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Biodiesel from Jatropha Curcas oil: A Comparative Study between Chemical and Bio catalytic Transesterification

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Abstract

Alternative energy sources are supposed to be the most challenging job of today's world. Among the alternative energy sources, biodiesel attracts considerable attention as it is renewable, non toxic, biodegradable and environmental friendly. Biodiesel is produced from different vegetable oils by transesterification method with alcohol in the presence of catalyst but Jatropha Curcas oil is supposed to be the most promising due to its higher oil content, non edible nature and possible cultivation of Jatropha Curcas plant in any land including barren land even in adverse environment. In the present investigation, a comparative study has been made between chemical (base) catalytic and enzymatic method using non-specific immobilized enzyme Novozyme 435 (Candida antarctica) for the preparation of biodiesel from Jatropha Curcas oil. Studies show 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. Moreover, recycling of enzyme is done in our experiment which reduces the cost of the transesterification process for the production of alternative energy sources. Our process may be implemented in industrial scale with an alternative solution of scarcity of energy resources in the near future.

Keywords: Biodiesel, Novozyme 435, Jatropha Curcas oil, alternative energy, transesterification.

Introduction

The declining nature of non renewable energy sources and its environmental tribulations in the present world demands a lot of attention to produce alternative, renewable energy sources for the future generations. Kyoto Protocol and Johannesburg Declaration also recommended reduced gas emissions and the development of renewable energy resources. Among the alternative energy sources, biodiesel (BD) has gained considerable attention which can be utilized as an environmental friendly, non toxic and biodegradable fuel. Studies show that the combustion products of biodiesel have reduced the levels of particulates, sulphur oxides, carbon oxides, nitrogen oxides and so significantly reduce environmental pollution[1,2]. Another major advantage of BD as an alternative fuel is that its energy content is similar to the conventional fuel and there is no need of altering existing energies[3].

BD can be produced from different vegetable oils or animal fats such as Rapeseed, Soybean, Palm, Sunflower, Cottonseed, Jatropha Curcas, Pongamia, Mahua, Beef, Tallow, Lard etc. Among all the sources, Jatropha Curcas oil (JCO) has been recognized as the most promising alternative in the present situation due to its higher oil content and non edible nature[4,5]. Jatropha Curcas plant can be cultivated on land where no other crops can grow and it can also be produced locally. It can be grown on barren land under simple conditions and can be cultivated on waste land also[6]. Due to its non edible nature, use of JCO for biodiesel production does not hamper the edible oils. So it has drawn attention across the developing countries, especially in India, as a feed stock for BD production[7] and has been chosen for the present investigation.

Production of BD from JCO is done by transesterification or alcoholysis reaction with alcohol in the presence of catalyst. A number of processes have been developed for BD production using either chemical catalyst or biological catalyst like immobilized or free enzyme[8-11]. It has been observed that alkali catalyzed transesterification reaction is much faster than acid catalyzed transesterification and is most often used commercially[12,13]. Sodium hydroxide, potassium hydroxide and carbonates are mostly used as alkali catalyst. Sulphuric acid and hydrochloric acids are the usual acid catalysts. Nakpong and Wootthikanokkhan[14] studied the production of BD from crude JCO using alkali catalyst and optimized the process. BD can be produced by using several chemical catalysts but there are several disadvantages in this catalytic process. The chemical catalytic process is energy intensive, recovery of by product glycerol and spent catalyst is difficult, alkaline wastewater generated from this process requires chemical treatment for environmental purpose and the presence of free fatty acids and water greatly interfere with the reaction. The free fatty acids or water contamination ultimately results in soap formation which makes the separation process more complicated[15,16]. On the other hand, bio catalytic method has several advantages over chemical catalytic method. Its reusability, specificity, ability to accept new substrates, thermo stability, mild reaction conditions, easy recovery of glycerol, complete conversion of

FFAs, no requirement of waste water treatment and environmental friendliness encourages enzymatic method of production of BD[17,18,19]. Enzymatic production of BD from different vegetable oils has been studied by several researchers. 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.[1] for determining the optimal conditions for BD production. Kumari et al.[20] used immobilized lipase from *Enterobactor aerogenes* using JCO in t-butanol solvent for BD production and they obtained 94% yield. Another study of Aransiola[21] 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.[22] 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%.

