MAJOR PROJECT – II

An experimental analysis of enzymatic biodiesel production from Linseed oil

Submitted in the partial fulfillment of the requirement for the award of degree of

Master of Technology
In
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Submitted by:

Onkar

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Under the guidance of

Dr. Amit Pal

Department of Mechanical Engineering and Production & Industrial Engineering

DELHI TECHNOLOGICAL UNIVERSITY,

Shahbad Daulatpur, Main Bawana Road, Delhi - 42

CANDIDATE'S DECLARATION

I hereby declare that the work, which is being presented in this dissertation, entitled **"An Experimental Analysis of Enzymatic Biodiesel production from Linseed oil "** towards the partial fulfillment of the requirements for the award of the degree of Master of Technology with specialization in Renewable Energy Technology, from Delhi Technological University Delhi, is an authentic record of my own work carried out under the supervision of Dr. Amit Pal, Associate Professor, Mechanical Engineering Department, Delhi Technological University, Delhi.

The matter embodied in this dissertation report has not been submitted by me for the award of any other degree.

Onkar
M. Tech. (Renewable Energy Technology)
2K14/RET/13
Place: Delhi
Date:

CERTIFICATE

This is to certify that the dissertation entitled “**An Experimental Analysis of Enzymatic Biodiesel production from Linseed oil**” submitted by Mr. Onkar, 2K14/RET/13, in the partial fulfillment for the award of Degree of Master of Technology in Renewable Energy Technology, Delhi Technological University, is an authentic record of the research work carried out by him under my guidance and supervision.

To best of my knowledge, the results contained in this dissertation have not been submitted to any university or institute for the award or any degree,

DR. AMIT PAL
Associate Professor
Department of Mechanical Engineering
Delhi Technolog1ical University
Delhi-110042

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M. Tech. (RET)

2K14/RET/13

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FAME	Methyl ester of fatty acids
FFA	Free fatty acid
COMR	Catalyst to oil molar ratio
MAOMR	Methyl acetate to oil molar ratio
SCM	Supercritical methanol
TA	Triacetin
MeOH	Methyl alcohol
ASTM	American Society for Testing and Materials
MTBE	Tertiary-butyl methyl ether
PEGK	Polyethylene glycol potassium
PEG 200	Polyethylene glycol with average gram molecular weight 200g
ETA	Ethyl acetate
S/N	Signal to Noise

Abstract

Biodiesel, being a green energy biofuel, has an outstanding potential as an alternative to petroleum diesel fuel. The reaction environment and other factors like catalyst cost prove the biodiesel from conventional methods more expensive than petroleum fuels. Beside all these requirements the conventional methods of biodiesel production lead to the formation of glycerol as by-product which is unavoidable. This crude glycerol is of no value unless purified, which indeed a very complicated process and thus costly too. It makes the pure glycerol produced by this method uneconomical. For the same reason, an economical method is needed to be developed which either can reduce the cost of pure glycerol produced or replace the glycerol as byproduct with one having better economic value than glycerol.

In this study, the ultrasonic aided inter-esterification reaction method using enzyme as catalyst, is investigated to convert fats and oils to biodiesel fuel i.e. fatty acids of methyl ester (FAME) and an additive triacetin (TA) completely. Linseed oil has been used as vegetable oil feedstock and methyl acetate as the reaction solvent.

The results obtained from experiments are optimized using Taguchi design methods. The 12:1 MAOMR and 1.25% catalyst loading at 30°C temperature are found as optimum process parameters.

Chapter 1

Introduction

A major part of energy consumed across the world is derived from sources such as petroleum, coal and natural gas. However, these fossil sources are limited, and on the verge of extinction. Therefore invention of alternative sources of renewable energy such as solar, wind, hydro, biomass, hydrogen fuel cell, nuclear and geothermal is of vital importance. Alternate new and renewable fuels have the potential to solve many of the current energy problems and concerns, like global warming air pollution, sustainability issues and environmental improvements can be solved by these alternative fuel sources. [1]

Vegetable oil has great potential as a renewable fuel. These show promising results due to the advantage of being derived from renewable sources and the environmental benefits. Apart this these can be converted to conventional fuel like diesel named as biodiesel in a considerably short span of time as compared to fossil derived diesel.

Vegetable oils are potentially in exhaustive and renewable with an energy content approximately equal to that of a diesel fuel. The vegetable oils as diesel fuel have following advantages [2] –

- Liquid nature portability
- Ready available
- Renewable
- High heat content (about 88% of diesel fuel)
- Negligible sulfur content
- Lesser aromatic content
- Biodegradable

In present day, vegetable oil as I.C. Engine fuel is not acceptable due to certain economic and operational drawbacks. Some disadvantages of vegetable oils as engine fuel are as follows [2,3] –

- More expensive than fossil diesel fuel.
- Higher carbon deposits during combustion.
- Higher smoke emissions.
- A series of modifications in existing I.C. engine.

Due to all these reasons, researchers world over had been forced to innovate another alternative which can remove all these drawbacks. And the answer lies in the term “Biodiesel”. Biodiesel is derived from vegetable oil or animal fat by the process of trans-esterification.

1.1 *Introduction to Biodiesel*

Biodiesel is similar to fossil derived diesel in combustion properties making it cleaner fuel then fossil derived diesel. Biodiesel is also known as methyl/ ethyl esters of fatty acids. The conventional production of biodiesel follows trans-esterification reaction of triglyceride with alcohol in the presence of a certain catalyst at suitable reaction environment. Biodiesel is a strong contender as a substitute for petro diesel fuel due to its renewable, green energy characteristics. The biodiesel can be used as a blended mixture with petro diesel in any proportion or as a standalone fuel in diesel engines [4]. The combustion properties of both biodiesel and petroleum diesel are also nearly similar. It has numerous advantages when used as fuel, some of them are listed below [4] –

- Biodegradable
- Enhanced lubricating properties
- Higher cetane number
- No sulfur content and non- toxic

Besides these advantages, they are some limitations too, like higher specific gravity, higher viscosity, higher flashpoint etc., as compared to petroleum diesel. The high specific gravity and viscosity make it difficult to atomize the vegetable oil after injecting to the cylinder and hence poor mixing with air increase the ignition delay time. To use vegetable oils as fuel, a very high compression ratio has to be employed along with the injection of fuel to the combustion chamber at a very high pressure. Apart these when the vegetable oils, when used as IC engine fuel problems like high particulate matter, unburned hydrocarbon emissions, high carbon deposit etc., has to be faced. A series of engine modifications have to be done for using vegetable oils as diesel engine fuel. But some minor modifications such as engine valve timings, compression ratio or mixing biodiesel and petroleum diesel etc., has neutralized a majority of these limitations. The following table shows a comparison of properties of diesel and biodiesel as per ASTM [5].

Table 1: Comparison of standards for diesel and biodiesel based on American Society for Testing and Materials (ASTM) [5]

Property	Diesel	Biodiesel
Standard number	ASTM D975	ASTM D6751
Composition	Hydrocarbon (C10–C21)	Hydrocarbon (C12–C22)
Specific Gravity (g/ml)	0.85	0.88
Flash point (K)	333-353	373-443
Cloud point (K)	258-278	270-285
Pour Point (K)	243-258	258-289
Carbon wt.%	87	77
Water vol. %	0.05	0.05
Cetane No.	40-55	48-60
Sulfur wt. %	0.05	0.05
Hydrogen wt. %	13	12
Oxygen wt. %	0	11

1.2 *Motivation for the present work*

Energy is the essential input to drive any process in the universe. Petroleum fuels either directly or indirectly are the major sources of energy for us to drive our major machines and equipment. With the intense growth and development in every sector resulted in the increased use of these fuels. But the stock of these petro fuels is very limited on the earth and their restock by natural process takes millions of years. Thus we need to develop some alternative energy source for petroleum fuels.

Biodiesel is an alternative to petroleum derived diesel fuel, which is produced from vegetable oils or animal fats by trans-esterification and inter-esterification processes. Thanks to our researchers that a number of trans-esterification and inter-esterification processes has been developed so far and still on the tracks of research to be more refine and saturate. Out of the several factors the major and most concerned is “COST” i.e., how much will it cost to replace the petro diesel with more environment friendly biodiesel? Enzymatic inter-esterification has shown great potential in the field of biodiesel production due to various advantages associated with it. The process is more environment friendly, safe, commercial, exhibit reuse of enzyme as a catalyst, carried out at normal temperatures and pressure etc.

The study is associated with the analysis of biodiesel fuel produced by enzymatic inter-esterification to compare it with that obtained from chemical trans-esterification process.

1.3 *Organization of the Report*

This study represents the work of my master’s thesis based on the enzymatic inter-esterification of Linseed oil aided by ultrasonic waves which is a part of the curriculum of the Master in Technology program. The study is correlated with the ultrasonic aided enzymatic inter-esterification process of biodiesel production to compare its fuel properties with biodiesel obtained from chemical trans-esterification process.

The report is subdivided into seven chapters; Chapter 1 deals with the introduction to biodiesel and its general properties and reason of motivation. In Chapter 2, a detailed review of literature has been arrayed. These literature depict the various methodologies of biodiesel production, analysis and factor affecting the yield. The literature covers almost all the methods available for the synthesis of biodiesel and development made in the field so far. Moreover, these build the basic knowledge of the process too.

After the brief literature review, the conclusion drawn and the objective associated with present work has been elucidated. Chapter 3 enlightens the various processes of trans-esterification of vegetable oils. It explains the basic methodology of trans-esterification process, ways in which trans-esterification can be carried out, factors influencing the processes and limitations etc. In chapter 4, various inter-esterification methods of biodiesel production have been discussed. The chapter elaborates how inter-esterification is different from trans-esterification, the process benefits, the process methodology, factors affecting the process and their limitations.

Chapter 5 deals with the ultrasonic aided enzymatic inter-esterification process, which my present work is based upon. It explains the detailed working process, equipment used & their setup, experiment procedure, calculations involved, etc. For the clear understanding aid of some neat diagrams of process and setup has been taken. Chapter 6 highlighted the results obtained in various experiments performed in association with this work. Finally, in chapter 7 conclusion drawn from the thesis work and scope for the future work are explained.

Chapter 2

Literature review

Enzymatic inter-esterification and trans-esterification methods of biodiesel production is an extensive area of research, where many researchers have contributed and many other are pampered in the same direction to develop an economical and cheap way to produce biodiesel on a commercial scale. A literature survey has been listed here to lighten it. The literature review has been classified into four groups viz. trans-esterification using chemical catalysts, supercritical trans-esterification, Inter-esterification using chemical catalysts, enzymatic inter-esterification.

Literature review classification

a) Trans-esterification using chemical catalysts
b) Supercritical trans-esterification
c) Inter-esterification using chemical catalysts
d) Enzymatic inter-esterification

2.1 Trans-esterification using chemical catalysts

R. S. Mishra et al. [30], presented the application of Taguchi design method to optimize the biodiesel production process parameters. The theory associated with Taguchi method was also discussed in detail. Neem oil was used as feedstock for trans-esterification with methanol and KOH as a catalyst. Among all parameters studied in this research viz. temperature, catalyst concentration, reaction time and methanol to oil molar ratio, the temperature has the maximum effect on the biodiesel yield because S/N ratio for 50^{o} C was maximum among all. The optimized parameters using Taguchi methods were found to be 50^{o} C process temperature, 1% KOH by wt., Methanol to oil molar ratio of 4.5:1 and reaction time of 75 min.

Ahyan Demirbas [7], investigated the production of biodiesel via various catalytic and non-catalytic trans-esterification methods. Vegetable oil besides being renewable, biodegradable and less polluting, cannot be used as internal combustion fuel due to its high viscosity of order 27.2

mm/sec^2 – 53.6 mm/sec^2. By trans-esterification of vegetable oils, the viscosity is brought down to a value of 3.59 mm/sec - 4.63 mm/sec^2 by converting vegetable oils to alkyl esters of fatty acids. Trans-esterification of vegetable oils using acid or alkali catalyst is a high yielding and very fast process. During the reaction 1 mole of triglyceride i.e. vegetable oil/fat reacts with 3 mole of alcohol say methanol and 3 mole of FAME (methyl ester of fatty acids) with 1 mole of glycerol are of obtained is products. The reaction is carried out in the presence of a catalyst at atmospheric pressure and a temperature close to the boiling point of alcohol used because at these reaction conditions the methanol will not react with triglycerides without a catalyst aid. Methanol is preferred due to its high reactivity and a low cost over ethanol beside its advantages like renewable as it is derived from agricultural sources, biologically less objectionable.

The alcohol with KOH catalyzed reactions is very sensitive to the free fatty acid concentration in the feedstock. Even an FFA concentration of 0.5% by weight decreases the biodiesel yield by a drastic amount. It is because of the presence of FFA in feedstock triggers the saponification reaction which consumes the metal hydroxide alkali catalyst. Apart this, the separation of products at the end turns complicated and costly due to the formation of gels, soaps etc.

The problem of saponification due to the presence of FFA in the feedstock is overcome by acid catalysts by a simultaneous esterification and trans-esterification reaction but with a limitation of having very slow reaction rate. Moreover, both acid as well as alkaline catalyzed trans-esterification biodiesel production method have a negative effect of the presence of water in the feedstock. Therefore a chemical trans-esterification process have drawbacks like decreased yield due to FFA and water content in the feedstock, inability to reuse the catalyst, costly separation process etc.

The optimum molar ratio of alcohol triglyceride depends on the reaction temperature, the type of catalyst used, vegetable oil composition etc. In the case of alkali catalyzed trans-esterification reaction, the optimum methanol to vegetable oil molar ratio of 6:1 has been investigated whereas in case of acid catalyzed reactions the optimum methanol to vegetable oil molar ratio reaches to 30:1. The amount and type of catalyst to be used depend on vegetable oil feedstock composition. In the trans-esterification process using alkaline catalyst is KOH the optimum catalyst loading is 1% by weight.

M. Canakci et al. [8], carried out a research on the acid catalyzed trans-esterification of vegetable oils. In this study, the effect of methanol to vegetable oil molar ratio and catalyst loading has been analyzed using H_2SO_4 (sulfuric acid) as catalyst. Different molar ratios of 3.3:1, 3.9:1, 6:1, 20:1 and 30:1 have been used in the reaction with 3% sulfuric acid by weight of oil at 60°C reaction temperature. A continuous increase in ester yield has been registered with an increase in the molar ratio. In ester yield of 98.4% has been investigated for a molar ratio of 30:1 and catalyst loading of the present in 48 hour reaction time. Three different catalyst loading of 1%, 3%, and 5% by weight of sulfuric acid had been analyzed and an increased ester conversion ratio had been noticed with the increase in catalyst loading. With the increase in the trans-esterification process temperature, the biodiesel yield got increased considerable amounts. The effect of free fatty acid concentration in the feedstock was also investigated with different FFA percentage viz. 5%, 10%, 15%, 20% and 33%. The ester conversion ratio is has dropped to below 90% for an FFA percentage of even 5%.

The presence of water even in a very small quantity (0.15%) In the feedstock considerably reduces the biodiesel yield to 73.2% from 95.4%. With 5% water by weight in feedstock reduces the ester yield to 5.6% only. The study concluded that optimum alcohol to triglyceride molar ratio is more in case of acid catalyzed trans-esterification is compared to alkaline catalyzed reactions moreover the reaction rate and catalyst also comparatively higher.

2.2 Supercritical trans-esterification

The supercritical trans-esterification of vegetable oils is carried out without any catalyst aid above a certain temperature and pressure combination called critical temperature and critical pressure respectively. The process is inert from the harmful effects of FFA and water presence in the feedstock. The supercritical process can be designed for a continuous flow type commercial production which is an added advantage.

Obie Farobie et al. [9], done a comparative analysis of biodiesel production by supercritical trans-esterification using methanol, tertiary- butyl methyl ether (MTBE) to investigate their reaction behaviour. The experiments had been carried out using a continuous reactor at various temperatures and a pressure of 20 MPa with fixed alcohol to go triglyceride molar ratio of 40:1. The biodiesel yield using supercritical methanol was found superior to that of supercritical ethanol and MTBE. However, an increase in product yield had been noticed with increase in temperature in each case. The yield was maximum at 350°C for supercritical methanol with a reaction time of 15 minutes. For the reaction kinetics is a nonlinear regression with least-squares of error method had been used to determine the error between experimental and calculating values. The forward reaction activation energy was found minimum for supercritical methanol followed by supercritical MTBE and ethanol. The optimum reaction time at three 50°C and 20 MPa were investigated as 10 minutes, 30 minutes and 30 minutes for supercritical methanol, ethanol and MTBE respectively.

Ayhan Demirbas [10], analyzed the trans-esterification of vegetable oils during supercritical methanol. The experiment was performed on six vegetable oils as viz. cottonseed oil, poppy seed oil, sunflower seed oil, safflower seed oil, hazelnut kernel rapeseed oil. The supercritical trans-esterification of hazelnut kernel oil had been carried out at different temperatures viz. 450 K, 493 K, 503 K, 513 K and 523 K with methanol is to triglyceride molar ratio of 41:1. The successive increase in biodiesel the yield had been noticed that with increase in temperature during the process. The trans-esterification of cotton seed oil using supercritical methanol had been performed at alcohol to oil molar ratio of 1:1, 3:1, 9:1, 20:1, 40:1. An increase the biodiesel yield had been investigated for an increase in the molar ratio. The reaction time had been decreased significantly when the alcohol to vegetable oil molar ratio what increased from 3:1 to 9:1. The viscosity of biodiesel from hazelnut kernel oil was found minimum i.e. 2.7 mm^2/s among all followed by safflower oil, cotton seed oil, sunflower oil, rapeseed oil and poppy seed oil.

Ayhan Demirbas [11], presented a non-catalytic biodiesel production and investigated its fuel properties. The experiments had been performed using supercritical methanol, ethanol, 1-propanol and 1-butanol at 575 K and found that biodiesel yield from the supercritical methanol was maximum. The effect of water and free fatty acid concentration was also studied for supercritical methanol, acid catalyzed method and alkaline catalyzed trans-esterification method. Method remained uninfected from the presence of water and FFA content. Whereas biodiesel yield was founded decreased in both acid as well as base catalyzed trans-esterification process.

