	13.93±0.182
Methyl sterate	4.87±0.039
Methyl oleate	41.45±0.203
Methyl linoleate	35.11±0.136

Table 3 shows that the characteristics of BD produced in our study are quite comparable with the BD standards and we got good results. It has been observed from Table 3 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 3 Jatropha Biodiesel Characterization And Biodiesel Standards

Properties	Jatropha biodiesel	Biodiesel standard	Diesel fuel	Test method
Acid number	0.23	0.5 max	-------	ASTMD-664-01
Kinematic Viscosity (mm^2/s)	5.7	1.9 to 6.0	1.3-4.1	ASTMD-445
Free glycerol, (% w/w)	0	0.2 max	------	------
Density at 15^0C, (kg/m^3)	880.1	865-900		ASTMD-4052-96
Flash point (^0C)	132	>120	60-80	ASTMD-93
Cetane number	52	40 min	50	ASTMD6751
Pour point (^0C)	4	-15 to 10	18	ASTMD-93
Specific gravity (15^0C)	0.87	0.86 to 0.90	0.82-0.95	-
Refractive index at 40 ^0C	1.43	1.34	1.45	-
Calorific value (MJ/kg)	38.9	33 to 40	45	ASTM-6751
Cloud point (^0C)	5.5	5	-	ASTMD-2500

V. CONCLUSION

Enzymatic production of biodiesel from Jatropha Curcas oil and methanol in solvent hexane has been studied in the present research investigation using non specific immobilized enzyme Novozyme 435 (*Candida Antarctica*) as catalyst. The effects of molar ratio of methanol to Jatropha Curcas oil, reaction temperature, enzyme concentration and stirrer speed have been analyzed for the optimum conversion of biodiesel. Studies show that at the reaction conditions of 4:1 molar ratio of methanol to Jatropha Curcas oil maintaining temperature at 50^0C and 600 rpm stirrer speed using 4% (w/w) concentration of Novozyme 435 for 4 hrs, maximum conversion (95.63%) of biodiesel was obtained. Cost is one of the determining factors for biodiesel production using enzyme catalyst but recycling of immobilize enzyme is helpful for reducing cost. Here, recycling of enzyme is done twenty times and the recycled enzyme gives significant conversion. So recycling or reusing of enzyme for biodiesel production is a cost effective approach. Characteristics of Jatropha biodiesel also match well with the biodiesel standard and diesel fuel indicating that lipase Novozyme 435 can be effectively utilized for biodiesel production from Jatropha Curcas oil as well as from other non edible oils. The excellent performance of the enzyme indicates that biodiesel from Jatropha Curcas oil can be produced in commercial scale with minimum process hazards. The results may be encouraging for the future researchers involved in biodiesel production enzymatically from other vegetable oils also. This process of production of biodiesel and recycling of enzyme may be an alternative solution for the present depleting condition of conventional fossil fuels for the future generations.

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