Production of BD from different vegetable oils using chemical or biocatalyst has been analyzed by different researchers but comparative study based on reaction parameters between chemical and enzyme catalyzed production of BD from JCO has not yet been made so far. Based on this aspect, in the present research investigation, a comparative study has been made by using sodium hydroxide as base catalyst and non specific enzyme Novozyme 435 (*Candida antarctica*) as enzyme catalyst for the preparation of BD from JCO. Studies show that enzymatic method is more effective than base catalytic method with regard to productivity, eco-friendliness, selectivity, purity of the product, minimum purification stage, low temperature requirement and reuse of catalyst. Recycling of enzyme is done in our experiment which reduces the cost of the transesterification process for the production of alternative energy sources. Comparative analysis indicated that enzymatic process may be implemented in industrial scale with an alternative solution of scarcity of energy resources in the near future.

Material and Methods

Chemicals: The JCO used in this study was provided by M/s Arora Oils Ltd., Burdwan, West Bengal, India. The enzyme used in the following study was non specific 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.

Transesterification method: For the transesterification reaction of BD production, 250 ml of 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, methanol was added maintaining a definite ratio. Catalyst was added in the reaction mixture in an appropriate proportion fitted with a water condenser and stirred by a magnetic stirrer at a suitable speed. Optimum reaction conditions were maintained for 2 hrs for the comparative analysis in the present investigation. Hexane was used as a solvent in enzyme catalyzed transesterification reaction. At suitable intervals, definite amount of samples were withdrawn and analyzed in our study.

After completion of alkali catalyzed transesterification reaction, the mixture was allowed to settle under gravity for 24hrs in a separating funnel. The upper layer consisted of BD, alcohol and some soap while the lower layer was a mixture glycerol, excess alcohol, catalyst, impurities. The upper layer was separated and washed with hot water several times to remove alcohol, soap and some trace catalyst. The separated BD was ready for characterization.

For enzymatic transesterification process, after completion of reaction, the mixture was centrifuged for 30 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 and reused for the next experiment.

Results and Discussion

Table 1 shows the characteristics of JCO which was used for the preparation of BD for both the cases. Its properties were established to ascertain the suitability for BD production using alkali and enzyme catalyst and to compare the parameters of production process.

Table-1
Analytical characteristics of Jatropha Curcas oil

Fatty acid (% w/w)	Density at 15^0C, kg/m^3	Free fatty acid (as oleic acid), (% w/w)	Kinematic viscosity (40^0C, mm^2/s)	Water content, (% w/w)
Palmitic acid : 12.22±0.180 Stearic acid : 6.97±0.037 Oleic acid : 40.43±0.216 Linoleic acid : 37.78±0.194	919.7±0.361	2.67±0.012	33.54±0.191	0.4457±0.002

Values are reported as mean ± s.d., where n=3 (n=no of observations).