When the fuel properties of biodiesel and diesel fuel were compared, the viscosity of biodiesel was compatible to that of diesel fuel and reused to a great extent after trans-esterification. The cetane number of biodiesel from various vegetable oil sources was in the range of 50-60. A reduced hydrocarbon, particulate matter, carbon monoxide and sulfur dioxide emission from vehicle exhaust had been reported when biodiesel was used as fuel because biodiesel is free from sulfur content. The peak torque was less for biodiesel fuel as compared to petro diesel fuel, but it was achieved at lower engine speeds.

2.3 Inter-esterification using chemical catalysts –

The trans-esterification process of biodiesel production involves the formation of glycerol as byproduct which is useful in certain applications viz. food industry, pharmaceutical's formulations, cosmetic industry etc. But before making use of it in about applications, it should have a very high purity. Crude glycerol obtained from trans-esterification has a typical and the cost oriented separation process, thus making it uneconomical. Inter-esterification of triglycerides yields triacetin along with the biodiesel which is a fuel solvent and enhances the IC engine fuel properties. Therefore triacetin and formed during the process need not be separated from biodiesel.

Abraham Casas et al. [14], carried out the study on chemical inter-esterification of sunflower oil using methyl acetate as acyl acceptor. The process is carried out at 50°C by making use of catalyst viz. potassium hydroxide, potassium methoxide and PEGK. The analysis was done on the biodiesel yield for each catalyst and various methyl acetate to vegetable oil molar ratios. The yield from PEGK is catalyst was found maximum with 15 minute reaction time whereas no ester yield was noticed in the case of potassium hydroxide as catalyst. The biodiesel yield reaches to equivalent to that of PEGK when CH_3OK well mixed with oil is used as catalyst instead of solid CH_3OK. A 0.2:1 catalyst to oil molar ratio was found the optimum for methyl acetate to vegetable oil molar ratio of 12. The water presence affects in a negative way as water reacts with potassium methoxide to produce KOH and CH_3OH. The presence of KOH along with CH_3OH inactivates the catalyst and lowers the yield. The methyl acetate to vegetable oil molar ratio of 12, 18, 24, 32, 50, 75 and 100 was investigated. An increase in yield was noticed with increase in molar ratio but increase is negligible beyond 24 molar ratio. Although a molar ratio of 50 is taken at optimum at a catalyst to oil molar ratio the 0.1:1.

Z. Sustere et al. [13], analyzed the inter-esterification of rapeseed oil with various acyl acceptor viz. ethyl acetate, methyl acetate, propyl acetate and isopropyle acetate. The inter-esterification was carried out at different temperatures corresponding to each alkyl acetate. It was 55°C for methyl acetate, 75°C for ethyl acetate, 70°C for propyl acetate and 87°C for isopropyl acetate.

These temperatures are kept close to the boiling point of corresponding alkyl acetate at an alkyl acetate to oil molar ratio of 18:1. Also an increase in ester yield has been noticed in every case with the increase in the catalyst to oil molar ratio of 0.1:1 after that no appreciable influence has been investigated. The process yield after 30 minutes of reaction time is noticed to 72% for both ethyl as well as isopropyl acetate at their respective boiling point whereas it was 63% for ethyl acetate at 55°C and 51% for isopropyl at 55°C. The biodiesel yield obtained is 93%, 89%, 87% and 88% for methyl acetate, ethyl acetate, propyl acetate and isopropyl acetate respectively at alkali acetate to oil molar ratio of 36:1.

Abraham Casas et al. [12], investigated methanol enhanced inter-esterification with methyl ethyl acetate. Solid CH_3OK has very low solubility in vegetable oil and methyl acetate resulting in slower reaction rate leading to an induction period in the first phase of the reaction. Potassium methoxide is completely soluble in methanol and the mixture is used as catalyst. The methanolic potassium methoxide increased the reaction rate as a catalyst when compared to that of solid potassium methoxide. Also an increased industrial process safety was aligned with inter-esterification using methanolic potassium methoxide is catalyst. A methyl acetate to oil molar ratio of 48:1 and catalyst to oil molar ratio of 0.1:1 was found the optimum for inter-esterification.

2.4 Enzymatic inter-esterification –

The enzymatic inter-esterification is strong alternative to the chemical inter-esterification due to simplified separation process, safe industrial working process, catalyst reusability and ability to use feedstock with the high FFA and moisture.

Ganesh L. Maddikeri et al. [15], carried out a comparative study of enzymatic inter-esterification of the waste cooking oil with methyl acetate. The yield was analyzed for various oil to methyl acetate molar ratios and different catalyst loading to optimize the process at temperature of 30°C, 40°C and 50°C. In the ultrasonic aided enzyme inter-esterification the increase in methyl acetate molar to vegetable oil molar ratio from 4:1 to 12:1 leads to increase the yield of 90% from the earlier value of 45%. But further increase in methyl acetate to oil molar ratio to 14:1 doesn’t show any significant increase in biodiesel yield. A catalyst loading ranging from 0.5% to 1.25% was investigated in the study. An increase in ester yield has been recorded for an increase in catalyst loading from 0.5% to 1%, but further increase in catalyst loading to 1.25% doesn't aid the biodiesel yield. Therefore 1% catalyst by weight has been taken as optimum catalyst loading.

When the process is carried out at various temperatures with 30°C, 40°C, 50°C, there has been an increase in ester yield for a temperature increase from 30°C to 40°C. But further increase in temperature to 50°C reduces the biodiesel yield due to the deterioration of lipase activity. During the study of the effect of variation in amplitude of ultrasonic vibrations, it has been found that there is an increase in esters yield for increasing amplitude from 40% to 60% whereas no increase in yield has been noticed for further increased the amplitude to 70%. Therefore as per the investigation enzymatic inter-esterification at 40°C temperature with enzyme loading of 1% by weight, 60% ultrasonic amplitude and methyl acetate to oil molar ratio of 12:1 are found to be the optimum parameters.

Preeti B. Subhedar et al. [16], carried out a study on the effect of variation in various parameters during ultrasonic assisted enzymatic inter-esterification on the biodiesel yield. Lipozym TL1M lipase enzyme was used as a catalyst. The investigation was done for different enzyme loadings for various methyl acetate to oil molar ratios, ultrasonic frequency, water concentration and reaction temperatures. For conventional enzymatic inter-esterification methyl acetate to oil molar ratio 12:1 is taken his optimum has increase in methyl acetate to oil molar ratio from 6:1 to 12:1 resulted in increased yield to 95 and 1% whereas further increase in MAOMR to 15:1 leads to a marginal decrease in ester yield. Whereas for the ultrasonic aided inter-esterification and MAOMR of 9:1 considered as optimum as further increase in molar ratio to 12:1 decreases the biodiesel yield. A biodiesel yield of 96.1% was investigated for MAOMR of 9:1 at 40°C for a reaction time of 3 hours. The increase in enzyme loading increases the yield but after enzyme loading of 3% further increase in biodiesel yield was negligible. Whereas in conventional enzymatic inter-esterification and enzyme loading of 6% by weight comes out to be of them at a reaction temperature of 40°C.

Roberta Claro da Silva et al.[16], investigated the enzymatic inter-esterification of lard and soybean oil blend using immobilized lipase Lipozym TL1M is a catalyst in a continuous packed bed reactor. A 70:30 and 80:20 blends of soybean oil and lard was used as feedstock. The process is carried out at a temperature of 60°C and a flow rate of 1 ml/min across the reactor. The temperature is kept constant using a salt bath for the reactor. The continuous glass by reactor had fixed bed to support the enzyme and equip with a peristaltic pump. A conversion ratio of 99.5 gm/100 gm was investigated during the process.

2.5 Conclusion from literature survey

1. The chemical trans-esterification is very sensitive to water and free fatty content in the feedstock. Thus can't be used for feedstock with high FFA content without pretreatment.
2. The separation of glycol from FAME in chemical trans-esterification is a cost oriented method. This increases the cost of glycerol produced and becomes uneconomic.
3. The inter-esterification is a good alternative to trans-esterification with reduced separation processes but catalyst involved are not soluble in the medium.
4. The supercritical method of biodiesel production is a promising alternative to catalyzed process of biodiesel production but the process involves high temperature and pressure. Thus the careful design of reaction equipment is necessary.
5. The enzymatic inter-esterification aided by ultrasonic waves is high yield and low energy input method. The catalysts involved can be reused for further production. The yield is independent on FFA and water content.

2.6 Research objective

The prime objective of the research work is to analyze the biodiesel yield from ultrasonic aided enzymatic inter-esterification. The biodiesel fuel properties like specific gravity, kinematic viscosity, cetane number, calorific value etc. would be compared with biodiesel ASTM D6751 standard. The number of experiments and process results will be optimized by using Taguchi design method. The optimum process parameters across all factors like process temperature, MAOMR, Reaction time, catalyst loading etc. are to be analyzed by Taguchi in order to maximize the biodiesel yield.

Chapter 3

Biodiesel synthesis via Trans-esterification

Biodiesel is generally produced by the trans-esterification of vegetable oil or animal fat feedstock. There are several methods for producing biodiesel; they are [17] –

- Trans-esterification of oil/fats using acid/alkali catalysts
- Trans-esterification in supercritical methanol
- Bio catalyzed trans-esterification
- Heterogeneous catalyst-based trans-esterification
- Supercritical trans-esterification
- Inter-esterification using acid/alkali catalyst
- Enzymatic inter-esterification
- Supercritical inter esterification

3.1 Trans-esterification

The trans-esterification of oils/fats proceeds with or without using a catalyst by making use of primary or secondary monohydric aliphatic alcohols with 1-8 carbon atoms.

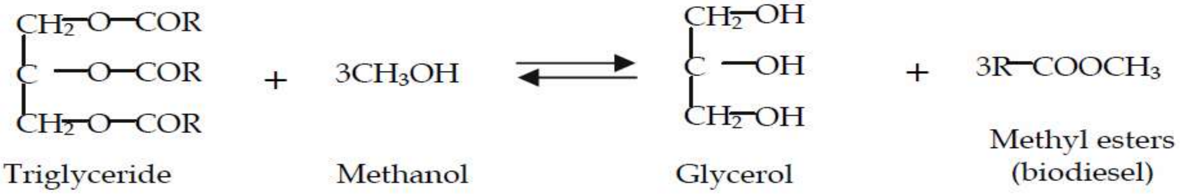

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Fig. 1 Trans-esterification reaction of triglycerides with methanol

Trans-esterification involves neutralizing free fatty acids (FFA), removing the glycerin from a triglyceride molecule or a complex fatty acid shown by the reaction in equation 1 [8]. A sequence of three reactions with monoglyceride and diglyceride intermediates, is involved in complete conversion of oils/fats to biodiesel.

Triglycerides + CH_3OH $\rightleftharpoons$ Diglycerides + $RCOOCH_3$

Diglycerides + CH_3OH $\rightleftharpoons$ Monoglycerides + $RCOOCH_3$

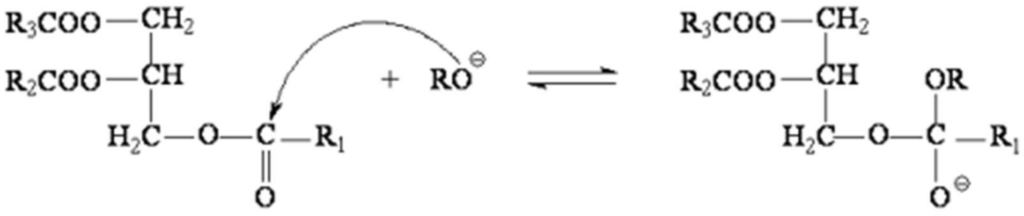
Monoglycerides + CH_3OH $\rightleftharpoons$ Glycerol + $RCOOCH_3$

The reaction mechanism of trans-esterification process using acid or alkaline catalysts can be shown as follows –

Base catalyst –

$$ROH + B \rightleftharpoons RO^{\ominus} + BH^{\oplus}$$

…1



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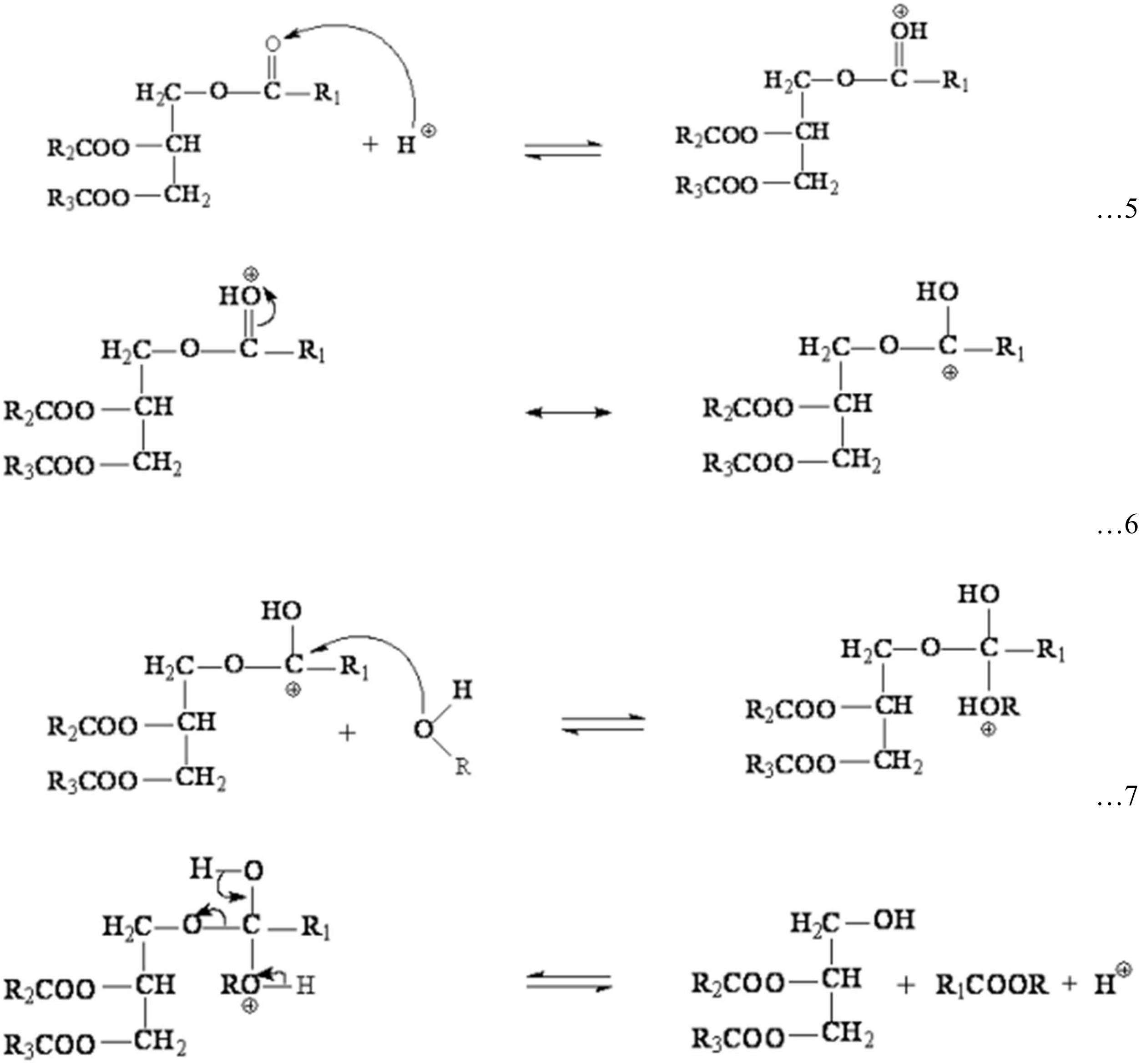
Acid Catalyst –



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…8

Fig. 2 Reaction mechanism of acid catalyzed and base catalyzed trans-esterification process

These reactions are reversible in nature, therefore we need to take the reactants i.e., alcohol in excess quantity to shift the equilibrium in forward direction and produce more alkyl esters as the desired final product. At present, vegetable oil from a variety of seeds of various species have been utilized for biodiesel production. The results are verging but promising. The oils used include soybean, rape seed, Jatropha, Jojoba, waste cooking oil, winter rape, sunflower seed, Curcas, linseed, mustered, cotton seed etc.[18]. Generally, methanol, ethanol, propanol and butanol are commonly used alcohols or the process. Methanol is the most commonly used alcohol in this process, reason being it's low-cost. However, ethanol can be used instead of methanol because it can be derived from agricultural products which are renewable and biologically least objectionable. But methanol is preferred over it because of its low-cost, its physical and chemical advantages as quick reaction with triglycerides because it is all in nature and short chain alcohol [19].

