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Enzymatic Transesterification of Rubber Seed oil using *Candida Antactica Lipase B*

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ABSTRACT

Biodiesel production from rubber seed oil containing high free fatty acid (FFA 52%) using *Candida Antarctica Lipase B (CAL B)* as biocatalyst was investigated in the present work. Immobilized *CAL B* was used as the catalyst. The effect of reaction parameters such as catalyst concentration, type of acyl acceptor, oil to acyl acceptor molar ratio were investigated against biodiesel conversion percentage. The reusability of the catalyst was also studied. Among the two acyl acceptors under study, methanol gave better results in comparison with ethyl acetate. The maximum biodiesel conversion was obtained as 81.23% at an oil to methanol molar ratio of 1:4 and enzyme concentration 10 (w/w) % of oil in a solvent free system. Reused *CAL B* has also shown good conversion efficiency.

Keywords - Biodiesel, *Candida Antarctica Lipase B*, Enzymatic transesterification, Rubber seed oil.

1. INTRODUCTION

Biodiesel is a renewable and environment-friendly alternative fuel, which comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats [1]. Non-edible oils like those derived from Jatropha, Pongamia, Rubber seed, Cashew nut shell oil etc. are promising feedstock for biodiesel production for a developing country like India where use of edible oils arouse a lot of moral issues [2-4]. Use of these low cost oils will further reduce the overall biodiesel production cost.

Among the different methods for biodiesel production, transesterification is the most accepted method. Transesterification is the reaction of a fat or oil with an alcohol to form esters (biodiesel) and glycerol. A catalyst is used to improve the reaction rate and hence to improve biodiesel yield. Sodium hydroxide (NaOH) or Potassium hydroxide (KOH) is the commonly used chemical catalyst. Research literatures are available with methanol, ethanol, propanol, butanol, methyl acetate, ethyl acetate, amyl alcohol etc. as alcohol reactant [5-7].

The widely used transesterification method is chemical transesterification in which an acid/alkali compound is employed as catalyst. In spite of its wide acceptance, there are several draw backs for this method as described below [8].

- Side reactions of saponification and hydrolysis affect biodiesel yield and its purity.
- The process, especially the acid pre-treatment step, is energy and capital intensive.
- Recovery and purification of catalysts and glycerol are expensive.
- Cost associated with treatment of waste water will add up to the total cost.

Since these issues are severe, the development of an alternate method for transesterification is essential. Enzymatic transesterification of oils is relatively a novel technique in which lipases (enzymes having hydrolysis capability on fats) are used as catalysts. This method has several advantages over chemical transesterification such as [9, 10]:

- Use of mild reaction temperatures.
- Broader substrate ranges due to ability to esterify both glycerides linked and non-esterified fatty acids in one step.
- Use of lower alcohol to oil ratios.
- Evading side reactions, easier separation and product recovery.
- Elimination of treatment costs associated with recovery of chemical catalysts.

- Enzyme biodegradability and environmental acceptability.
- Opportunity for enzyme reuse and improved stability through enzyme immobilization.

Several researchers around the world are exposed to this new method of biodiesel production with different vegetable oils. Some of their work details are referred in Table 1.

Table 1 Comparison of enzymes based on the yield

Enzyme	Oil	Acyl acceptor	Solvent	Yield %	Reference
Pancreatic Lipase	Cotton seed oil	Methanol	Water	80	[11]
Candida Antarctica B	Rubber seed oil	Methanol	Water	85	[12]
Steapsin	Rubber seed oil	Methanol	No solvent	39	[13]
Pseudomon-as Lipase	Sunflo-wer oil	Methanol	Petroleum Ether	79	[14]
Lipozyme	Sunflo-wer oil	Ethanol	No solvent	83	[15]
Pseudomon-as Fluoresecens	Jatrop-ha oil	Methanol	n-hexane	72	[16]

Based on the literature survey *Candida Antarctica Lipase B (CAL B)* was selected as catalyst for the present experiments. Considering the availability of rubber seed oil (RSO) in Kerala, it was selected as feed stock.

2. MATERIALS AND METHODS

2.1 Materials

Rubber seed oil was purchased from Virudhnagar, Tamilnadu. By following standard AOCS Ca 5a-40 procedure, free fatty acid (FFA) content of the oil was found as 26%. Catalyst *Candida Antarctica Lipase B* (a sample pack of 50 g) was received from Fermenta Biotech Ltd. (Thane) as a gift. Methanol, ethyl acetate and other solvents used were of analytical grade and were purchased from a multi-brand supply unit Chemind Chemicals, Kozhikode. The other major instruments used for experiments and testing include incubated shaker, centrifuge, micro-pipette, micro-weighing balance, Brookfield viscometer and thermometers.