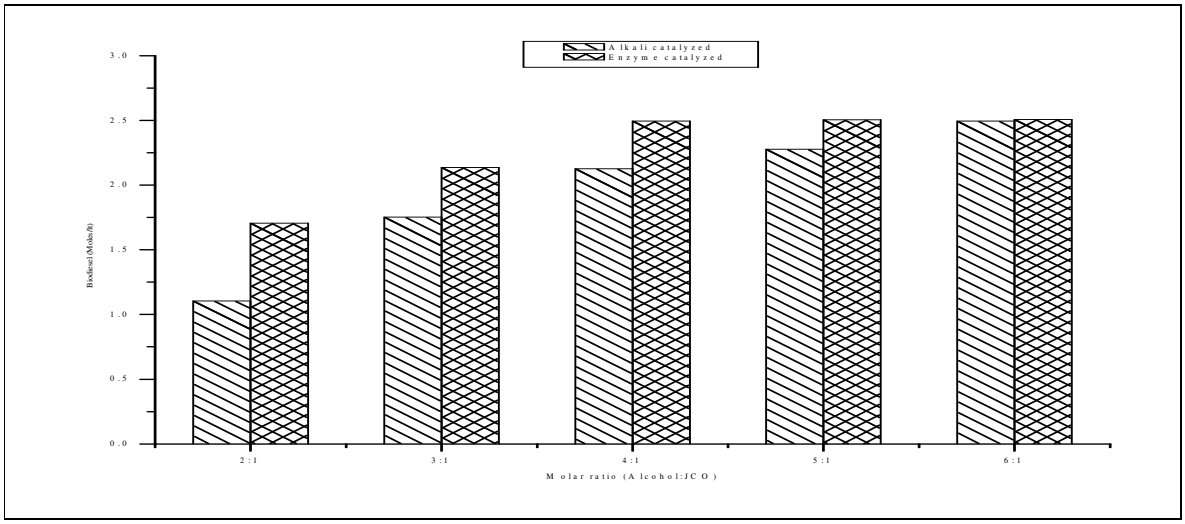

Figure-1
Molar ratio analysis for alkali and enzyme catalyzed transesterification reaction of BD production

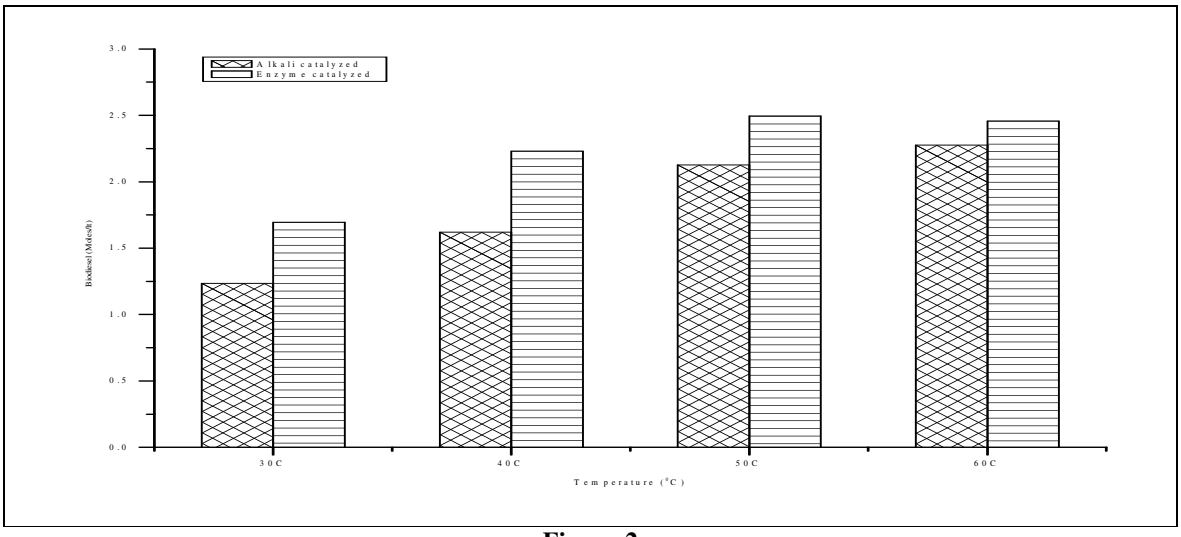

Figure-2
Temperature analysis for alkali and enzyme catalyzed transesterification reaction of BD production

Comparative analysis with respect to molar ratio: For the comparative analysis with regard to alcohol to oil molar ratio in alkali and enzyme catalyzed transesterification reaction, reaction conditions maintained were temperature 50^0C with a stirrer speed of 600 rpm using 2% catalyst for 2 hrs. It has been observed from figure 1 that for enzyme catalysis, alcohol to oil molar ratio 4:1 contributed 83.16% conversion after 2 hrs but maintaining the same molar ratio, alkali catalyzed reaction produced 70.86% BD. It has also been obtained from figure 1 that nearly 83% conversion has been achieved for alkali catalyzed reaction only when a molar ratio of 6:1 (alcohol: oil) was maintained during the same time interval. So, enzyme catalyzed transesterification reaction for BD production is more efficient than alkali catalyzed reaction with regard to alcohol to oil molar ratio.

Comparative analysis with respect to temperature: For the comparative analysis of transesterification reaction for BD production with regard to temperature, reactions conditions were alcohol to oil molar ratio 4:1 maintaining a stirrer speed of 600 rpm using 2% catalyst for 2 hrs. It revealed from figure 2 that after 2 hrs, enzyme catalyzed transesterification reaction contributed a maximum conversion at 50^0C (83.16%) while alkali catalyzed reaction resulted a maximum conversion at 60^0C (70.86%) for the same reaction conditions though it was less than enzyme catalysis. So, more energy was needed for alkali catalyzed transesterification reaction than enzyme catalyzed reaction for the production of BD. Therefore, for BD production from JCO, enzyme catalyzed reaction is more suitable and energy saving than base catalyzed reaction.