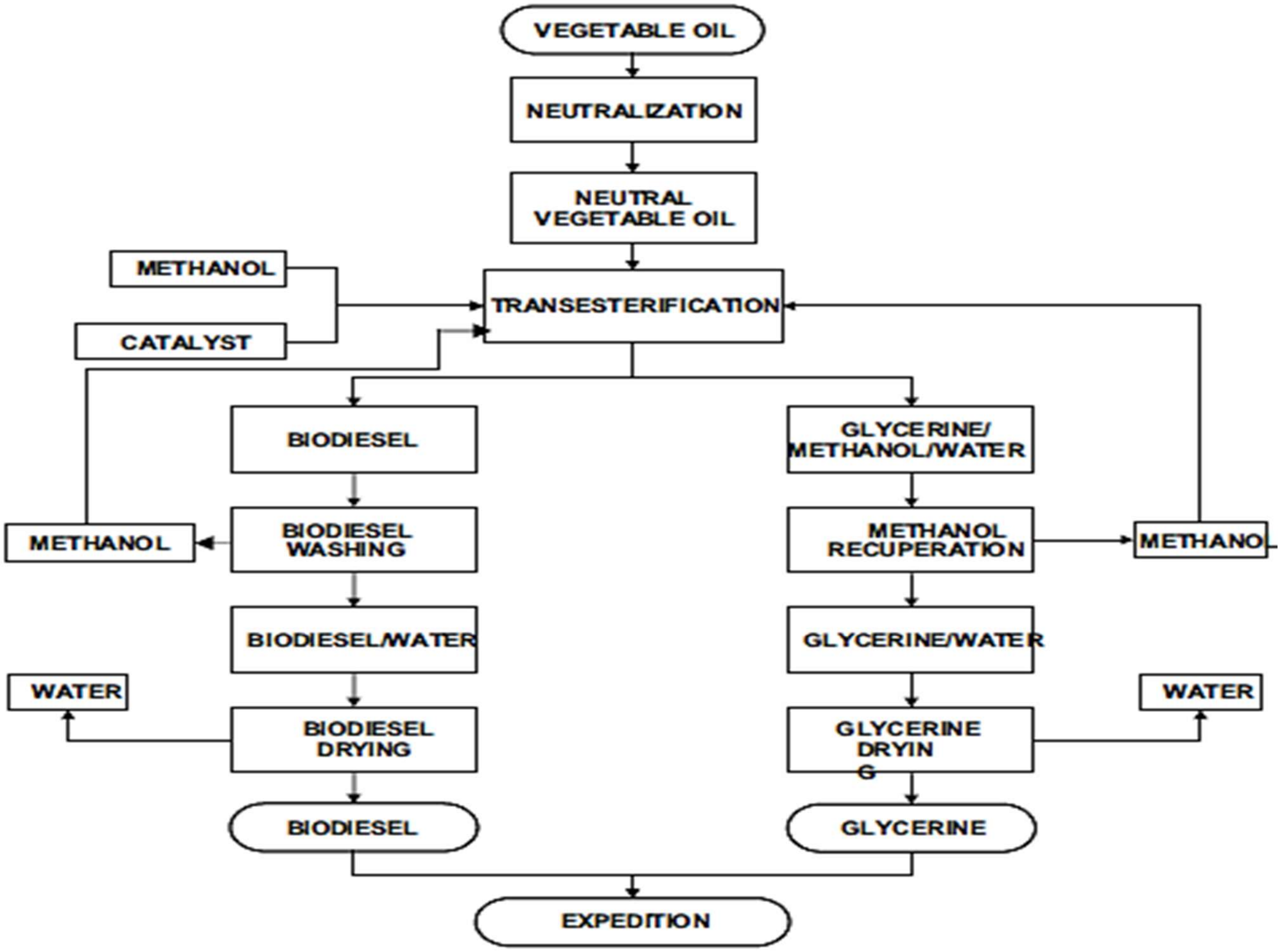


Fig. 3 Block diagram of Biodiesel production process [18]

In the conventional production of biodiesel, free fatty acids and water always produce negative effects during trans-esterification since the presence of free fatty acids and water causes soap formation, consumes catalyst and reduces the effectiveness of catalyst which results in a low conversion ratio. A block diagram of trans-esterification process will lighten the steps involved.

Trans-esterification process can be broadly classified into categories

a) Non-catalytic trans-esterification and super critical conditions

b) Catalytic Trans-esterification

3.2 Non-catalytic trans-esterification in supercritical conditions –

The trans-esterification by supercritical alcohols like methanol, ethanol etc. has shown the promising results in the field of biodiesel production. The process involves the reaction take place at certain temperature and pressure called critical temperature and critical pressure respectively below which the reaction will not proceed at all. Different alcohols have different critical temperature and critical pressures as listed in the table.

Table2 Critical Temperatures and critical pressures of various alcohols [11]:

Sr. No.	Alcohol	Critical Temperature (K)	Critical Pressure (MPa)
1	Methanol	512.2	8.1
2	Ethanol	516.2	6.4
3	1-Propanol	537.2	5.1
4	1-Butanol	560.2	4.9

The process doesn’t use any catalyst and a non-catalytic biodiesel production process. It removes all the steps involved for removing catalyst from biodiesel yield by conventional catalytic biodiesel trans-esterification. The supercritical trans-esterification involves high yield because a simultaneous esterification of fatty acids and trans-esterification of triglycerides occurs during the process [11]. The reaction temperature and alcohol to vegetable oil molar ratio are the most affecting variables on biodiesel yield during supercritical trans-esterification reaction other variables affecting the yield are reaction time, pressure etc. Fig. 2 shows the graph for the change in fatty acids alkyl ester conversion from triglycerides in supercritical treatment with different alcohols at 575 K.

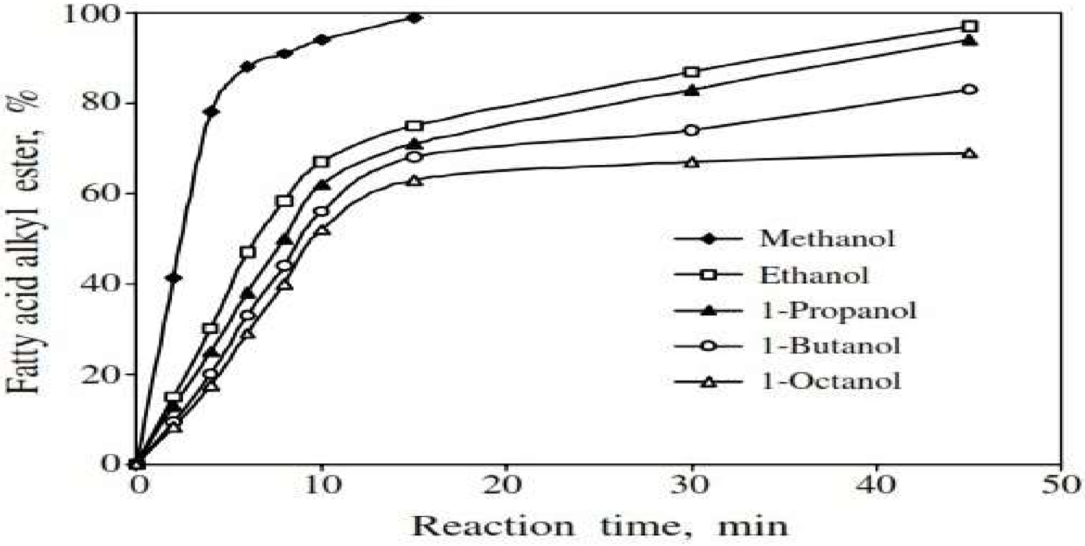


Fig. 4. Plots for the change in fatty acids alkyl ester conversion from triglycerides in supercritical treatment with different alcohols at 575 K [20]

3.3 Effect of process variables

3.3.1 Effect of molar ratio

The stoichiometric alcohol to vegetable oil molar ratio is 3:1 to yield 3 moles of alkyl ester and 1 mole of glycerol. Higher the alcohol to vegetable oil molar ratio higher will be the biodiesel yield. Alcohol to vegetable oil molar ratio from 6:1 to 40:1 has been investigated and results are shown in fig.5 [8].

3.3.2 Effect of reaction temperature

The reaction temperature affects the biodiesel yield in the very similar manner as in case of catalytic trans-esterification process. A higher biodiesel yield has been noticed with an increase in reaction temperature and restrained after reaching the temperature near to 520 K. Apart this there is a minimum temperature requirement for the supercritical trans-esterification reaction to occur

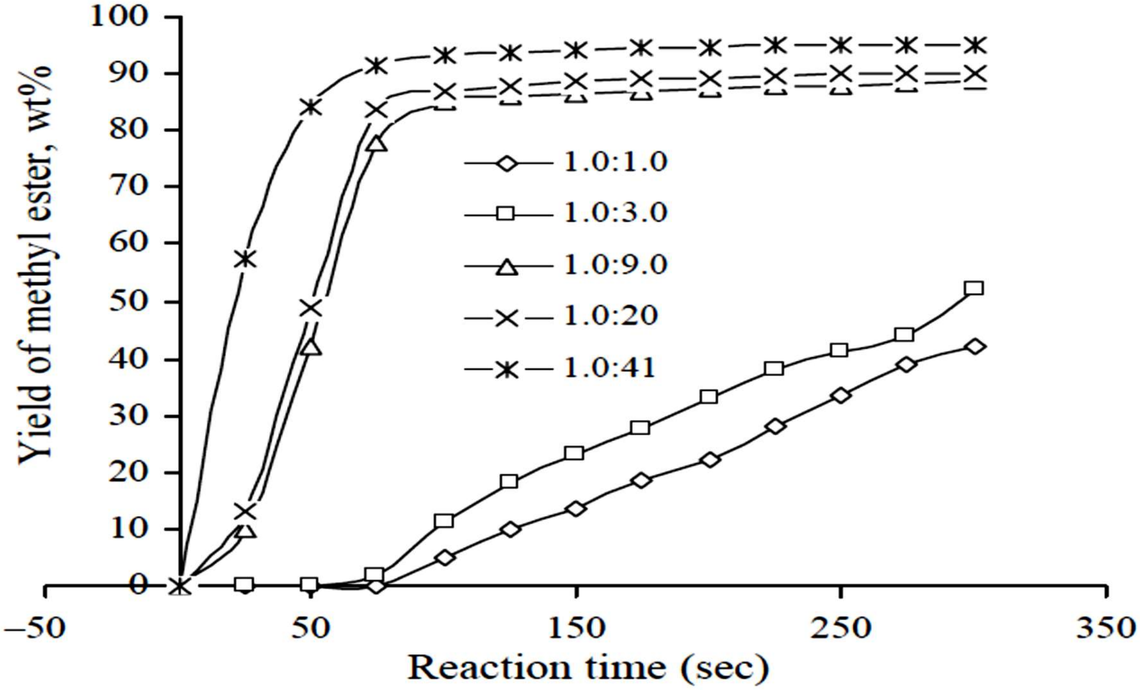


Fig. 5 Variation in biodiesel yield with oil to methanol molar ratio at 513K [8]

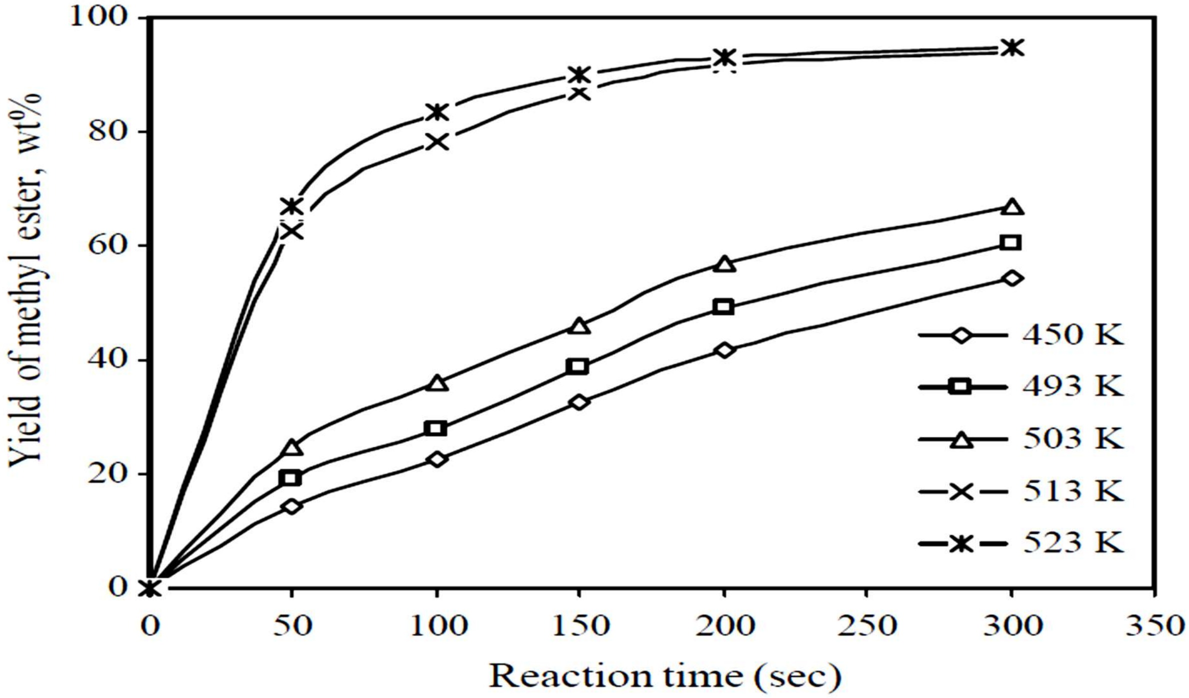


Fig. 6 Variation of biodiesel yield with reaction temperature at Oil to methanol molar ratio 1:41 [8]

which largely depends on the alcohol used followed by the reaction pressure and vegetable oil feedstock. Figure 6 shows the graph indicating the variation of biodiesel yield with reaction temperature at Oil to methanol molar ratio 1:41. The yield approached 51 % after 300 seconds of reaction time at temperature 450 K which further increases by small value after increasing reaction temperature to 493 K and 500 K. But with further temperature increase to 513 K, a significant increase in the biodiesel yield has been noticed after 300 seconds of reaction time [8].

3.3.3 Effect of free fatty acids concentration

Unlike the conventional catalytic trans-esterification process, trans-esterification with supercritical alcohols has negligible effect of free fatty acids concentration on the biodiesel yield. **Figure 8** shows the variation of biodiesel yield with respect to free fatty acid concentration in the feedstock in various biodiesel production processes. The biodiesel yield is negligibly affected in case of supercritical trans-esterification while the yield decreases with increasing in free fatty acid concentration in case of catalytic trans-esterification. The decrease in yield is more in case of acid catalyst as compared to alkaline catalyst trans-esterification [8]. These facts made the supercritical trans-esterification process, a promising alternative of catalytic trans-esterification if used cooking oil is being utilized as feedstock.

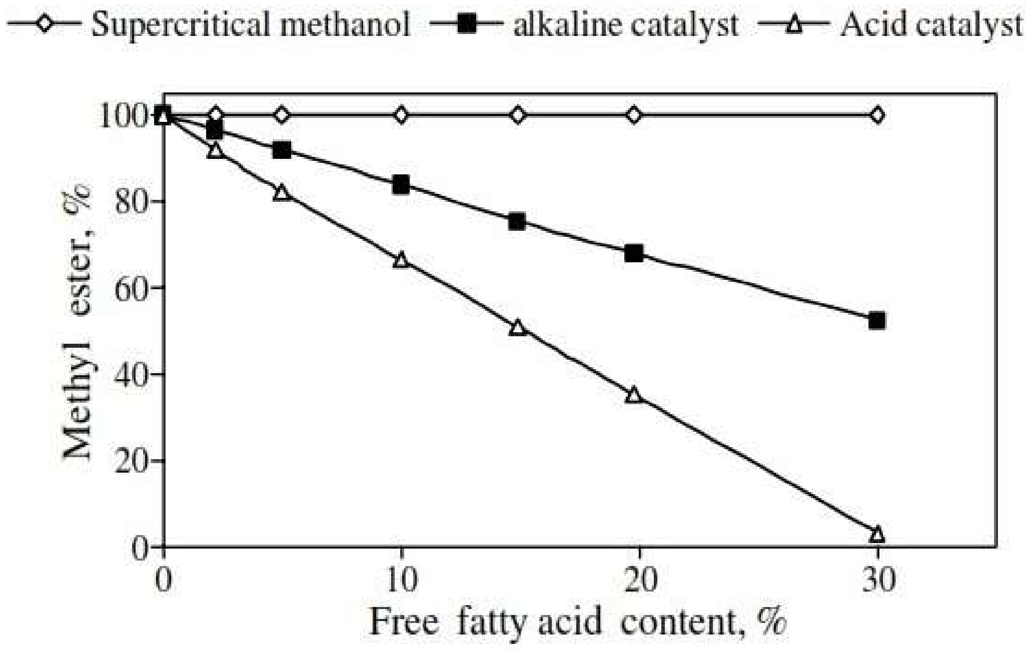


Fig. 7. Variation in the yield of methyl esters as a function of free fatty acid (FFA) % content [8]

3.3.4 Effect of water content

In the conventional trans-esterification process of biodiesel production presence of water in feedstock always have negative effects on yield. The presence of water and free fatty acids leads to the formation of soap, consumes catalyst and cause reduced catalyst activity. But in case of supercritical trans-esterification no saponification reaction occurs due to presence of water and free fatty. Further, there is no effect on the yield of biodiesel due to water and free fatty acids presence. Therefore the vegetable oil as feedstock need not be pretreated unlike conventional catalytic trans-esterification processes [8].

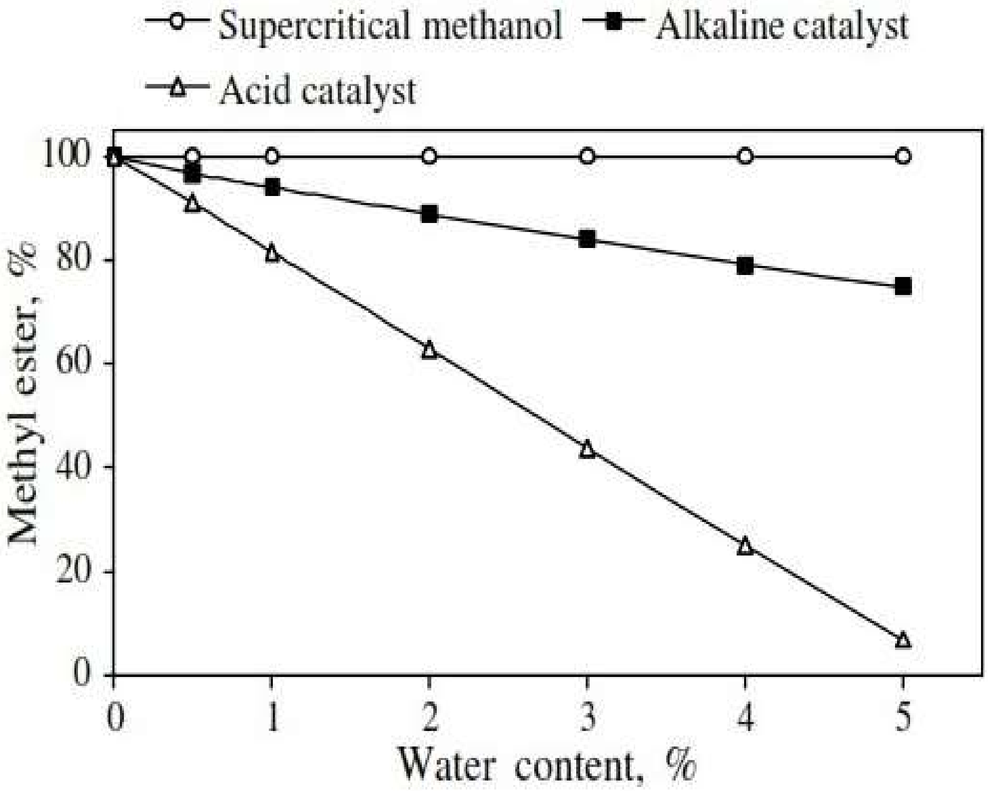


Fig. 8 Variations in the biodiesel yield in trans-esterification of vegetable oils/fats as a function of water content [8]

A tabular comparison of catalytic trans-esterification and supercritical trans-esterification is shown in the table below –

Table 3 Comparison between catalytic methanol (MeOH) process and supercritical methanol (SCM) method for biodiesel from vegetable oils by trans-esterification [11]

	Catalytic MeOH process	**SCM process**
Methyling agent	Methanol	Methanol
Catalyst	Alkali	None
Reaction temperature (K)	303-338	523-573
Reaction pressure (MPa)	0.1	10-25
Reaction time (min)	60-360	7-15
Methyl ester yield (wt. %)	96	98
Removal for purification	Methanol, catalyst, glycerol, soaps	Methanol
Free fatty acids	Saponified products	Methyl esters, water
Continuity easiness	Discontinue	Easy continuity

3.4 Catalytic trans-esterification

A catalyst is used to enhance the reaction rate and the product yield. Both homogeneous as well as heterogeneous catalyst can be utilized in trans-esterification reactions. In turn, the homogeneous catalysts comprises of acids and alkali. Sodium hydroxide, sodium methoxide, potassium hydroxide etc. are the widely used alkaline catalysts while sulfuric acid (H_2SO_4), hydrochloric acid and (HCL) and sulfonic acid are most preferred acid catalysts [21]. Finally, the heterogeneous catalysts consist of enzymes like lipase, titanium silicate, alkaline earth metal compounds like, anion exchange resins etc.