2.2 Experimental procedure

10 ml sample of rubber seed oil was used for every set of experiments. The oil was poured into a clean and dried Erlenmeyer flask of 50 ml volume. Measured quantity of acyl acceptor was added to the oil and mixed well. Acyl acceptors used in the experiments were methanol and ethyl acetate. Molar ratios followed were 1:4, 1:5 and 1:6 when methanol was used and 1:4, 1:7, 1:9 and 1:11 were followed when ethyl acetate was used. Three step addition was employed for methanol addition in order to avoid inhibition of enzyme activity. However, ethyl acetate was added in single step as it did not inhibit enzyme activity [17]. Measured quantity of enzyme was added to the mixture and placed in a reciprocating shaker. Enzyme quantity was expressed as percentage weight of oil (i.e. 10ml oil has a weight of 9.1 g, so 1 (w/w) % of oil is 0.091 g). Solvent was also added in three steps at equal time intervals. Shaking frequency was maintained at 170 rpm and temperature as 37°C. After completion of the reaction, the mixture was filtered to separate enzyme. The filtered sample was centrifuged at 5000 rpm for 15 minutes to separate glycerol and traces of enzyme powder from the produced biodiesel. The separated mixture was water washed twice to remove the unreacted acyl acceptor and other impurities and heated to 110°C to remove the water content. Viscosity of each sample was measured using a Brookfield viscometer. After the reaction, enzymes were washed by distilled water and n-hexane, dried and stored below 8°C temperature for further use.

2.3 Influencing parameters

From literature review, the major parameters influencing the reaction were identified as:

i. Catalyst concentration
ii. Molar ratio
iii. Type of acyl acceptor
iv. Percentage of solvent added
v. Reusability

3. RESULTS AND DISCUSSION

Ten sets of experiments were carried out with different enzyme weight, molar ratio and solvent concentrations. Stepwise addition of methanol was followed to avoid enzyme activity inhibition. The best biodiesel conversion was quantified as 81.23% at an oil to methanol molar ratio 1:4, enzyme concentration of 15 (w/w) %, and after 48 hours.

3.1 Effect of enzyme concentration

The variation in conversion of biodiesel was analysed with different enzyme concentrations. Enzyme weight was varied as 5, 10 and 15 (w/w) % of oil and the maximum conversion was obtained at 15 (w/w) %. The effect of enzyme concentration on biodiesel conversion efficiency is plotted in Fig. 1. It can be seen that the conversion rate increases with increase in enzyme concentration. This trend was predominant in lower enzyme concentration levels (i.e., change from 5% to 10%) and less in higher concentration levels (i.e., change from 10% to 15%). The maximum conversion obtained was 81.23% at a molar ratio 1:4. However, the optimum concentration of enzyme is recommended as 10 (w/w) % of oil considering the enzyme cost.

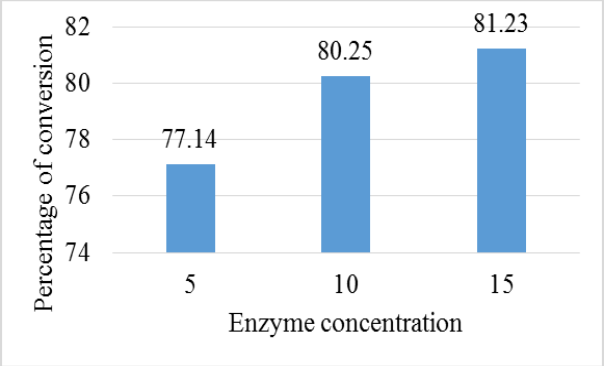

Fig. 1 Effect of enzyme concentration on biodiesel conversion

3.2 Effect of molar ratio

The oil to methanol molar ratio was varied from 1:4 to 1:6. The maximum conversion was achieved as 80.25 % at a molar ratio of 1:4. When molar ratio increased from 1:4 to 1:6, a slight reduction in percentage of conversion was observed which is due to inhibitory action of methanol on enzyme activity. But on comparison with free enzymes used (in earlier studies) effect of methanol inhibition was less on immobilized *CAL B.*

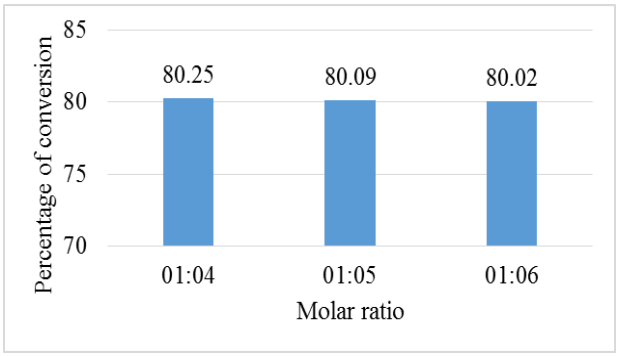

Fig. 2 Effect of molar ratio on biodiesel conversion

3.3 Effect of Acyl acceptor

To avoid the inhibitory effect of methanol on enzymes, Modi et al. recommended ethyl acetate as a potential acyl acceptor for lipase mediated transesterification of jatropha, karanja and sunflower oils [17]. Ethyl acetate was taken as an alternate to methanol in the present experiments and their reactivity towards transesterification process was compared. At a molar ratio of 1:4 methanol gave better conversion than ethyl acetate. For methanol, the percentage conversion obtained was 80.25% whereas for ethyl acetate it was only 57.26% (Fig. 3) at an enzyme concentration 10 (w/w) % of oil. So for the rest of the experiments methanol was used as acyl acceptor.