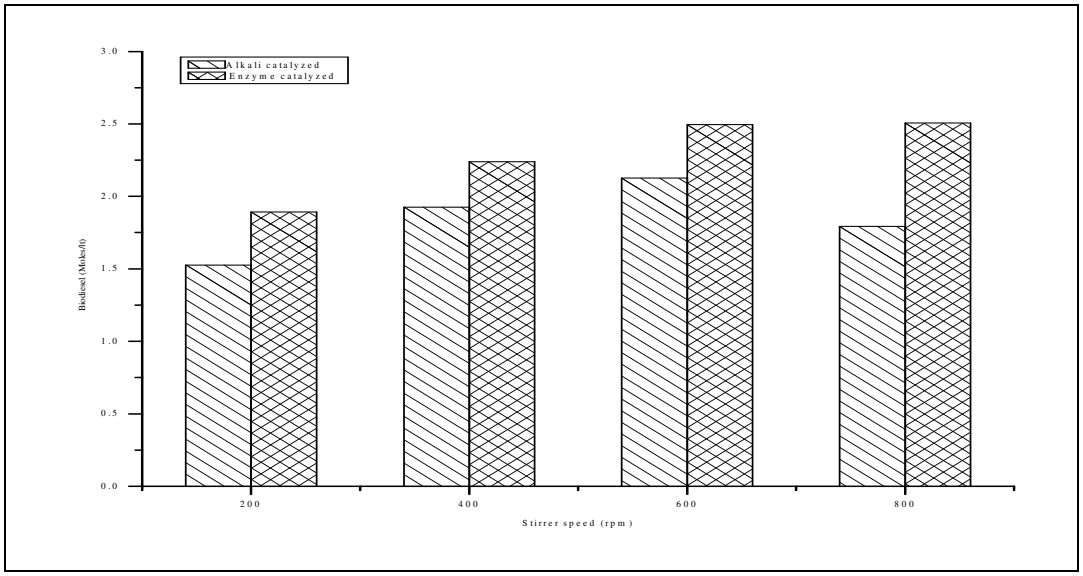

Figure-3
Stirrer speed analysis for alkali and enzyme catalyzed transesterification reaction of BD production

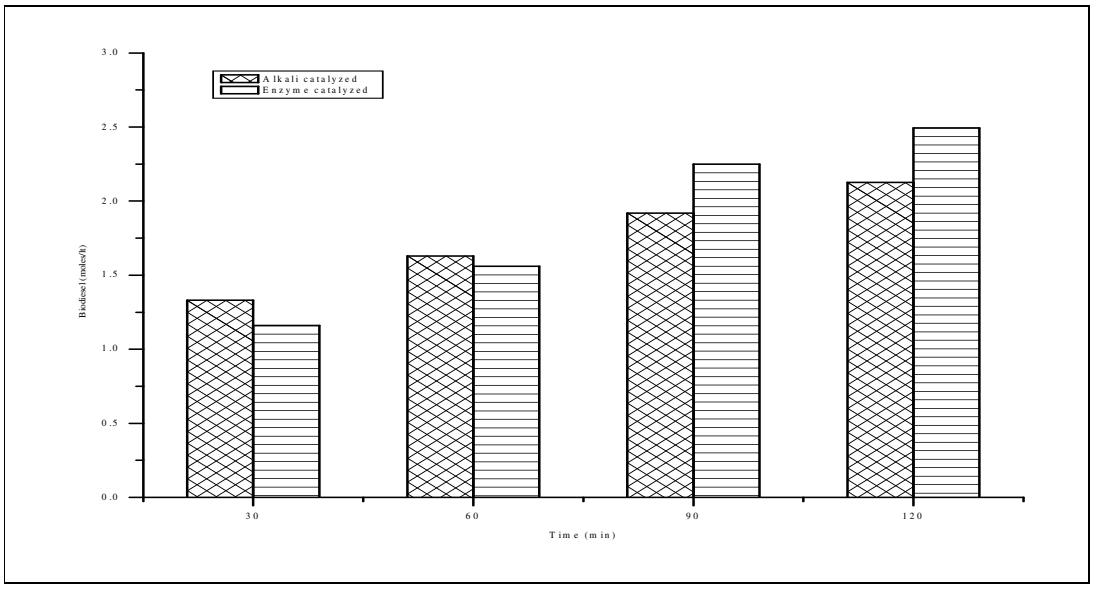

Figure-4
Reaction time analysis for alkali and enzyme catalyzed transesterification reaction of BD production