The catalytic trans-esterification starts with the heating of vegetable oil to remove the moisture. The particular amount of alcohol to oil molar ratio is taken and the catalyst is dissolved in it by vigorous steering for about 20 – 30 minutes. The moisture free oil simple is heated to a temperature of 50 – 55°C and transferred to biodiesel reactor. The alcohol – catalyst mixture that added to the reactor gently. The final mixture is then stirred vigorously maintaining the temperature of 50 – 55°C for 30 minutes to 2 hours depending on the alcohol to oil molar ratio taken.

After trans-esterification process got completed, the two liquid products are formed i.e., ester and glycerol. Ester being lighter floats above crude glycerin (dark brown color liquid). The phase separation can be noticed even after 10 minutes, but complete separation will take 4-10 hours depending on the volume. After complete settling, water washing is done to remove excess alcohol and catalyst present in crude biodiesel. Or water, 25 – 30% by volume of oil is added to the separated crude biodiesel and stirred gently for a small time. The mixture is then allowed to separate for 4-5 hours and biodiesel floor on the top. The separated biodiesel is then further washed with 28% (by volume) of water [10]. After final washing and settling the biodiesel is separated followed by heating to remove the water content present in biodiesel and a pure, clear biodiesel is obtained.

Catalysts used in biodiesel production process are broadly classified in two categories –

i) Homogeneous catalysts
ii) Heterogeneous catalysts

3.5 Homogeneous catalysts

Homogeneous catalysts, generally liquids, are dissolved in alcohol in proper proportion which leads to a homogeneous alcohol – catalyst mixture. Homogeneous catalysts are further classified as –

3.5.1 Alkaline-based catalyst

Sodium hydroxide, potassium hydroxide, sodium methoxide, or sodium peroxide etc. are the widely used alkaline catalysts for trans-esterification. The alkaline base catalytic trans-esterification reactions are generally faster than the acid base reactions.

The reactor is charged with the vegetable oil and heated to about 60 to 70°C with moderate steering. Meanwhile, about 0.5 to 1% (w/w) and anhydrous catalyst (KOH or NaOH) is dissolved in methanol of desired a molar ratio [22].

3.5.2 Acid-based catalyst

The commonly used acid catalyst of our hydrochloric acid, sulfuric acid and phosphoric acid. Free fatty acid concentration in used cooking oils, nonedible oils etc. is generally more than 2% and in animal fats FFA is approximately 5 – 30%. Very low quality feedstock, like trap grease may contain 100% free fatty acid. Free fatty acids or FFA when of oil is further increased by the presence of water or moisture in vegetable oil feedstock. It has been seen that the alkaline catalyst reacts with the high FFA feedstock to produce soft and water. The alkaline catalyst can be used up to 5% FFA however, to compensate for the catalyst loss in side saponification reaction, and additional catalyst amount is taken for the reaction to complete. When the free fatty acid value in vegetable oil exceeds 5%, soap formation inhibits the methyl esters separation from the glycerol and forms an emulsion during water wash. For these particular cases, acid catalysts are utilized to esterify the FFA methyl esters [22].

The standardized conditions for the trans-esterification are 60°C of reaction temperature, 3% sulfuric acid, 6:1 molar ratio of methanol to oil and a reaction time of 48 hours. The ester conversion percentage is increased to 95.1% from the previous value of 87.8% if the reaction time was increased to 96 hours and instead of 48 hours. The drawbacks associated with acid esterification are formation of water and a longer reaction time. The increase in the reaction temperature also decreases the specific gravity of ester. Esterification conversion efficiency is shown in the figure below with respect to water percentage in the oil. Even a very small percentage of water addition (0.1%) reduces the ester yield. With further addition of water in vegetable oil feedstock is significant decrease in the biodiesel yield can be noticed during the reaction process. If a water content more than 0.5% is present in feedstock the biodiesel yield would be below 90%.

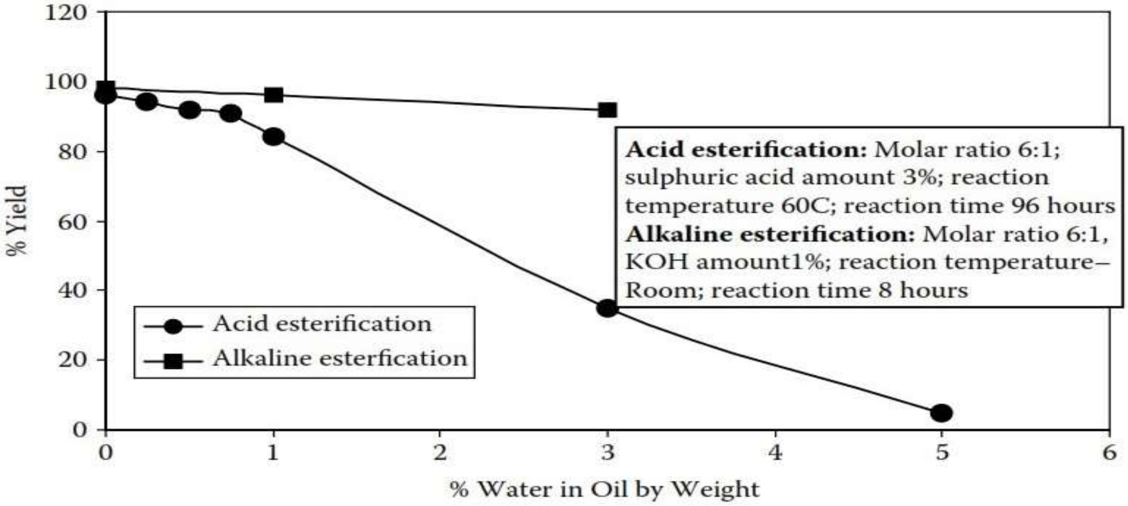


Fig. 9. Effect of water content % in vegetable oil on the biodiesel yield [15]

3.6 Factors affecting catalytic trans-esterification

Major factors affecting the trance esterification yield and reaction rate is discussed below –

3.6.1 Alcohol to vegetable oil molar ratio

The 6:1 alcohol to vegetable oil molar ratio gives the highest yield (greater than 98% by weight). Lower molar ratios lead to a longer reaction time to complete trans-esterification process. Even high year molar ratios increase the reaction rate but lead to difficulties associated with the glycerol separation. Only at a molar ratio of the process yields higher biodiesel and easier glycerol separation [22]. The type and quality of the vegetable oil used decides the optimum alcohol to oil molar ratio for the reaction.

3.6.2 Catalyst molar ratio

The concentration of catalyst is a critical parameter in the trans-esterification reaction is a small increase or decrease in catalyst concentration during the reaction will lead to a drastic variation in biodiesel yield. The amount of catalyst to be added in the trans-esterification reaction depends on the type of feedstock used and also on the type of catalyst used. Alkali catalyst increases the reaction rate several times faster than acid catalysts. With an increase in concentration from 0.5 to 1% of the alkaline catalyst by weight yield increases from 94 to 99%. Further increase in catalyst concentration does not increase the yield, but it adds the cost and makes the process of separation more complicated. The concentration of catalyst in case of acid catalyzed trans-esterification is more than that of base catalyzed reaction. Moreover, a high (12:1) alcohol to vegetable oil molar ratios have to be used for a high reaction yield.

3.6.3 Reaction temperature

The trans-esterification reaction rate is strongly influenced by the reaction temperature. Generally, the reaction is carried out close to the boiling point of alcohol used at atmospheric pressure. With a further increase in temperature they are all more chances of loss of alcohol, but an increase in temperature and increases the reaction rate. The base catalyzed trans-esterification is most commonly performed at a temperature of 55°C – 65°C depending on the type of alcohol used. Whereas in case of acid catalyzed trans-esterification the reaction temperature is kept in range of 80°C – 100° C.

3.6.4 Mixing intensity

The effect of mixing intensity is more effective during the slow rate region of the trans-esterification reaction. When a single phase got established the mixing becomes insignificant. Understanding the effects of mixing on the trans-esterification process kinetics is a precious tool in the process design and scale up. Generally, a stirring for 5 to 10 minutes after adding alcohol – catalyst mixture to oil promotes a higher rate of ester conversion.

3.6.5 Purity of reactants

The ester conversion levels are significantly affected by the impurities present in the feedstock. The vegetable oil (reined or crude oil) occurs before the trans-esterification reaction. During storage in a tank, the oil settled at the bottom would give lower biodiesel yield because of the settling of impurities such as wax at the bottom.

3.7 Heterogeneous catalyst

Heterogeneous catalysts are generally in solid-state and can be categorized as below –

- Solid acid catalysts
- Solid base catalysts
- Alkaline metals salt on porous support
- Ion exchange resins

3.7.1 Solid acid catalysts –

A huge potential has been shown by the heterogeneous catalyst to replace the homogeneous catalyst and circumvent the problem occurring in conventional trans-esterification method. Solid acid catalyst simultaneously catalyze the esterification and trans-esterification reactions. Both Bronsted as well as Lewis acid sites can catalyze the esterification reaction. Of the most reviewed materials, sulfate materials have shown the highest catalytic activity, but due to poisoning effect these are ordered. Many heterogeneous catalysts are unable to achieve the conversion of FAME as per ASTM standards. Several catalysts that are highly active, are subjected to sulfate leeching and deactivation with successive reactions. Zirconia alumina, supported heteropoly acids, Zirconia titania are the most active and stable catalysts [23].

Solid acid catalysts, including 10 list and oxides, tin oxides, zeolites, heteroploy acids and sulfated zirconia, have been revealed so far. A interconnect large porous texture with a high acid side concentration and a hydrophobic surface should be possessed by an ideal solid acid catalyst. A hydrophobic surface promotes the adsorption of oily hydrophobic part on the surface of catalyst and to avoid the deactivation of catalytic sites by adsorption of all bicarbonates like water, glycerol etc. and thus essential.

The trans-esterification using TPA (12-tungstophosporic acid) is supported on hydrous zirconia at a temperature of 200°C with 9:1 alcohol to vegetable oil molar ratio and 3% catalyst by weight have a biodiesel yield of 90% by weight. Also there is no loss in activity of catalyst with successive use during the process [23].

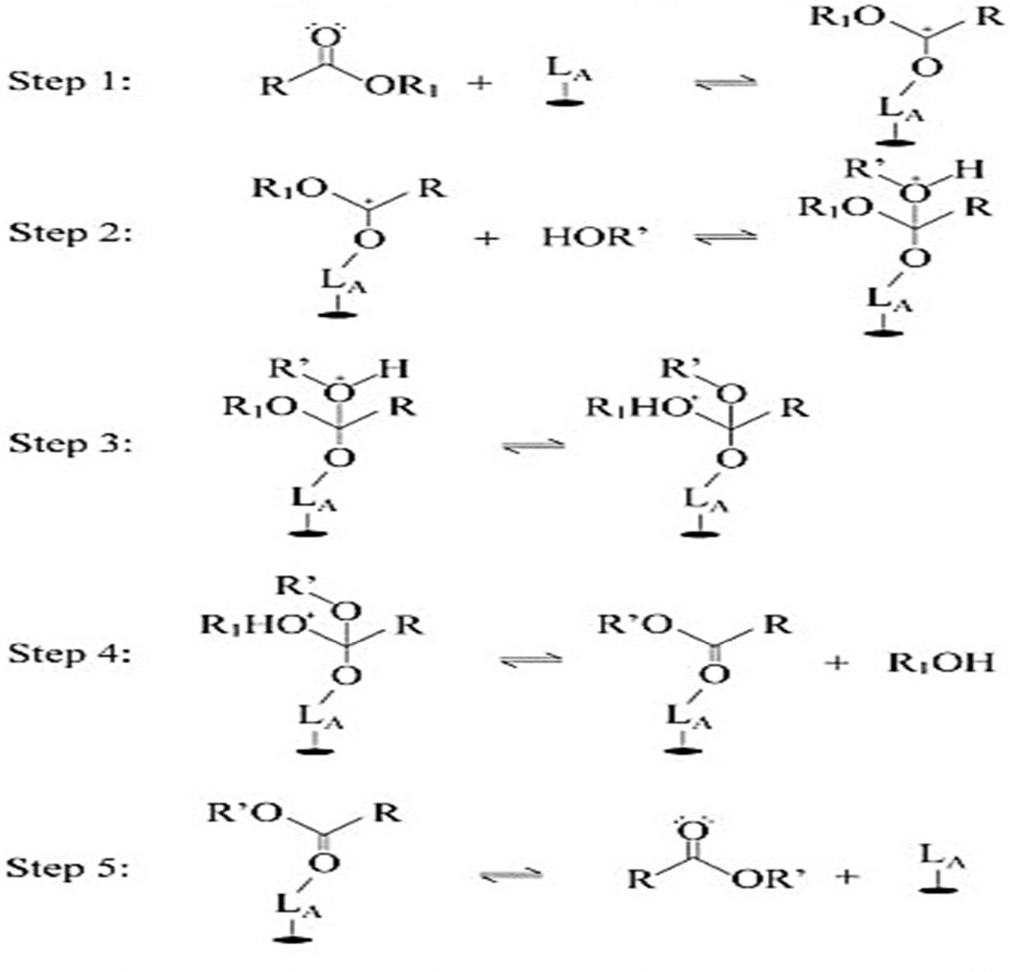


Fig. 9 Reaction mechanism by acid catalyzed simultaneous trans-esterification and esterification [24]

3.7.2 Alkali base solid catalysts –

Solid alkaline catalysts have lower reaction times as compared to the solid acid catalyst. Beside this the solid alkaline catalyst is a strong contender because of numerous disadvantages of homogeneous alkaline catalysts. The homogeneous alkaline catalysts consume a high energy contest for the process. Also the purification of crude biodiesel and separation of the catalyst is from the product mixture makes the process very costly. Generally, in case of solid alkaline catalysts can be separated from the reaction final product mixture with ease and reused. The reaction temperature is kept between 60 – 65°C with an optimized catalyst loading of 5% by weight and alcohol to vegetable oil molar ratio of 24:1.

The physical surface properties like active side concentration, surface area, pore volume and pore size are part of the catalyst activity deciding factors. Most heterogeneous alkaline catalysts are tested at a temperature near to a boiling point of alcohol. The reaction mechanism of heterogeneous base catalyzed trans-esterification is similar to that of the homogeneous base catalyzed trans-esterification reaction, relying on the formation of alkoxydecation (CH_3O-) bonded to the surface of catalyst [24].

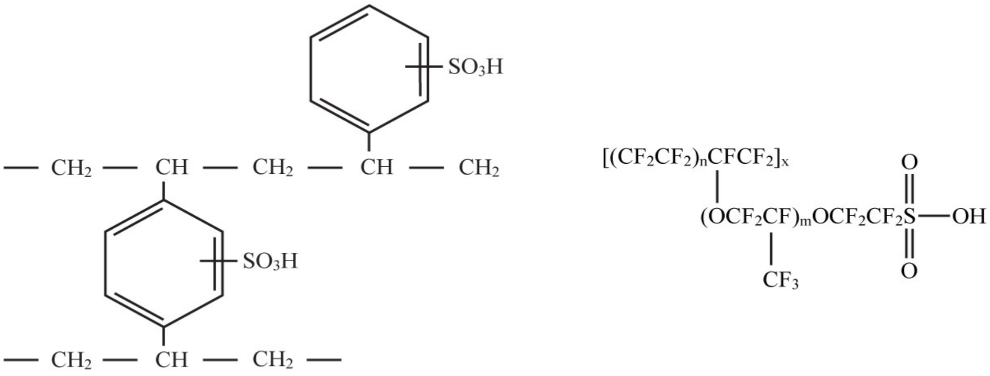


Fig. 10 Structural composition of sulfonic acid styrene-divinyl benzene IER (left) and Nafion SAC-13 (right) [24]

Majority of catalyst is achieved a conversion about 90%. Zabeti report a conversion ratio of 98% using calcium methoxide ($Ca(OCH_3)_2$) is catalyst 2% by weight at the temperature of 65°C with alcohol to vegetable oil molar ratio 1:24. In 99% conversion has been reported using calcium oxide on calcium carbonate at a temperature of 65°C and reaction time of two hours. Lipase catalysts are the promising alternative for the future research in biodiesel production as biodiesel yield of greater than 94% has been reported using the lipase as catalyst. Metal salts like lithium nitrate ($LiNO_3$), sodium nitrate ($NaNO_3$), potassium carbonate etc. doped on alumina (Al_2O_3) can also be used as catalyst with catalyst loading of 5% by weight and THF (tetrahydrofuran) as co-solvent at a temperature of 60°C and one hour reaction time.

3.7.3 Ion- Exchange resins

Ion exchange is termed as a reversible exchange of ions between a solid ion exchange material and liquid which exhibits no permanent change in solid structure. These are the most commonly used in water treatment and also a separation method in various non-water processes. The major advantage rests with the reusing ability of ion exchange resin material. These provide basic and acidic functional groups during a chemical reaction to catalyze the reaction. The ion exchange resin efficacy depends on Lewis and Bronsted acid sites for an acid catalyzed reaction. Considering the sulfonic acid (HSO_3) as a functional group, an H^+ ion is available over the reaction. Most of Ion-Exchange resins are stable at 120°C. Harmer and Sun (2001) studied some commercially available resins [24].

