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DOE Analysis Applied for Enzymatic Transesterification of High FFA Rubber Seed Oil

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Abstract— Biodiesel production from non-edible oils with high free fatty acid content using biocatalyst was investigated in the present study. Pancreatic Lipase in the form of free powder was used as catalyst for the transesterification of rubber seed oil. The effect of reaction parameters such as catalyst concentration, water concentration and oil to acyl acceptor molar ratio were studied. The experiments were designed and analyzed using the statistical method Design of Experiments. The analysis shows that water concentration has significant effect on percentage of biodiesel conversion from vegetable oil.

Index Terms— biodiesel, enzymatic transesterification, pancreatic lipase, rubber seed oil, high free fatty acid

1 INTRODUCTION

Biodiesel is an environment-friendly alternative fuel which is comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats. The diminishing fossil fuel reserves and the increasing pollution standards are making us to think more about biodiesel [1]. The difficulty made by the high viscosity of vegetable oils to use directly as fuels in engines would be the root cause of beginning of reasearchers in biodiesel.

There are several methods for producing biodiesel in which emulsification, pyrolysis and transesterification are the most discussed methods [2]. Transesterification is the widely accepted method for biodiesel production due to certain advantages such as increased energy efficiency, quality of biodiesel produced and stability of biodiesel. In transesterification of vegetable oils, a triglyceride reacts with an alcohol in the presence of a catalyst, producing a mixture of fatty acid alkyl esters and glycerol [3]. Chemical transesterification method is the industrially accepted method for biodiesel production because of its low production time and high conversion rate [4]. In this method acid or basic chemical compounds or metal oxides are used as catalyst for transesterification reaction. Still this process has several disadvantages related to environmental unfriendliness and energy intensive processing steps [5]. Here comes the importance of enzymatic transesterification.

Enzymes are biological molecules which can act as catalyst for certain processes. Lipases are the enzymes having potential to hydrolyze fats. Many results in recent researches show that enzymes are preferred over chemical catalysts for transesterification reaction. Major advantages of lipase catalysts over chemical one for biodiesel production are listed below [2], [6]:

1. Feed stocks with high Free Fatty Acid (FFA) content can be used.
2. Elimination of post process treatment costs associated with recovery of catalysts.
3. Transesterification in a single step process.
4. Oils can be used without any pretreatments like degumming, refining etc.
5. Room temperature reaction condition.
6. Biodegradability and environmental acceptance.

A few literatures are available on transesterification of vegetable oils using enzymes as catalysts. Some enzymes with their biodiesel conversion efficiency are listed in Table 1.

TABLE 1
COMMONLY USED BIOCATALYSTS

Substrate	Enzyme	Yield (%)	Reference
Soyabean oil	Novozym 435	97	[7]
Plant oil	ROL	90	[8]
Soyabean oil	Candida Rugosa	80	[8]
Crude palm oil	Lipozyme RM IM	12	[9]
Rubber seed oil	Steapsin	39	[10]
Cotton seed oil	Pancreatic Lipase	80	[11]
Palm oil	PS 30 Lipase	72	[12]
Soyabean oil	Pseudomonas Fluorescens Lipase	80	[13]
Sunflower oil	Thermomyces Lanuginosus Lipase	35	[14]

Pancreatic lipase is one of the low cost as well as commonly available enzyme in both industrial and laboratory scale. Soraya Ebrahimi et al. [15] used Pancreatic Lipase for transesterification of Soyabean oil and obtained a maximum conversion of 34.1% in a solvent free system when Methanol was used as acyl acceptor. The molar ratio at optimum condition was 1:3, enzyme concentration was 5% weight of oil, and the frequency used was 180 rpm. Pancreatic lipase was selected as the catalyst for the experimental works in this paper.

Concentration of enzyme, oil to alcohol molar ratio, water concentration etc. are some critical parameters which have

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great influence on biodiesel yield. These parameters are depenedent each other so that there can be combined effects of some of them. The statistical method of Design of Experiments (DOE) is applied to study the significance of these influencing parameters on biodiesel production yield in this work.

The oil used in the experiments is high FFA contained rubber seed oil (RSO). RSO was selected due to ample availability of rubber seeds in India. Also, the naturally produced rubber seed is heavily under utilized [16]. Therefore RSO can be considered as a potential feedstock for biodiesel production.

2 Materials And Methods

2.1 Materials

Rubber seed oil was purchased from Virudhnagar, Tamilnadu and its viscosity was measured as 47.1 cP using Brookfield viscometer. The FFA content of the oil was obtained as 26% using standard AOCS (Americal Oil Chemists Society) method. The properties and fatty acid compositions of the oil are listed in Table 2. The catalyst Pancreatic Lipase (a sample pack of 100 g) received from Biolaxi Private Limited, Bhiwandi was used for biodiesel production.