Comparative analysis with respect to stirrer speed: Stirrer speed or mixing intensity has a vital role for proper conversion of product as it helps to collide between the reactants in a suitable way. In case of enzyme catalyzed reaction, proper contact between active sites of enzyme and reactants occur only by applying suitable stirrer speed. Figure 3 shows a comparative stirrer speed analysis of enzyme catalyzed and alkali catalyzed transesterification reaction for BD production at alcohol to oil molar ratio 4:1 with a temperature of 50^0C maintaining a catalyst concentration of 2%. It has been observed from figure 3 that increasing stirrer speed from 200 to 800 rpm increases the BD production up to a certain limit for both the enzyme and alkali catalyzed reactions. Maximum conversion of BD was obtained at a stirrer speed of 600 rpm for both the cases but the production of BD was low in case of alkali catalyzed reaction. So, enzymatic approach was more effective than alkali catalyzed approach at a definite mixing intensity as evidenced from figure 3. Enhancement of stirrer speed did not increase the production for alkali catalyzed reaction beyond 600 rpm rather conversion was decreased probably due to the formation of emulsion due to presence of soap in the reaction mixture.

Comparative analysis with respect to reaction time: Time study analysis in the present investigation has been done for BD production from JCO maintaining 4:1 alcohol to oil molar ratio with a temperature of 50^0C using 2% catalyst at 600 rpm. It has been observed from figure 4 that though initially (up to 1 hr) conversion of BD was high in case of alkali catalyzed reaction but finally, enzyme catalyzed reaction produced more BD than alkali catalyzed reaction. This may be due to the fact that initial contact between the active sites of enzyme and reactants take some time, so the conversion was low. But after that, conversion of BD increases at a definite rate and contributes maximum yield.

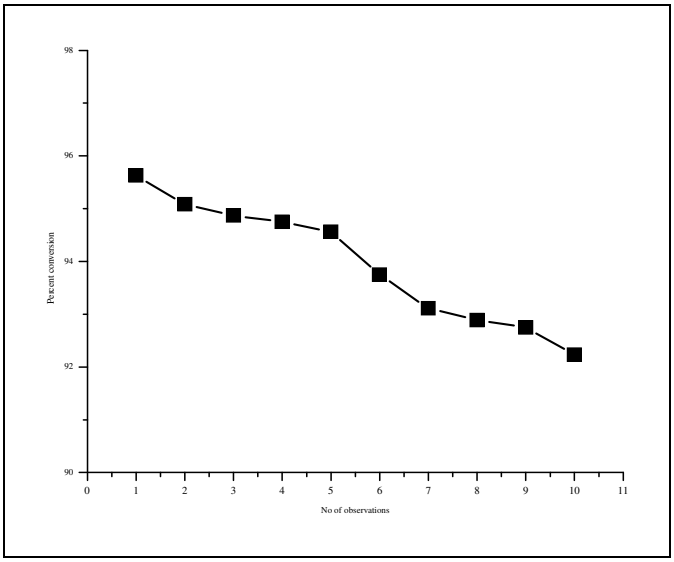

Figure-5
Percent conversion of biodiesel by recycling of enzyme Novozyme 435 (Alcohol to oil molar ratio: 4:1, temperature: 50^0C, time: 4 hrs, stirring: 600rpm)

Recycling of immobilized enzyme Novozyme 435 (*Candida antarctica*): Cost of the enzymatic transesterification process for BD production can be reduced by reusing or recycling the enzyme several times. In the present study, recycling of enzyme has been successfully done 10 times. Figure 5 shows that after recycling 10 times, the enzyme is still active for further transesterification reaction. It has been observed from figure-5 that initial BD conversion was 95.63% (1^{st} batch) but after 10 times, conversion was 91.72% (10^{th} batch). The trend of BD conversion from 1^{st} batch to 10^{th} batch was declining in nature as observed from figure 5. This may be due to the fact that separation and isolation of enzyme after each batch probably includes very little loss. Another reason is the moisture content of enzyme which varies after each batch. Moisture content of enzyme plays a significant role about its activity.