3.7.4 Enzymatic trans-esterification

The commercial practice of biodiesel production in industries uses alkali and acid catalyzed trans-esterification. However, the catalyst removal through neutralization and separation of the salt from biodiesel is difficult to achieve. The physiochemical synthesis may lead to undesirable side reactions due to presence of moisture and high free fatty acid (FFA) content. The enzymatic conversion of the vegetable oil/fat has been cited a promising alternative to conventional physicochemical methods. The use of lipase is a catalyst for biodiesel production should be preferred as compared to chemical methods using alkali or acid catalysts due to the reason that no complex operations are needed for the separation of glycerol and also for the elimination of the catalyst and salt from the final product mixture. The key step involved is to immobilize the enzyme successfully. This will allow its recovery from the product mixture for its further use in process.

The typical method using lipase is a catalyst for biodiesel production uses a methanol oil molar ratio of 8:1, 5% of water by weight, immobilized lipase catalyst loading of 20 – 30% by weight at 40°C and 650 rpm for the reaction time of 30 – 50 hours. The immobilized enzyme then washed with water and the final mixture is than kept for gravity separation followed by water washing. It will take 6 to 8 hours for the final separation of biodiesel and glycerol in gravity settling. Glycerol is drained out from bottom in another container and sent for further purification. The remaining crude biodiesel is a washed with hot water several times until clear biodiesel is not obtained. When trans-esterification process is carried out by the enzymatic means, a yield of greater than 90% of obtained with the reaction time of 30 – 50 hours. This much long reaction time limits the industrial production of biodiesel using enzyme as catalyst. To increase the yield and decrease reaction time if alcohol to vegetable oil molar ratio increased it may lead to the inactivation of enzyme by blocking the immobilize enzyme. The main advantages of the enzymatic trans-esterification process are –

- Enzyme reusability
- Simplified product separation process
- Higher yield

Apart of this there are many disadvantages which are –

- Very high catalyst cost
- Long reaction time
- Decreased catalyst activity in the successive reuse

Chapter 4

Biodiesel synthesis via inter-esterification

The process of biodiesel synthesis from triglycerides by reaction with alkyl acetates producing alkyl esters of fatty acids i.e. biodiesel along with a byproduct triacetin instead of glycerol, is termed as inter-esterification. The byproduct triacetin need not to be separated out and it is indeed a fuel solvent for FAME unlike glycerol.

The trans-esterification of biodiesel production involves the formation of glycerol as byproduct which is useful in certain applications viz. pharmaceuticals formulations, cosmetic industry, food industry etc. But before making use of it in above applications, it should have a very high purity [27]. Crude glycerol obtained from trans-esterification has a typical and cost oriented separation process, thus making it uneconomical.

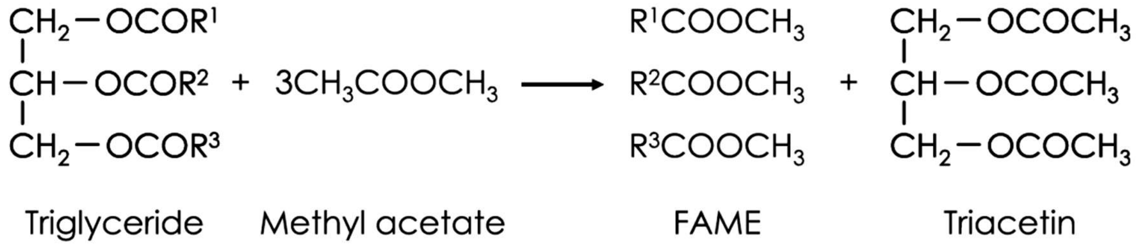


Fig. 11 Inter-esterification process reaction between triglyceride and methyl acetate [25]

4.1 Inter-esterification using chemical catalysts

The inter-esterification by chemical means can be carried out by using a number of catalysts viz. sodium methoxide, potassium methoxide, PEG 200 (polyethylene glycol with average molecular weight 200) etc. The alkyl acetate as reactant is instead of alcohol changes the nature of reaction mixture from polar to nonpolar and the catalyst remains partially insoluble. The chemical inter-esterification is favored because of high cost associated with the enzymatic and supercritical means. The sodium methoxide is most widely used as catalyst for inter-esterification in industrial synthesis of biodiesel. But the limited solubility of it limits its application in industrial inter-esterification. The use of poly ethylene glycol complexed with potassium (PEGK) has improved the solubility of methoxide during the process. Generally, the use of PEG is done as phase transfer catalyst for the reactions where catalyst and reagents are insoluble. The first phase of inter-esterification reaction using sodium methoxide as catalyst is assumed as pre reaction phase because during this course of time the added catalyst reacts with oil to act as real catalyst for successive inter-esterification reaction. The phenomena can be identified by the change of oil color to reddish brown. Although this has not been proven yet and still an assumption [14]. Like the chemical trans-esterification, inter-esterification by using chemical catalyst is also dependent on several factors as the reaction temperature, methyl acetate to triglyceride molar ratio, amount of catalyst used etc.

4.1.1 Effect of methyl acetate to triglyceride molar ratio

The conversion of vegetable oil to FAME during chemical inter-esterification is a reversible process. Moreover the products, intermediates like diglycerides, monoglycerides etc. and reagents are completely miscible.

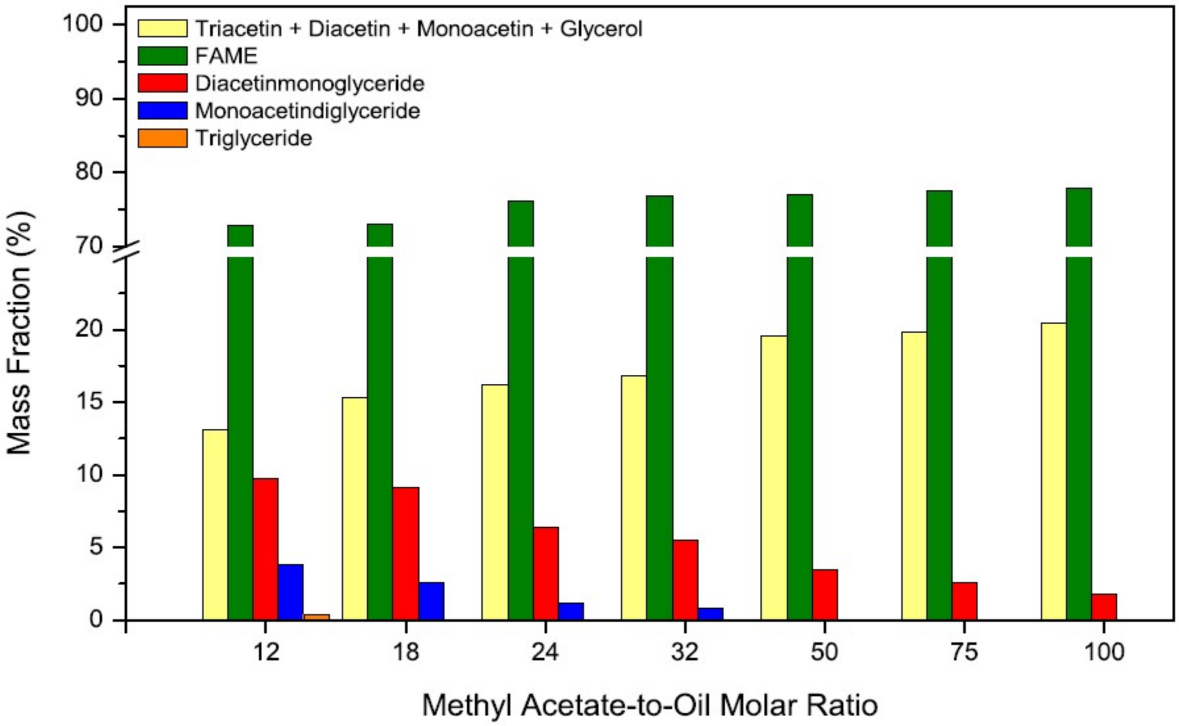


Fig. 12 Variation in mass fraction of different inter-esterification products with methyl acetate to oil malar ratio for COMR 0.5 and reaction temperature of 50°C [14]

Therefore to shift the equilibrium in forward direction for maximum yield and minimize the unwonted intermediates, we need to add more methyl acetate as compared to the stoichiometric amount. The figure shows the variation of inter-esterification products for different molar ratios of methyl acetate to oil. The 50:1 methyl acetate to oil molar ratio has been selected as optimum at 50°C and CEO and COMR 0.5 [14].

4.1.2 Effect of catalyst to oil molar ratio

A significant effect of catalyst loading on inter-esterification has been observed. It can be seen that for the catalyst to oil molar ratio (COMR) of 0.05 no biodiesel yield is obtained but a slight increase in COMR to 0.1 shows a significant FAME yield. The reaction equilibrium has been obtained in just 10 minutes for COMR 0.2 at a temperature of 50°C. Whereas the reaction time further reduces with the increase in COMR.

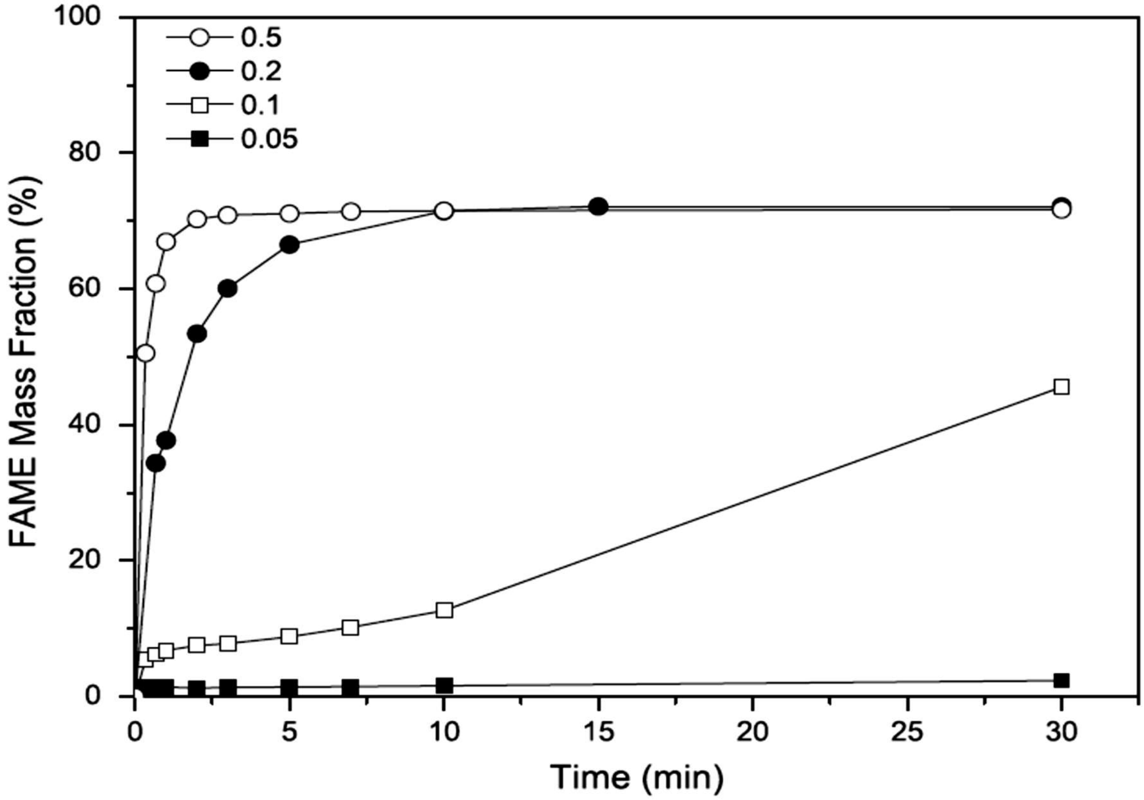


Fig.13 Plots showing the variation in FAME mass fraction with reaction time (min) at different catalyst to oil molar ratio (COMR) [14]

4.1.3 Effect of catalyst type

Although a lot of catalyst choices are available for chemical inter-esterification process but choice of an ideal catalyst is always a key task. As it can be clearly seen that the reaction completion time as well as biodiesel yield are different for different catalysts. Out of all, PEGK and CH_3OK has shown the maximum yield at all temperatures. The difficulties like insolubility in oil, explosive nature etc. associated with CH_3OK use diverted towards the use of PEGK as catalyst. A yield of 78% approx. has been achieved for only 10 min. of reaction time [14].

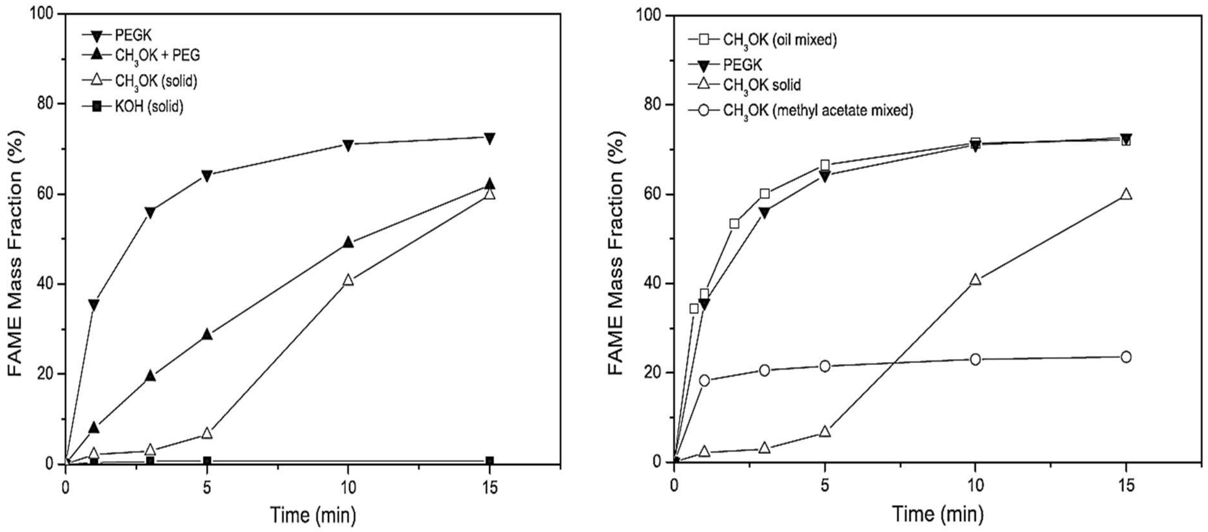


Fig. 14 Effect of different catalyst is used in inter-esterification on FAME mass production for CRM are 0.5 and 50°C temperature [14]

4.2 Supercritical Inter-esterification

The supercritical inter-esterification process is a catalyst free production of biodiesel fuel using alkyl acetate such as Methyl acetate or ethyl acetate at high temperature and high pressure. In the temperature of the process generally remains greater than 300°C for a methyl estate at 20 MPa as below this temperature the reaction between triglycerides and methyl acetate does not occur at all.

The process can be carried out in batches or a continuous flow type system. A continuous flow conversion system can be employed for industrial scale production, is an added advantage of this method. A system involves a high pressure pump to pump the raw materials (methyl acetate and triglyceride mixture) at designed pressure, a reaction tube, a salt bath for maintaining the desired temperature, required for supercritical inter-esterification.

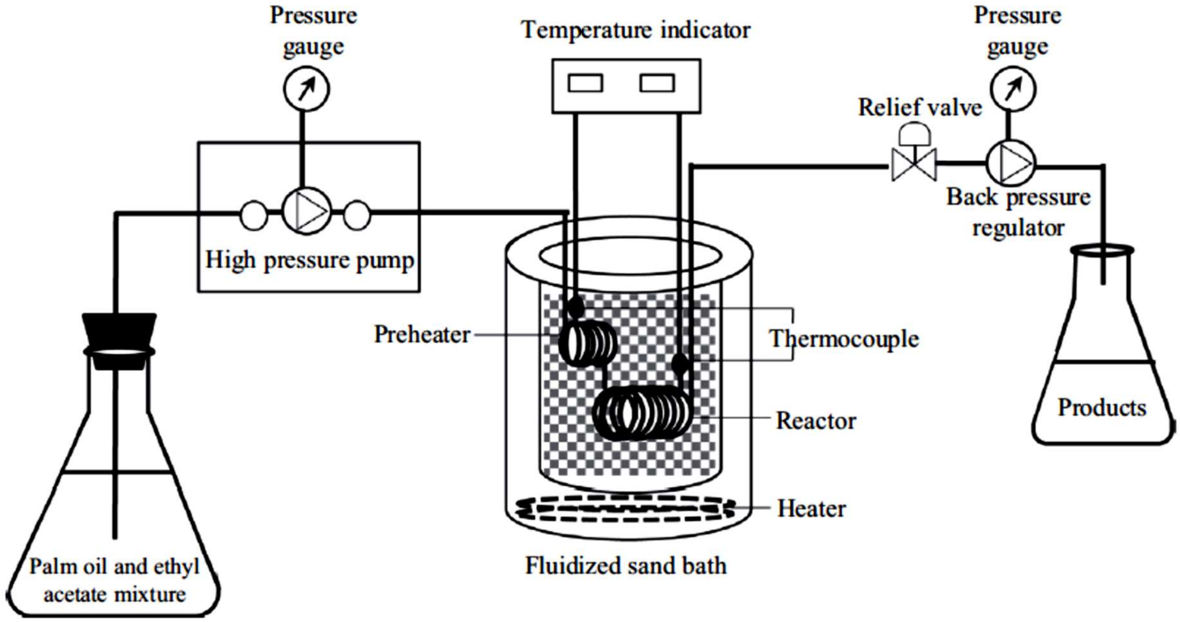


Fig. 15 Schematic diagram of continuous supercritical inter-esterification biodiesel production process [27]

Generally, the reaction is carried out at a pressure of 15 – 20 MPa at a temperature of 350 – 400°C with vegetable oil to alcohol molar ratio of 1: 30 – 1:35. In the length and diameter of reaction tube is designed in such a way that it provides the necessary holding time or the reaction to occur [27].

A number of factors like the reaction temperature, pressure, oil to alcohol molar ratio, water concentration etc. significantly affects the reaction time and biodiesel yield.