TABLE 2
COMPOSITIONAL CHARATERISTICS AND PHYSICO-CHEMICAL PROPERTIES OF RSO

Property	Value
Fatty acid composition (%)	
Palmitic acid $C_{16:0}$	10.2
Stearic acid $C_{18:0}$	8.7
Oleic acid $C_{18:1}$	24.6
Linoleic acid $C_{18:2}$	39.6
Linolenic acid $C_{18:3}$	16.3
Specific gravity	0.91
Viscosity (mm^2/s) at 30 °C	47.1
Flash point (°C)	210
Acid value (mgKOH/g)	52

2.2 Design of Experiments

Experiments were designed according to Design of Experiments method and a 2^3 full factorial design was used for response analysis. Experiments were conducted by taking various combinations of parameters to satisfy 2^3 full factorial design and the corre sponding percentage of conversion was recorded (Table 4).The tool used for DOE analysis was Design Experts Ver.10. The parameters considered for the experments were enzyme concentration, molar ratio and percentage of water added.

TABLE 3
LEVELS OF VARIABLES IN ENZYMATIC TRANSESTERIFICATION

Variable	Low	High
Percentage of water addition in (v/v) %of oil	0	5
Molar ratio	1:4	1:6
Enzyme concentration (w/w) % of oil	5	15

2.3 Experimental Procedure

10 ml of oil and acyl acceptor was mixed in a 25 ml Erlenmayer flask. Methanol was the acyl acceptor used in this experiment. Depending on the molar ratio followed quantity of the acyl accepter was calculated. 1:4, 1:5 and 1:6 were the molar ratios followed (1:1 molar ratio means for 1 ml of oil 400 μl of methanol). Three step addition (535 μl in each step for 1:4) was employed to avoid the chance of inhibition of enzyme activity by methanol (after 0 h, 8 h and 16 h, respectively). Measured quantity of enzyme (Pancreatic Lipase) was added and kept in a reciprocating shaker. The quantity of enzyme was expressed as the percentage of weight of oil (10 ml oil has a weight of 9.1 g, so 1 (w/w)% of oil means 0.91 g). Shaking frequency was 180 rpm and temperature was fixed as 37 °C. After completion of the reaction (24 hours), the mixture was filtered to remove the enzyme. The mixture was water washed twice to remove the unreacted acyl acceptor and traces of enzyme. Centrifugation was done at 5000 rpm for 15 minutes to separate the product from impurities. Then the mixture was heated to 110 °C to remove the water content and to denature any remaining enzyme in the produced biodiesel.

2 Results And Discussion

Enzymatic transesterification of rubber seed oil was done using Pancreatic Lipase as catalyst. The influencing parameters on conversion percentage were identified as concentration of enzyme, oil-acyl acceptor molar ratio and percentage of solvent added. However, in order to understand the significance of each of the variables on conversion percentage ANOVA table (Table 5) was created using the software Design Experts Ver. 10.

3.1 Effect of enzyme concentration

ANOVA results show that there was not much significant effect for enzyme concentration (p-value>0.05) on percentage of conversion. It can be assumed that any effect of enzyme concentration may be nullified by the major effect of solvent addition. But there was a trend of increasing conversion percentage with increasing enzyme concentration (see Fig. 1). This is due to increase in the forward reaction with increase in the catalyst concentration. But considering the cost of enzyme higher concentration can not be recommended.

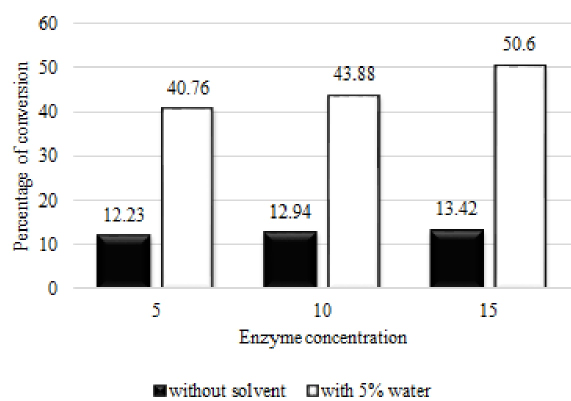

Fig. 1. Effect of enzyme concentration on percentage of conversion (with and without solvent)

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3.2 Effect of molar ratio

Even though methanol and ethanol are the widely used acyl acceptors for transesterification reaction, they have some inhibition action on enzymes. So it is expected that with increase in the quantity of methanol (molar ratio) percentage of conversion should be reduced. The experimental results also showed the same trend. With increase in oil-alcohol molar ratio from 1:4 to 1:6 there is a slight reduction in conversion percentage, which could be due to the inhibitory effect of short chain alcohols on enzyme. From ANOVA table, it is clear that molar ratio is not a significant parameter (between 1:4 to 1:6) as the p value calculated is greater than 0.5. It can be assumed that any effect of molar ratio may be nullified by the major effect of solvent addition.