Table 2 shows comparative analytical results of alkali vs enzyme catalyzed BD production from JCO. It has been observed from the results that maintaining same molar ratio of alcohol to oil and a temperature of 50^0C, enzyme catalyzed BD production was higher than alkali catalyzed BD. This may be due to the fact that due to presence of alkali catalyst, sodium hydroxide, some amount of methanol may be consumed due to formation of sodium methoxide. This ultimately hampers the proper molar ratio of alcohol to oil in alkali catalyzed reaction which finally reduces the conversion of BD. Again after completion of reaction, it has been observed from table 2 that total processing time was very high (30±2 hrs) in alkali catalyzed reaction than enzyme catalyzed reaction (4±0.5 hrs). The main cause here is that settling time of reaction mixture and soap separation consume much time in case of alkali catalyzed BD production. So it is time consuming process.

Table-2
Results of comparative analysis (Reaction time 2hrs, catalyst concentration 2%)

Variables		Alkali catalyst (NaOH)	Lipase catalyst (Novozyme 435)
Molar ratio (Methanol:JCO:: 4:1)		70.86% biodiesel	83.16% biodiesel
Reaction temperature (50^0C)		70.86% biodiesel	83.16% biodiesel
Total processing time		30±2 hrs	4±0.5 hrs
Final product composition	Biodiesel	70.86 ±1.012 %	83.16 ±1.64%
	DG	12.35 ±0.737%	8.75 ±0.096%
	MG	9.69 ±0.685%	7.25 ±0.089%
	FFA	3.67 ±0.12%	Nil
	Soap	1.74 ±0.078 %	Nil
	Others	1.67±0.011%	0.84 %
Recycling		Not possible	Several times
Free fatty acids in raw materials		Saponified products	Methyl esters or BD only
Water in raw materials		Unfavourable	Favourable
Recovery of glycerol		Difficult	Ease
Purification of methyl ester		Repeated washing	None
Production cost		Cheap	Normal when recycled
Quality of BD		Normal	Very good

Product purity was somewhat less in alkali catalyzed reaction than that of enzyme catalyzed reaction. Table 2 shows that alkali catalyzed transesterified BD contains 3.67 ±0.12% FFA and 1.74 ±0.078 % soap which are undesirable in the final product. This may be due to some undesirable side reactions which occur during alkali catalyzed transesterification reaction. Here, some amount of diglycerides and monoglycerides are present along with BD as evidenced by thin layer chromatography. Presence of moisture hampers the rate of alkali catalyzed transesterification reaction but in case of enzyme catalyzed reaction, small amount of water is necessary for the catalytically active conformation of enzymes which enhances the rate of reaction. Glycerol recovery was quite difficult in case of alkali catalyzed reaction due to presence of soap in the reaction mixture but it was easier for enzymatic reaction. In addition to the purity of product, recycling of enzyme was done for transesterification reaction which was not possible in alkali catalyzed reaction.

Conclusion

Jatropha Curcas oil is the most significant raw material for the production of alternative energy sources like biodiesel for the future generations. Therefore, identification of suitable process technology for the production of biodiesel is necessary and urgent. Here, a comparative study has been made for the production of biodiesel and the present study revealed that enzymatic transesterification process using nonspecific lipase Novozyme 435 is a better option for this purpose and is more effective than alkali catalysed process using sodium hydroxide. The enzyme catalysed transesterification process is energy saving and renders higher conversion of biodiesel with minimum process hazards. Isolation of by product glycerol and enzyme from the reaction mixture are simple. Recycling of enzyme is successfully done in our study and enzymatic approach can be effective in industrial scale by reusing enzymes several times. Process cost can only be minimized by utilizing this recycling method. Therefore, enzymatic transesterification process can be adopted in bench scale to obtain maximum productivity and is advantageous for the production of alternative energy sources compared to chemical catalytic process. Our results may be helpful for the future researchers in better perceptive of the process technology for the biodiesel production as well as recycling of enzymes in different chemical and biochemical field.

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