4.2.1 Effect of temperature

With the increase in the reaction temperature above 300°C the reaction rate of inter-esterification increases considerably but at a temperature below 380°C, there is hardly any production of FAME. Also when the temperature is increased from 350°C to 380°C there is a decrease in the amount of FAME produced. This can be due to the fact that at temperature more than 350°C the unsaturated fatty acids break down and a lower yield is obtained even after a reaction time of 45 minutes. The effects of temperature can be represented as below [28].

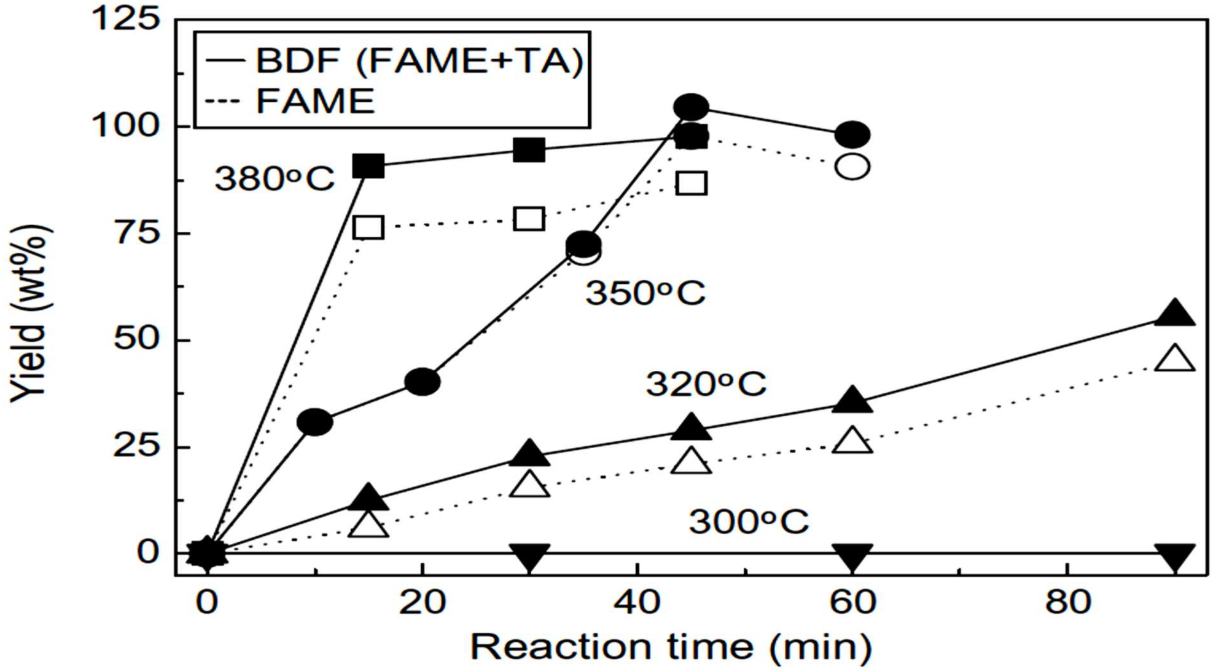


Fig. 16 Plot showing the biodiesel yield at different reaction temperatures for methyl acetate to oil molar ratio 42:1 and reaction pressure 20MPa[28]

4.2.2 Eff1ect of pressure

The higher reaction pressure results in the increased colleges in rate between the reactants. Therefore, with the increase in reaction pressure from 12 MPa to 18 MPa the reaction time further increased. The pressure is kept constant in the reaction tube using a back pressure regulator. The rate of triacetin production increases as we increase the pressure from 14 MPa to 16 MPa beyond this an increase in pressure has negligible effect on the TA yield. But FAME production percentage get noticeably reduced after a pressure increase beyond 16 MPa. The effect of pressure variation can be seen as depicted by the figure 17 [27].

4.2.3 Effect of triglyceride to methyl acetate molar ratio

Like temperature and pressure the triglyceride, tile acetate molar ratio has a significant effect on the yield (FAME+TA). It has been seen that a molar ratio of 1:30 has the maximum yield. Initially by increasing the triglyceride to methyl acetate molar ratio there is an increase in the yield. This can be due to the fact that higher the molar ratio higher will be probability of collisions between

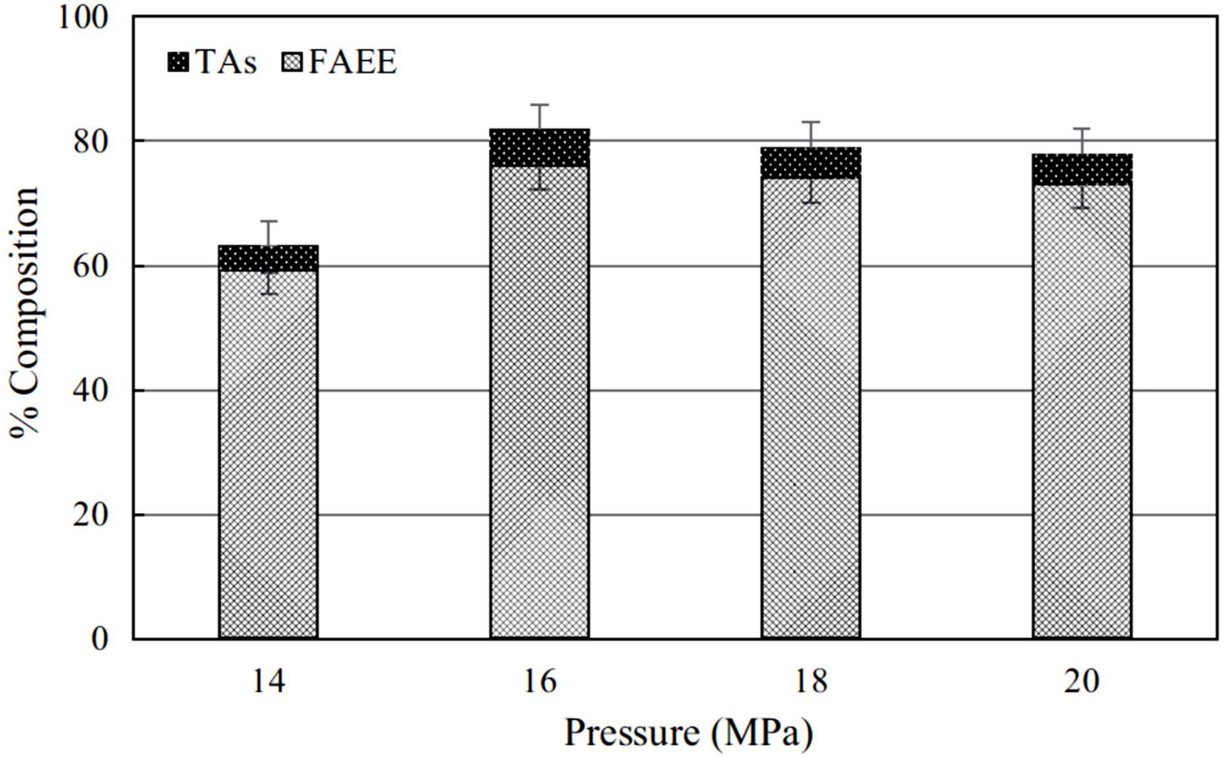


Fig. 17 Variation in FAME & TA yield at different reaction pressures for 350°C temperature and 30:1 ethyl acetate to oil molar ratio [27]

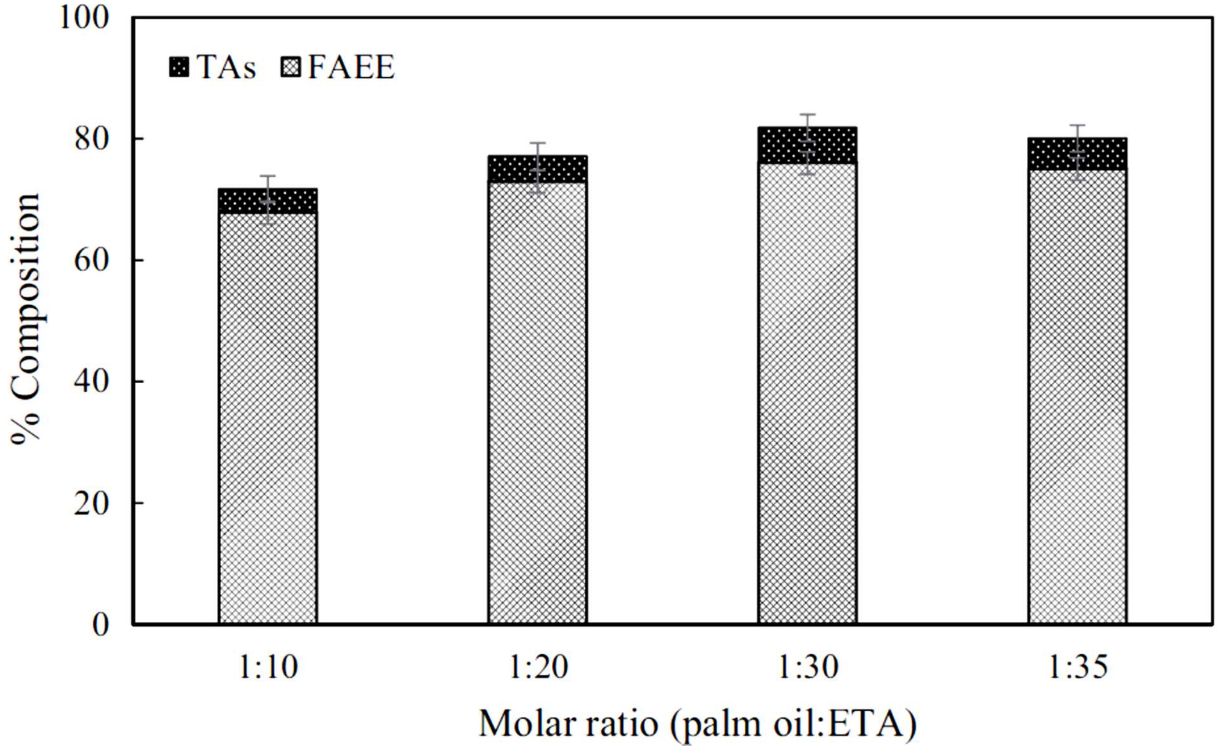


Fig. 18 Variation of biodiesel yield with change in ethyl acetate molar ratio at 350°C and 16 MPa [27]

triglyceride and methyl acetate, therefore higher will be the reaction rate [27]. But beyond molar ratio of 1:30 is slight decrease in yield has been noticed as shown in the figure 18.

4.2.4 Effect of water molar ratio

In the supercritical methanol trans-esterification we have seen that there is no effect of moisture presence in feedstock on the biodiesel yield but in case of supercritical methyl acetate it has been seen that water presence effects significantly the biodiesel yield. The presence of water has no effect on the TA yield but with increase in molar ratio of oil to water and increase in FAME yield has been noticed. Yield is maximum for oil to water molar ratio of 1:10 but further in a creasing the molar ratio leads to the reduction of yield [27]. This can be depicted by the figure 19.

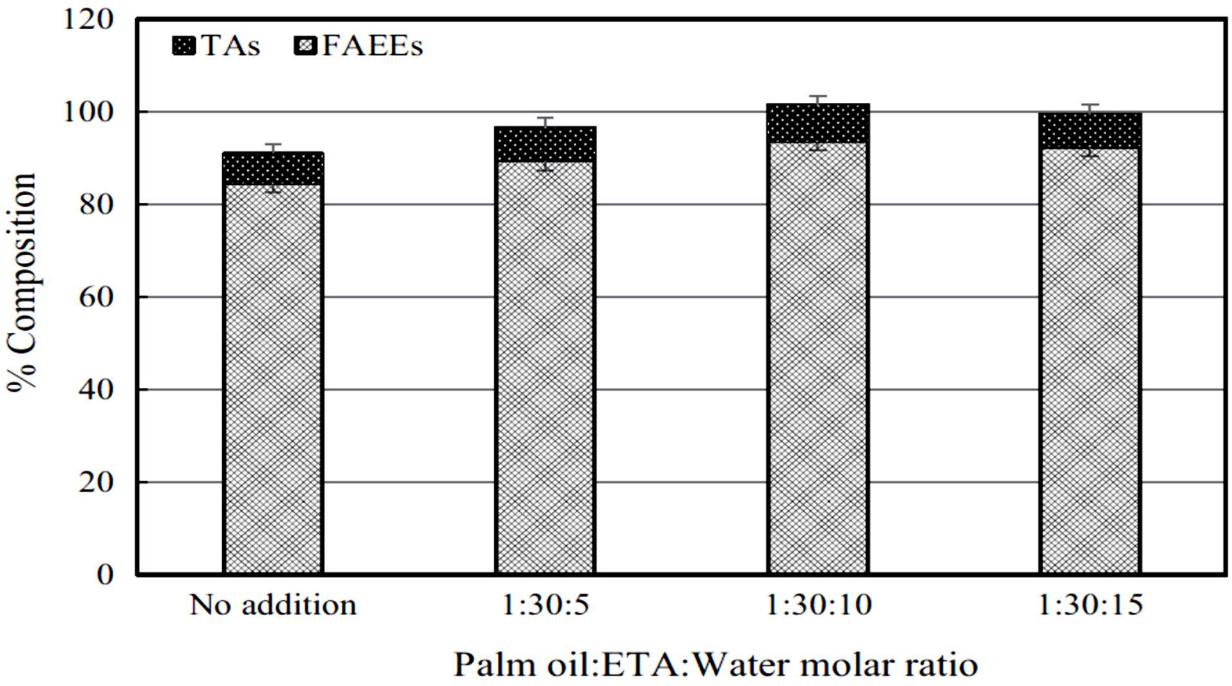


Fig. 19 Effect of water concentration on biodiesel (FAME + TA) yield at temperature 350°C and 16 MPa pressure [27]

4.3 Enzymatic Inter-esterification

The biodiesel production using lipase is enzyme is gaining the interest nowadays. Its main objective is to cover the drawbacks of conventional biodiesel production processes that involves the recovery and treatment of reaction products for purification and separation which indeed is a complex and cumbersome practice. The trans-esterification using enzyme as catalyst is performed at room temperature say 40°C. It can be done with ultrasonic or hydrodynamic cavitation aid in order to decrease the reaction time by a drastic factor. The enzyme catalyzed inter-esterification is more advantageous then the chemical synthesis route in a way such as moderate reaction environment, catalyst reusability, easy catalyst separation, simplified crude biodiesel production etc. the only drawback is long reaction time due to slow process and the higher cost of a mobilized enzyme.

Short chain alcohols are mostly used during the enzyme catalyzed trans-esterification, however the excess quantity of methanol in the reaction causes enzyme deactivity and thus in inhibits the process. This limits the use of enzyme catalyzed process in the commercial scale production [16]. Replacing the short chain alcohols like methanol, ethanol etc. with methyl acetate or ethyl acetate as acyl acceptor are so most of these issues inherited with enzyme catalyzed biodiesel production. The new process is called inter-esterification.

Using with methyl acetate as acyl acceptor byproduct formed is triacetin instead of glycerol as in case of conventional trans-esterification. As compared to glycerol triacetin has great commercial value due to the fact that it is widely used as additive in pharmaceutical, tobacco andis cosmetic industry. Moreover, if triacetin is not separated and left as it is in the final reaction product, it doesn’t degrades the biodiesel fuel properties much. Also, no adverse effect of triacetin has been noticed on enzyme activity [16].

Another advantage of enzyme catalyzed inter-esterification is that the waste cooking oil is feedstock can also produce higher yields. This significantly reduce the biodiesel cost to a huge extent if waste cooking oil is used as feedstock.

Enzyme catalyzed inter-esterification can be performed in two ways –

- Enzyme catalyzed inter-esterification without ultrasonic aid
- Ultrasonic assisted enzyme catalyzed inter-esterification
- Inter-esterification without ultrasonic aid

4.3.1 Enzyme catalyzed inter-esterification without ultrasonic aid –

The conventional method of enzyme catalyzed inter-esterification involves the reaction of triglycerides with methyl/ ethyl acetate at 35 – 40°C and atmospheric pressure. The reaction is catalyzed by a mobilized lipase enzyme. The lipase is immobilized so that it can be easily separated from the reaction products and reused again. The only drawbacks of the matter are its longer reaction time and cost of lipase enzyme.

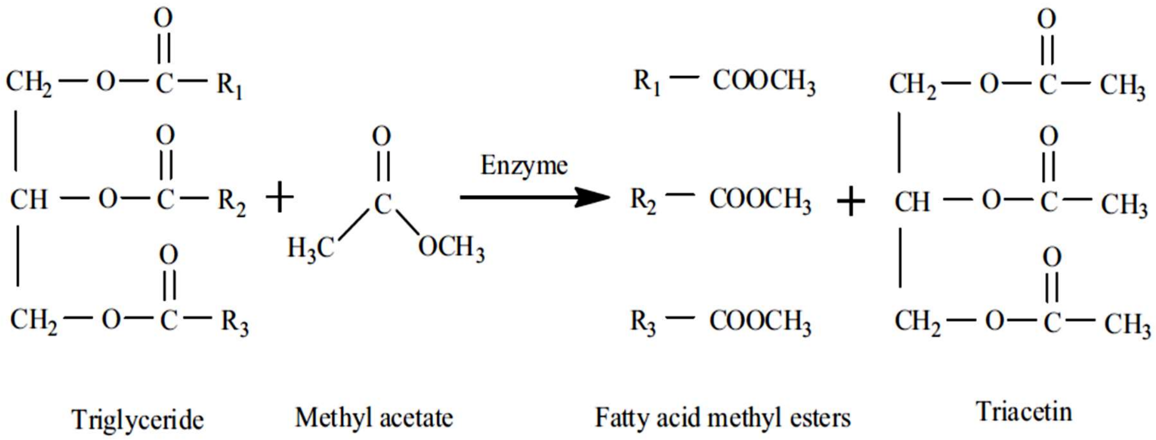


Fig. 20 Reaction involved during enzymatic inter-esterification [16]

Conventional approach involves a reactor equipped with the magnetic stirrer and few baffles fixed to avoid formation of vortex. A water bath is used to maintain the constant temperature during the process. Lipase enzyme is loaded to the methyl acetate and oil mixture after reaching the desired reaction temperature [16]. The reaction is then stirred for hours say 30 – 35 hours to achieve the complete conversion of triglycerides.