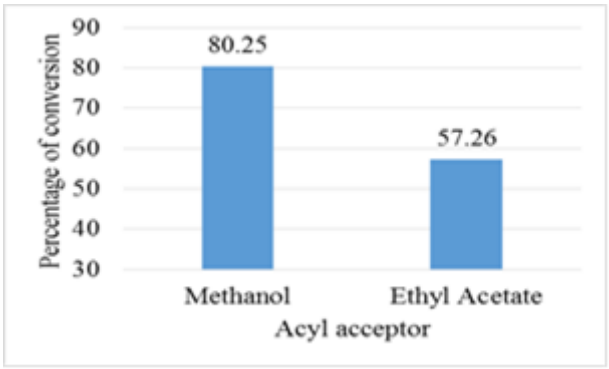

Fig. 3 Effect of acyl acceptor on biodiesel conversion

3.3 Effect of solvent addition

It is well known that the lipase does need a minimum water content to maintain its active conformation to catalyse reactions [11, 18]. In the present work, water is added as a solvent to enhance lipase activity and to avoid the resistive effect of glycerol on lipase. Solvent concentrations were 2.5% and 5% of v/v of oil. But the effect was indifferent and was seen that with increase in water concentration the conversion of biodiesel was reduced (Fig. 4). Therefore, it can be concluded that addition of water as solvent for enzymatic

transesterification of high FFA contained RSO is not recommend.

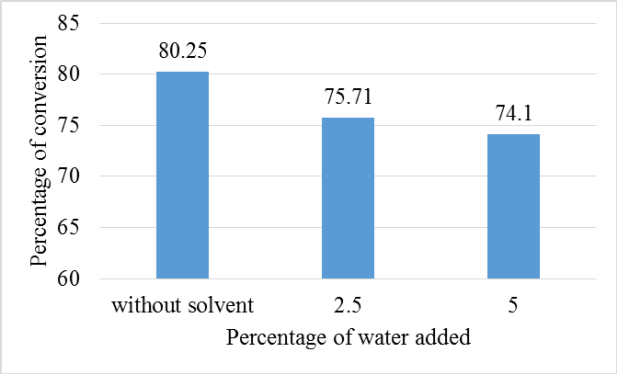

Fig. 4 Effect of solvent addition on biodiesel conversion

3.4 Reusability

Higher thermal stability and reusability are the two major advantages of immobilized enzymes over free enzyme. When *CAL B* was used for second time the percentage of conversion was reduced only by two percentages (Fig. 5) compared to the fresh *CAL B*. This shows that the immobilized enzyme *CAL B* is reusable, which can reduce the production cost.

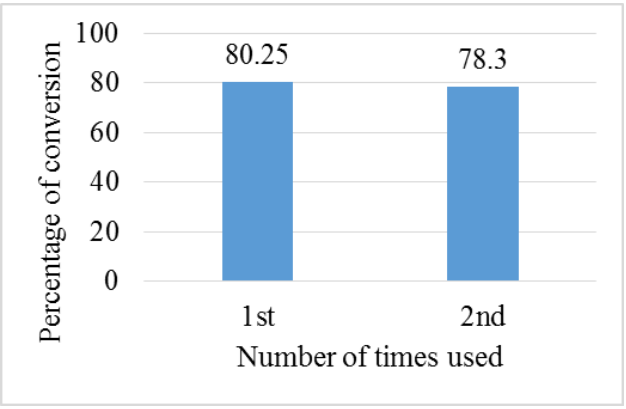

Fig. 5 Reusability assessment of enzyme

4. CONCLUSION

Biodiesel production from high FFA rubber seed oil was investigated in this study. Biodiesel was produced by enzymatic transesterification using immobilized *CAL B* as catalyst. The reaction is mainly influenced by catalyst concentration, type of acyl acceptor used and molar ratio. It was also found that water as solvent for enzymatic transesterification of high FFA contained RSO is not recommendable.

The maximum conversion of biodiesel was obtained as 81.23 % at an oil to methanol molar ratio of 1:4 and enzyme concentration 10 (w/w) % of oil, in a solvent free system. Reused *CAL B* was also showed a good percentage of conversion which confirms the potential of immobilized *CAL B* for repeated use for biodiesel production.

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