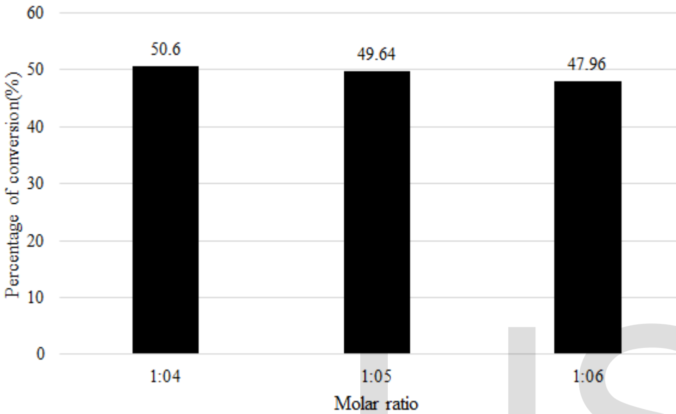

Fig. 2. Effect of molar ratio on percentage of conversion (enzyme concentration 15(w/w)% of oil, 5% (v/v) of water

3.3 Effect of solvent addition

Figure 1 reveals the heavy influence of solvent addition on percentage of conversion. The conversion percentage has increased almost five times as a result of five percentage solvent addition.

As p value for the variable reduces, significance of variables on the response variable increases. ANOVA result shows that water addition was significantly affected the percentage of conversion (p value < 0.05). The byproduct glycerol which is produced during transesterification has some inhibitory effect on enzyme activity. Water can dissolve a part of glycerol produced during reaction which could keep the enzyme activity without significant degradation. In addition, lipases act efficiently at oil water interface, which can again increase the conversion percentage.

3.4 Analysis on Design Expert

The software tool recommended the order and combinations of experiments based on DOE analysis (Table 4). From experimental results a regression equation was developed to predict the response variable (percentage of conversion) within the range of experiments.

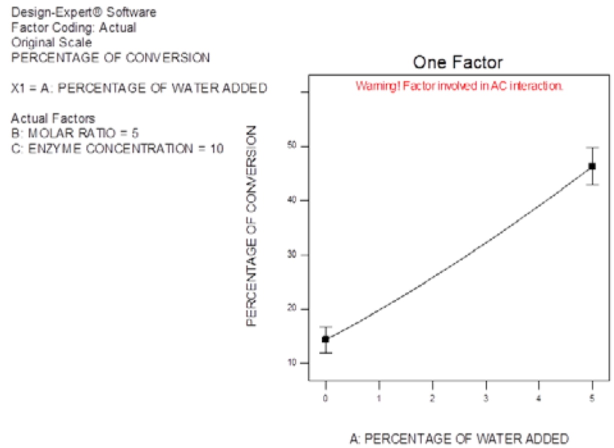

Fig. 2. Effect of solvent addition alone on percentage of conversion

$$P^{0.7} = +1.94354 + 1.4295*A+0.85608*B+0.28739*C+ 0.021145*A*C -0.052841*B*C$$

Where
P – Percentage of conversion
A – Percentage of water addition in (v/v)% of oil
B – Molar ratio (oil to methanol)
C – Enzyme concentration (w/w)% of oil

TABLE 4
PERCENTAGE OF CONVERSION FOR VARIOUS COMBINATIONS OF VARIABLES

		Factor 1	Factor 2	Factor 3	Response 1
Std	Run	A	B	C	PERCENTAGE OF CONVERSION
4	1	5	6	5	40.2
2	2	5	4	5	40.76
7	3	0	6	15	16.07
3	4	0	6	5	15.82
8	5	5	6	15	47.96
1	6	0	4	5	12.23
6	7	5	4	15	50.6
5	8	0	4	15	13.42

TABLE 5

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ANOVA TABLE

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	137.77	5	27.55	111.70	0.0089	significant
A-Percentage of water added	134.64	1	134.64	545.82	0.0018	significant
B-Molar ratio	0.86	1	0.86	3.48	0.2030	
C-Enzyme concentration	1.16	1	1.16	4.69	0.1627	
AC	0.56	1	0.56	2.27	0.2712	
BC	0.56	1	0.56	2.26	0.2713	
Residual	0.49	2	0.25			
Cor Total	138.26	7				

4 Conclusion

Production of biodiesel from rubber seed oil was carried out using Pancreatic Lipase and the major process parameters were optimized for maximum conversion. Three parameters like enzyme quantity, oil to alcohol molar ratio and water concentration were tested. DOE analysis shows that increase in molar ratio is the least significant in biodiesel conversion within the range of operations. A maximum conversion of 50.6% was obtained at an enzyme quantity of 15 (w/w)% of the oil. The analysis also reveals that the most significant variable on biodiesel production rate is concentration of water in the reaction medium. The maximum conversion was obtained at oil to methanol molar ratio 1:4 and at 5% water concentration.

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