Effect of various reaction parameters

4.3.1.1 Oil to methyl acetate molar ratio – the triglyceride to methyl acetate molar ratio just enough to complete the reaction is 1:13. But the reaction is reversible in nature and to shift the equilibrium in forward direction extra mole of methyl acetate is are added to the reaction. It has been noticed that with increase in molar ratios the reaction rate increases and so the yield. But such increase is limited to a particular triglyceride to methyl acetate molar ratio and beyond this the yield de and a creases significantly. Generally, a 12:1 oil to ethyl acetate molar ratio is found optimum but in more cases it may increase to 18:1 [16]. A bar chart showing the variation of yield at various vegetable oil to methyl acetate molar ratio in case of rapeseed oil is shown in figure below.

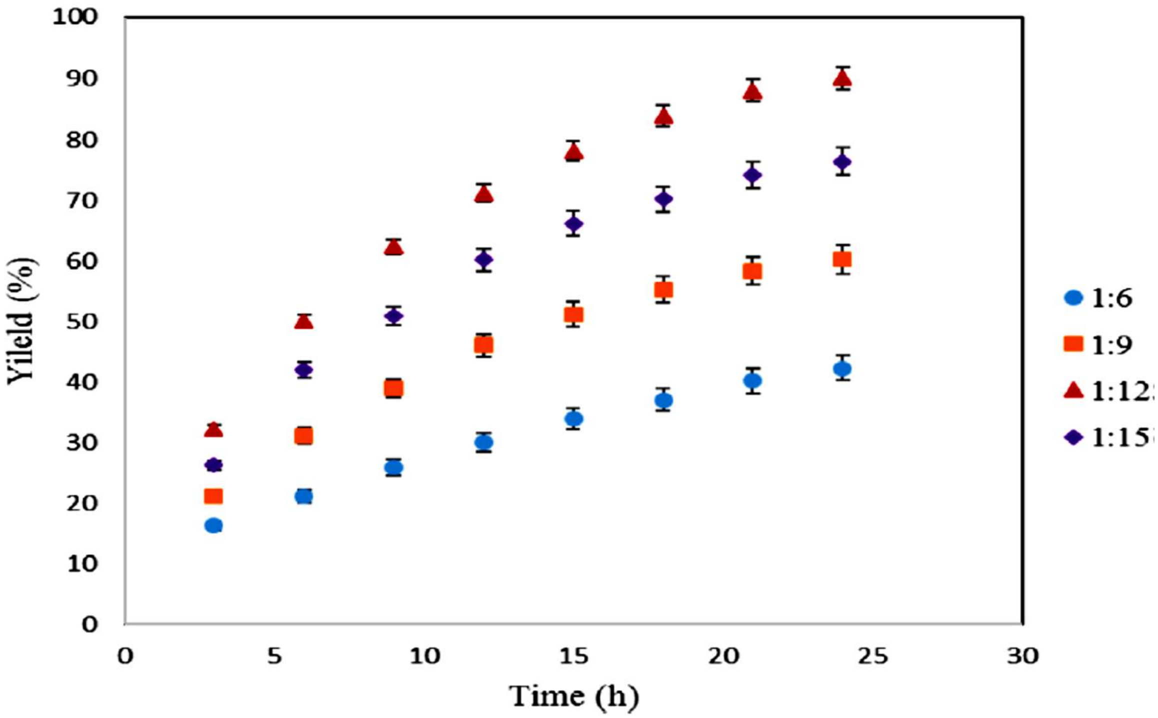


Fig. 21 Effect of methyl acetate to oil molar ratio on biodiesel yield [16]

4.3.1.2 Effect of temperature – reaction temperature is also the most affecting factor on the biodiesel yield. The optimum temperature is found to be 40°C. With increase in temperature the energy of molecules got increased and thus the collision frequency. But all this happen in this way up to 40 – 45°C depending on the species of lipase enzyme used is catalyst. As the temperature increases it starts damaging the enzyme by changing its structure and this will lead to reduced activity of enzyme. The temperature effect can be depicted in a graphical manner by the figure 22 [16].

4.3.1.3 Effect of enzyme loading – enzyme loading refers to the amount of enzyme added per unit of feedstock. Increasing the enzyme loading increases the number of active sites which leads to increase in biodiesel yield. This increases in yield is not taste up to a certain value beyond this increased enzyme loading has no effect on yield. Generally enzyme loading varies between 2% to 8%. Optimally 6% enzyme loading is found to be most economical with a yield greater than 90%.

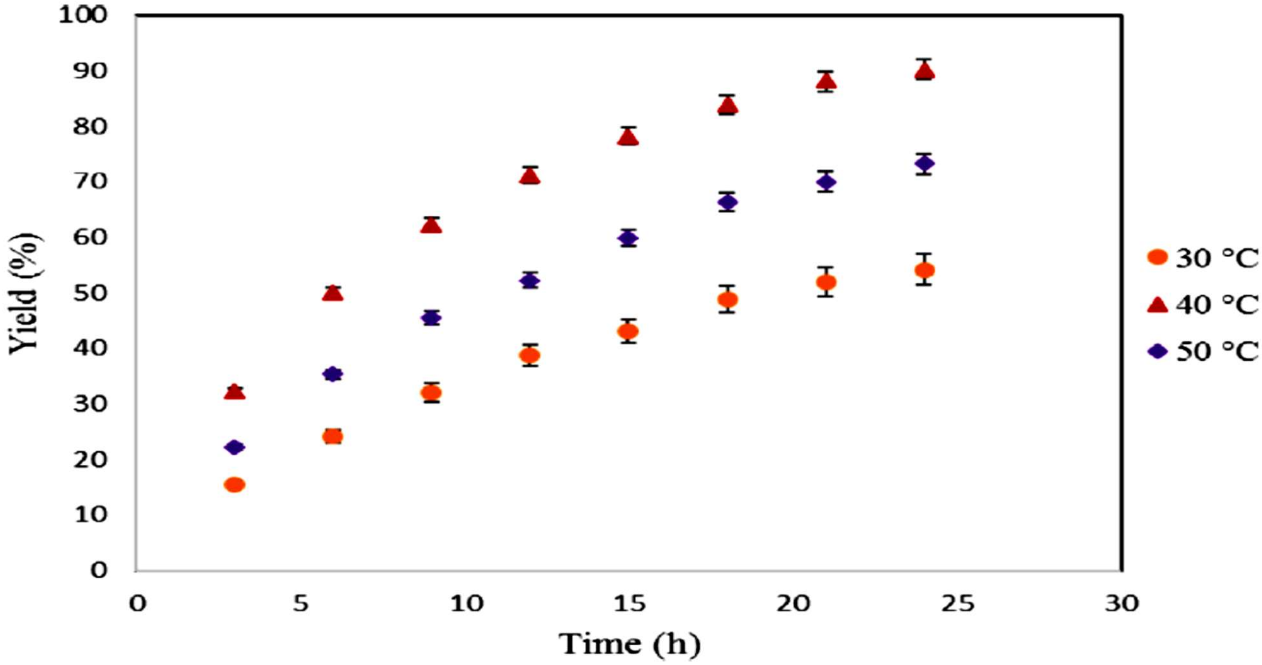


Fig. 22 Effect of process temperature on biodiesel yield [16]

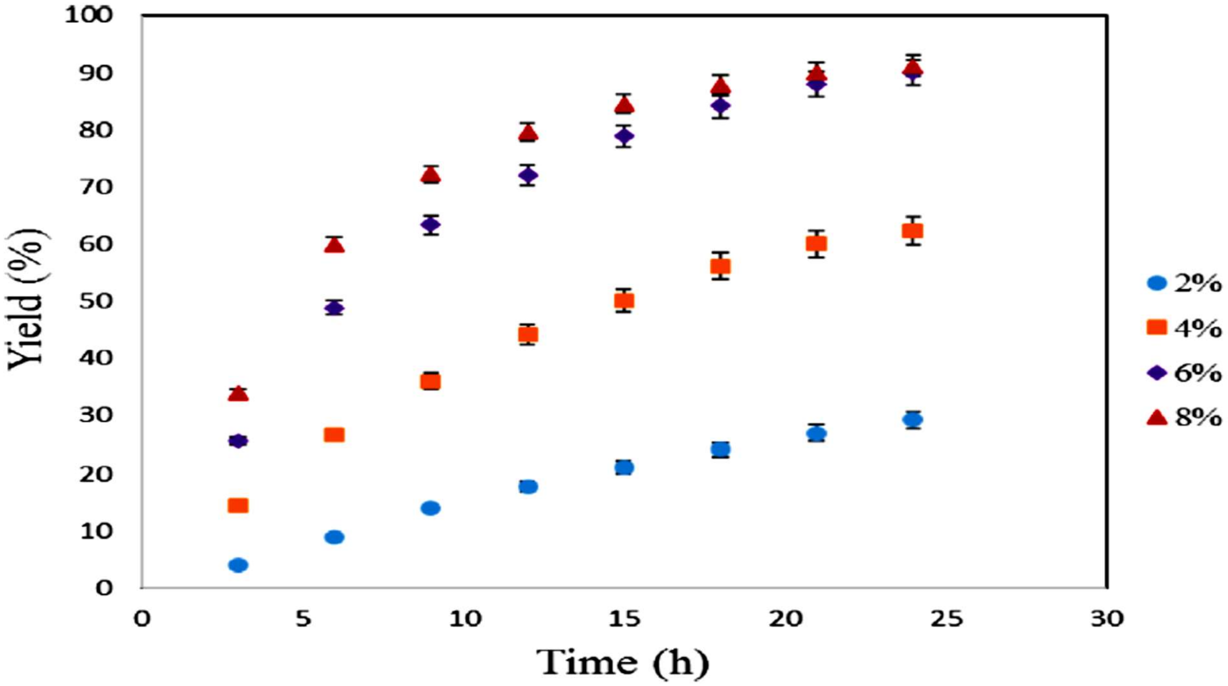


Fig. 23 Effect of enzyme loading on biodiesel yield [16]

Chapter 5

Ultrasonic assisted enzymatic inter-esterification

The use of ultrasonic cavitation is an effective tool to decrease the reaction time of enzyme catalyzed inter-esterification products of biodiesel. The ultrasonic waves produced by generator horn induces the intense turbulence on microscopic level across the reaction medium and increases the frequency of collisions among reactants. Thus this induced turbulence leads to a shorter reaction time for ester conversion. Whereas in case of conventional enzymatic synthesis of biodiesel no such turbulence is introduced. However turbulence is there due to magnetic stirrer but on a macro scale [16]. Thus even after mechanical stirring in conventional enzymatic inter-esterification the reaction rates are very high.

5.1 Equipments employed

5.1.1 Experimental setup for ultrasonic assisted enzymatic inter-esterification

The experiments for ultrasonic assisted inter-esterification were performed using a setup which is nothing but an electrical - mechanical transducer arrangement which converts the electrical signal to ultrasonic waves. The setup comprises of following equipments –

a) **Ultrasonic generator** – A 120 Watt ultrasonic generator manufactured by *Thoroughclean Ultrasonic (India) Pvt. Limited* has been used to perform the experiments. The generator was equipped with a power regulation knob with markings from 10% - 100% of rated power to adjust the working power for generating ultrasonic waves. The generated is designed to generate the ultrasonic waves of frequency 20 KHz. A timer is also mounted to adjust the duty cycle of generator. For eg. a duty cycle of 60% means that the generator remains ON for 60% of the total time say for 3 minutes out of 5 minutes of total time. After 5 minutes the machine has to be started again by pressing a red colored starting button in the front.

b) **Ultrasonic Horn** – The generator is connected to an ultrasonic horn via a cable which transmits the signal to the horn. The horn is a rod shaped metallic bar mounted on a stand with a provision of height adjustment. The bottom end of the horn is little protruding radially along circumference to transmit the ultrasonic waves into the reaction medium. The upper part of the ultrasonic horn is fixed in a hollow metallic housing on the top of which a fan is mounted for warm air exhaust.

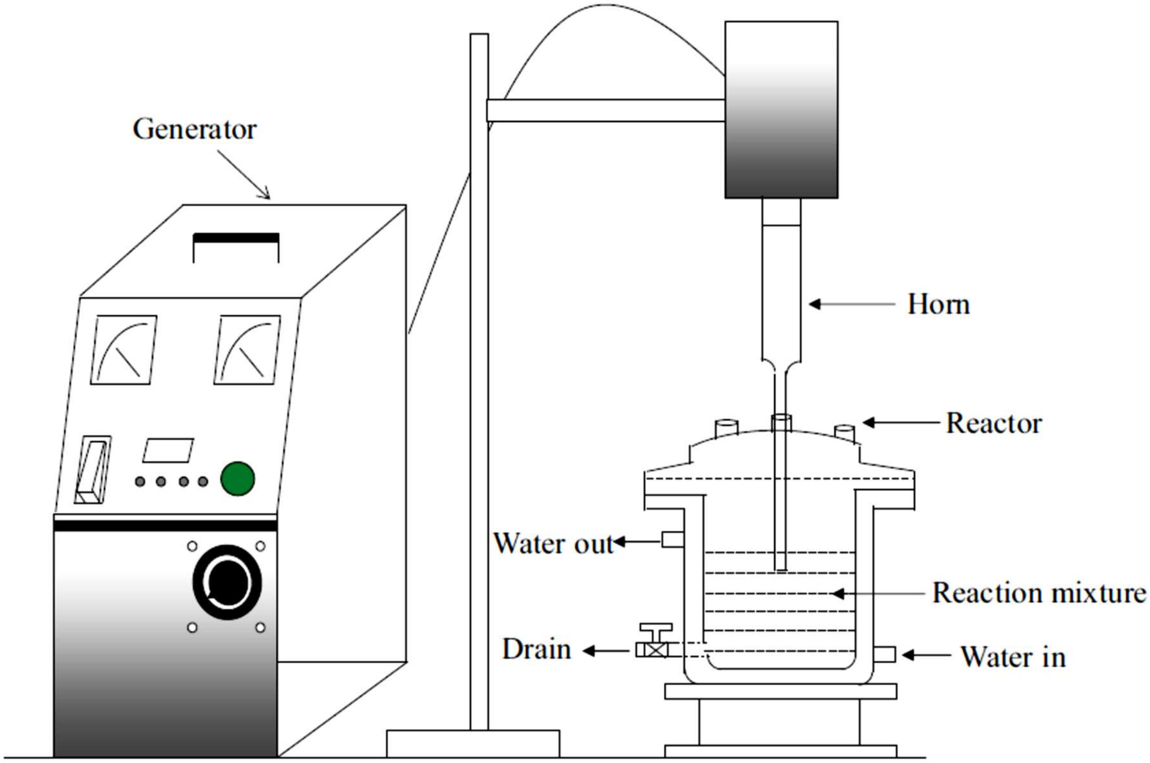


Fig.24 Experimental equipment setup of ultrasonic assisted enzymatic inter-esterification process in laboratory

c) **Adjustable reactor stand –** A stand with height adjustment function was there to support the reactor. The height is adjusted such that the horn remains dipped for about 0.75” - 1” in the reactant mixture all the time.

d) **Reactor with water bath –** A 100ml Borosil reactor facilitated with water bath is used to carry out inter-esterification reaction. The water bath is used to maintain a constant process temperature. The process temperature has to be maintained content as due to ultrasonic wave the temperature of reaction medium rises which can cause the enzyme deterioration.

The reactor was sealed by a thermo coal seal to make it air tight. This was done to prevent the volatile methyl acetate to evaporate during the process.

e) **Digital Thermometer –** A digital thermometer is mounted on the reactor to measure the reaction medium temperature precisely.

5.1.2 Hydrometer

An Anton Paar DMA 4500 hydrometer was used to measure the specific gravity of biodiesel produced. A 15ml sample is injected into the apparatus after cleaning the tubing with acetone. The specific gravity of biodiesel was measured taking water as standard fluid.

Fig. 25 Hydrometer

5.1.3 Kinematic viscometer

Petrotest Viscobath manufactured by SCHOTT Instruments, Mainz is used to measure the kinematic viscosity of liquids. The specification of capillary tube used is designated as *527 13/Ic.*

The kinematic viscosity of biodiesel was measured at 40° C. A 20ml biodiesel sample liquid column was allowed to pass through a narrow capillary opening vertically and time was measured by stopwatch. The kinematic viscosity was calculated by using empirical formula explained later in this section.

Fig. 26 Kinematic viscometer

5.1.4 Bomb calorimeter

Parr 6100 Calorimeter was used to find the calorific value of the fuel. The measured mass of biodiesel fuel was burned in supply of oxygen at suitable pressure and the rise in water temperature was noticed. The calorific value of the fuel was calculated by empirical formula explained later in the section.

$$\text{Calorific value} = m_w \times c_p \times \Delta t$$

Fig. 27 Bomb Calorimeter

Where m_w is mass of water taken (*generally 2 kg)*

c_p is specific heat of water at cont. pressure

Δt is the temperature difference of water

5.1.5 Pour point and cloud point cryostat

A cryostat is used to measure the pour point and cloud point of a fuel. The pour point is the temperature at which the fuel ceases to flow i.e. below this temperature fuel can't flow. The cloud point for any fuel is the temperature below which wax in a fuel forms a cloudy appearance.

5.1.6 Rotary evaporator

Heidolph rotary evaporator was used to separate excess methyl acetate from the biodiesel and triacetin mixture. The final product mixture was heated to 60° C in vacuum for 1 hour in rotary evaporator and the excess methyl acetate was evaporated out.

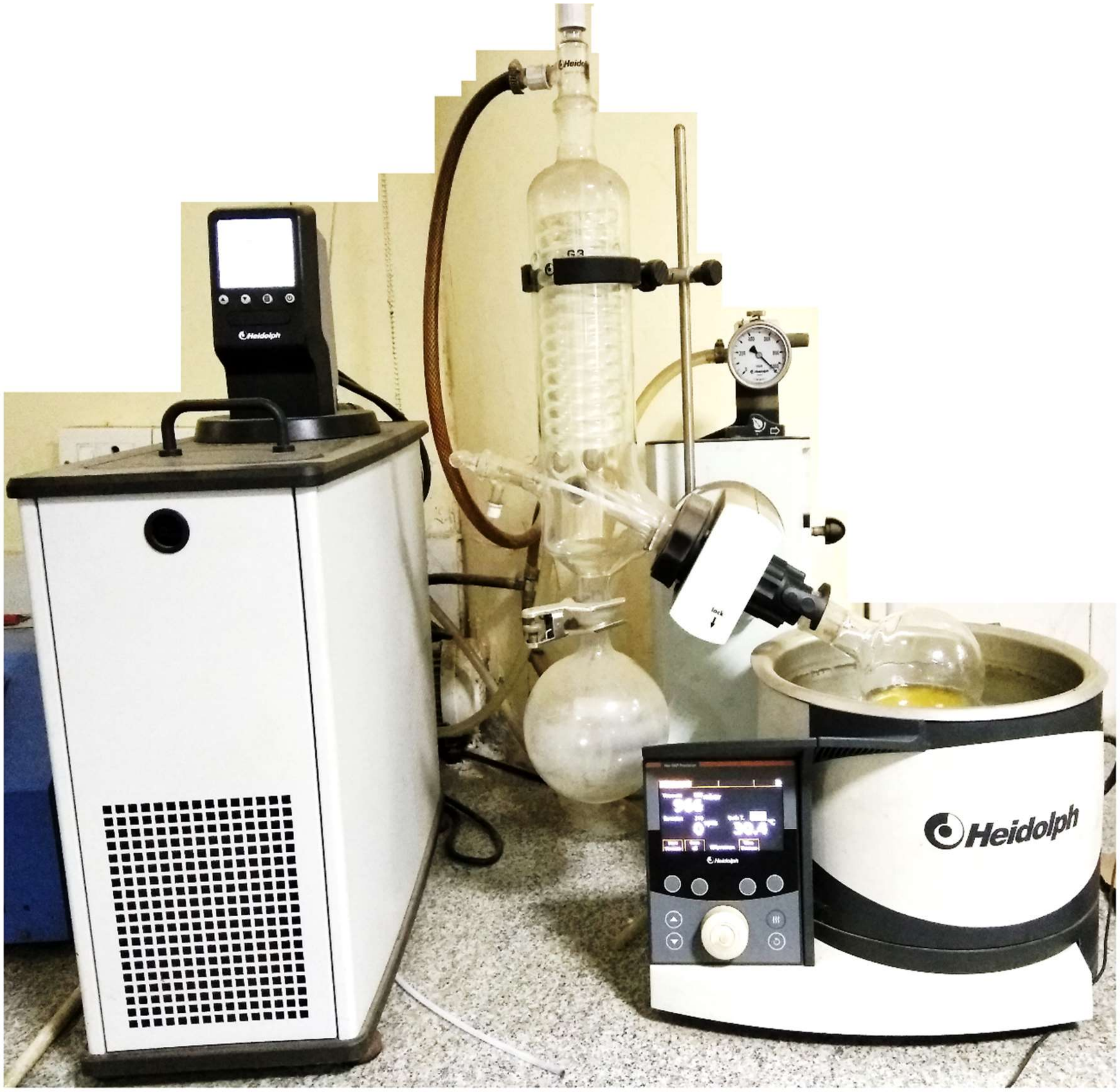


Fig. 28 Rotary Evaporator

5.2 Methodology

The detailed procedure is divided in two parts viz. preparation of phosphate buffer solution and biodiesel synthesis. A pH 8 phosphate buffer solution was prepared to maintain the constant pH of the reactant mixture throughout the reaction period.

Preparation of buffer solution for enzyme –

Step 1 – The 0.667 g of 99% Na_2HPO_4 and 0.0414 g of 99.2% $NaH_2PO_4.2H_2O$ was weighed.

Step 2 – Both the salts are added to 100 ml of distilled water.

Step 3 – The mixture is than stirred gently for 5-6 min. to obtain 0.1 M pH 8 phosphate buffer.

Step 4 – The pH of the solution is checked with pH meter.

The experiment for biodiesel synthesis by ultrasonic enzymatic inter-esterification is stepped as follows –

Step 1 – The oil sample was filtered to remove the solid impurities and other contaminants.

Step 2 – Oil sample was heated to 130^{o} C to remove the moisture from the oil.

Step 3 – A 50 g oil sample is weighed in 200 ml glass beaker.

Step 4 – Methyl acetate of desired methyl acetate to oil molar ratio is taken.

Step 5 – Buffer solution 10% by volume of oil is taken and the enzyme is added to it.

Step 6 – The buffer and enzyme solution was added to methyl acetate and kept for 15 min.

Step 7 – Measured oil sample at a particular temperature is taken in the reactor flask and mixture of methyl acetate, enzyme and buffer are added to it.

Step 8 – The ultrasonic generator was set at desired power level and duty cycle.

Step 9 – The mixture was allowed to keep under the ultrasonic generator for measured reaction time.

Step 10 – The temperature was kept constant by using a water bath.

Step 11 – The samples of 0.5 ml were frequently drawn as analyzed using gas chromatography for the analysis of reaction progress.

Step 12 – After measured reaction time the sample was filtered by filter paper.

Step 13 – The methyl acetate is removed filtered sample by using vacuum rotary evaporator for 60 minutes.

Step 14 – The final mixture was analyzed using gas chromatography.

5.3 Calculation and Empirical relations

- **No. of mole calculation**

$$\text{No. of mole of substance} = \frac{\text{Weight of substance sample taken in grams.}}{\text{Gram molecular weight of substance}}$$

Gram molecular weight of Linseed oil sample was taken as 887 g/mol.

- **Density calculation**

The density calculation was done using Aston Paar DMA 4500. The density readings were obtained in g/cm^3.

- **Kinematic viscosity calculation**

$$\text{Kinematic viscosity} = \underline{K \text{ x } t} \quad mm^2/sec.$$

Where, K = Capillary constant = 0.02672 mm^2/sec.^{2}
t = time (in sec.)

- **Calorific value calculation**

The calorific value was measured by Aston Paar 6100 bomb calorimeter. The 0.8 gm weighed biodiesel sample was used for calorific value measurement. The fuel was burned in pressurized supply of oxygen. The calorific value obtained is lower calorific value of the biodiesel fuel. The readings were displayed

5.4 Taguchi design method

Taguchi method is based on the statistical methods for the evaluation of the sensitivity of a set of response variables or output to a set of independent variables (control parameters). This can be done by setting the experiments in an orthogonal array aiming to attain the optimum setting of control parameters. Orthogonal arrays help us to obtain the best set of balanced (minimum) experiments. The table 4 shows the standard orthogonal arrays with respect to a various number of factors and number of columns. For ex. L_4 orthogonal array has 4 rows and 3 'Level 2' columns. Similarly, L_9 orthogonal array has 9 rows and 4 'Level 3' columns. The number of rows represents the minimum number of experiments required to perform the calculations. The number of rows must be equal to the DOF associated with control variables.

Generally,

$$\text{DOF (Degree of Freedom)} = \text{No. of levels} - 1$$

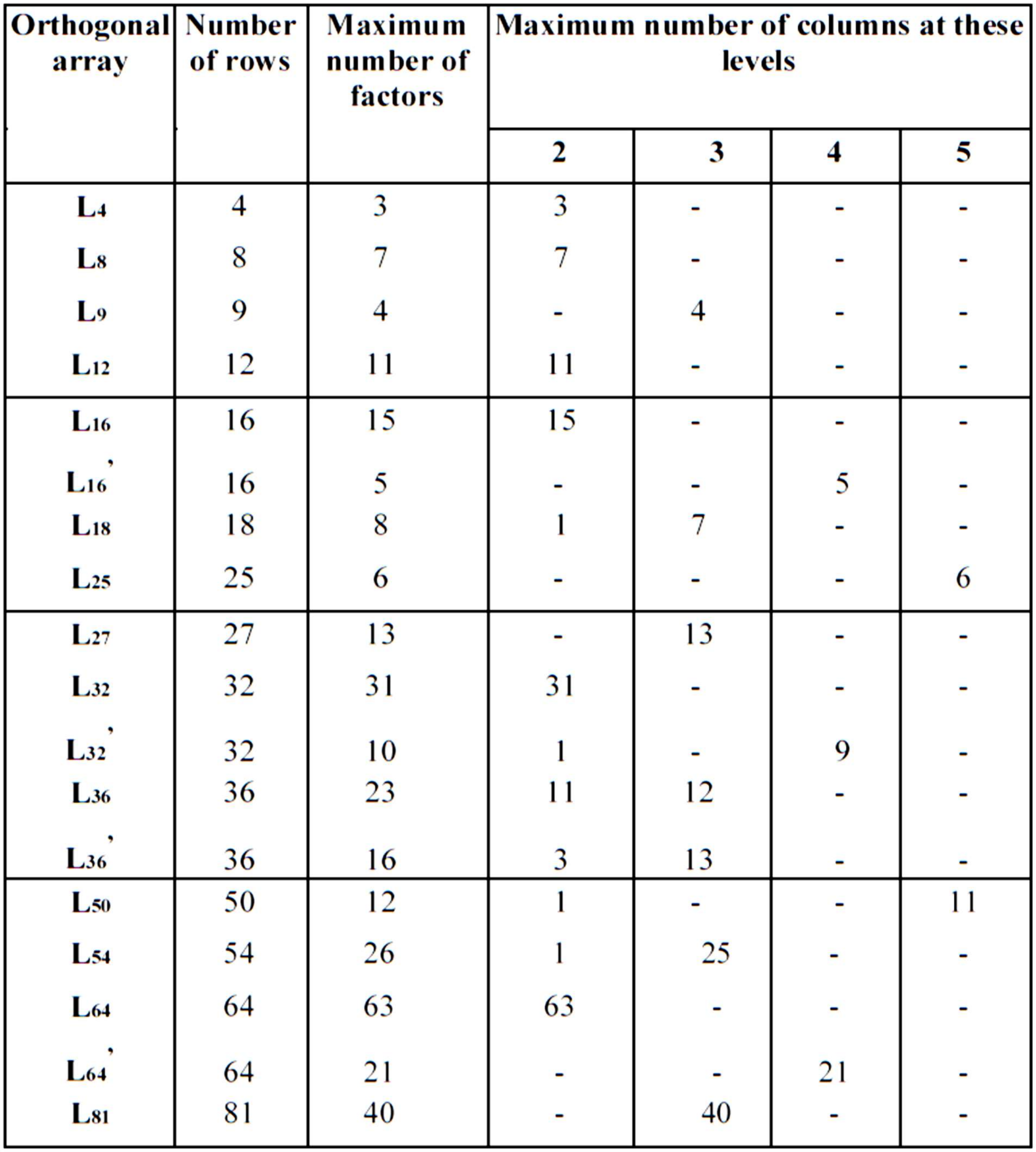
The number of columns in an array represents the maximum limit of factors that can be studied with the selected array.

The S/N ratios (signal to noise ratio) serve as the objective function. These are the log function of our desired output result. There are two types of optimization problems i.e., static problems and dynamic problems.

5.4.1 Static problem

When a number of control factors influence the process to be optimized and in turn directly decides the target value of the output. The optimization involves evaluating the best levels of control factors so that the output comes as the target value. Such a problem can be termed as '*Static problem'* which can be better explained using a P-Diagram (process diagram). The noise can be present during the process but will certainly have no effect on the output function, which is the prime objective of Taguchi method. That is why this method sometimes called ‘ROBUST Method’.

Table 4 Standard orthogonal arrays



Orthogonal array	Number of rows	Maximum number of factors	Maximum number of columns at these levels			
			2	3	4	5
L_4	4	3	3	-	-	-
L_8	8	7	7	-	-	-
L_9	9	4	-	4	-	-
L_{12}	12	11	11	-	-	-
L_{16}	16	15	15	-	-	-
L_{16}'	16	5	-	-	5	-
L_{18}	18	8	1	7	-	-
L_{25}	25	6	-	-	-	6
L_{27}	27	13	-	13	-	-
L_{32}	32	31	31	-	-	-
L_{32}'	32	10	1	-	9	-
L_{36}	36	23	11	12	-	-
L_{36}'	36	16	3	13	-	-
L_{50}	50	12	1	-	-	11
L_{54}	54	26	1	25	-	-
L_{64}	64	63	63	-	-	-
L_{64}'	64	21	-	-	21	-
L_{81}	81	40	-	40	-	-

5.4.2 Dynamic problem

In a dynamic Taguchi design problem, the quality characteristics or the output function operates along the range of values or in other words if the output has a signal which directly affect the output. In such a problem design our goal is to improve the relationship between the output function and a signal factor.

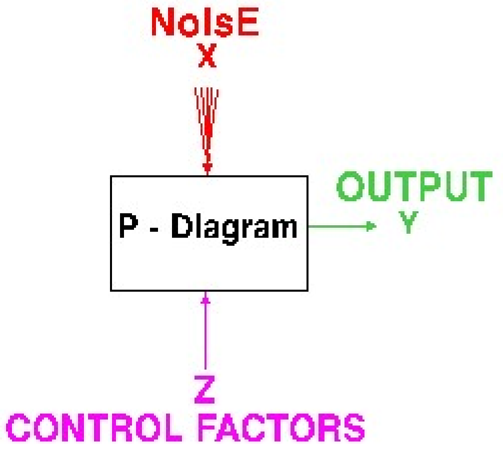


Fig. 29 P-Diagram for Static problem

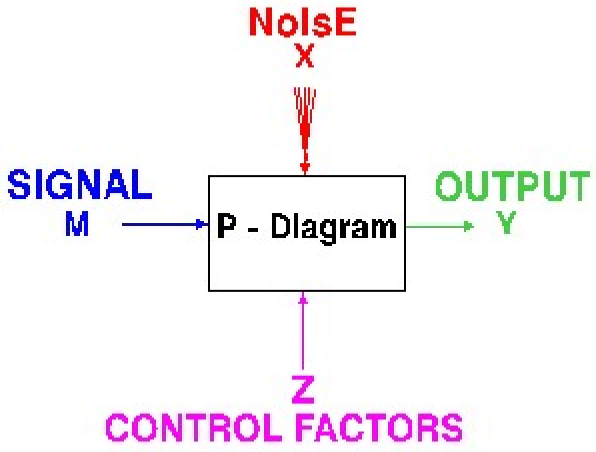


Fig. 30 P-Diagram for Dynamic problem

S/N ratio (Signal to Noise ratio)

There are three types of S/N ratios commonly used for the optimization of static problems. We'll discuss here only the static problems of Taguchi methods as related to our research project.

1. Smaller-the-better
2. Larger-the-better
3. Nominal-the-best

5.4 Application of Taguchi method

Generally, a static Taguchi problem can be solved in following steps –

- i) Identify the objective function, side effects and failure mode
- ii) Identify the testing conditions and quality characteristics
- iii) Identify the control factors and their levels
- iv) Select the orthogonal array matrix experiment
- v) Conduct the matrix experiment

vi) Analyze the data, predict the optimum levels and performance

Our objective function is to maximize the biodiesel yield. Reaction temperature (° C), MAOMR, catalyst loading and reaction time (min.) were selected as the four process control factors. The L9 orthogonal array was selected in Minitab for the process control factor optimization of maximum biodiesel yield.

Table 5: Factors and their levels

Factors		Levels		
		1	2	3
A	Reaction Temperature (° C)	30	40	50
B	MAOMR	4:1	6:1	9:1
C	Catalyst loading	0.5%	1.0%	1.25%
D	Reaction time (min.)	60	90	120

Table 6: L_9 array matrix experiment table

Exp. No.	**Column no. & factor assigned**				**Kinematic Viscosity k (mm^2/sec.)**
	Reaction Temperature (° C) (A)	**MAOMR (B)**	**Catalyst loading (C)**	**Reaction time (min.) (D)**	
1	30	6	0.5%	60	12.5
2	30	9	1.0%	120	10.2
3	30	12	1.25%	180	5.13
4	40	6	1.0%	180	7.50
5	40	9	1.25%	60	8.60
6	40	12	0.5%	120	8.10
7	50	6	1.25%	120	6.20
8	50	9	0.5%	180	8.10
9	50	12	1.0%	60	6.40

Chapter 6

Result and Discussion

The various methods of biodiesel synthesis has been discussed in chapter 3 and chapter 4. The ultrasonic aided inter-esterification of vegetable oil using enzyme as catalyst, was chosen for the current study. The detailed methodology of different experiments performed and equipment used for the same has been elaborated in chapter 5.

The biodiesel was synthesized from Linseed oil as feedstock by ultrasonic aided enzymatic inter-esterification. Lipoprotein lipase from Pseudomonas sp. has been used as catalyst. The experiment was performed at different reaction parameters and the result was compared to analyze the effect. Taguchi design method is used to analyze the effects of different parameters on biodiesel yield. The biodiesel was also synthesized by chemical trans-esterification of Linseed oil. The fuel properties of biodiesel obtained from chemical trans-esterification and enzymatic inter-esterification were compared. The results obtained are discussed below.

6.1 Result Validation

The experimental results have been validated for the ultrasonic assisted enzymatic inter-esterification. The kinematic viscosity is taken as the indication of triglyceride conversion to biodiesel. The results obtained are tabulated below in Table 4.

Table 7 Properties of biodiesel fuel (FAME+TA) from enzymatic inter-esterification

Property Description	**Unit of measurement**	**Experimental value**	**Literature's value****
Specific gravity	-	0.8903	0.921
Density	g/cm^3	0.8895	0.92
Kinematic viscosity	mm^2/sec.	5.13	4.5
Cetane Number*	-	-	64.5
Pour point	ºC	-12	-18
Cold filter plug point	ºC	-10	-17
Calorific value	Cal/g	9414.95	-

Table 8 Properties of biodiesel obtained from chemical trans-esterification of Linseed oil

Property Description	Unit of measurement	Experimental value	ASTM D-6751 specification[29]
Specific gravity	-	0.8782	-
Density	g/cm^3	0.8774	-
Kinematic viscosity	$mm^2/sec.$	3.1942	1.9-6.0
Cetane Number*	-	-	≥ 47
Pour point	ºC	-16	-
Cloud point	ºC	-15	-
Calorific value	Cal/g	9045.94	-

*Cetane number is taken from literatures and not experimentally calculated.

**The data is corresponding to biodiesel from rapeseed oil

6.2 Parametric study

Based on the experiments performed, a study of various parameters on the biodiesel kinematic viscosity has been done by using Taguchi design methods. The detailed parametric study is presented here.

6.2.1 Effect of methyl acetate to oil molar ratio

The methyl acetate to oil molar ratio (MAOMR) is a major governing factor for the biodiesel yield. The MAOMR is varied from 6:1 to 12:1 as 6:1, 9:1 and 12:1. The corresponding viscosity has been investigated and plotted as shown in fig. 31. The viscosity gradually decreases with increase in MAOMR reason being the increased molar ration increases the collision frequency among reactants. But after 12:1 MAOMR the increase in yield was insignificant. The S/N ratio curve and mean viscosity for various molar ratios have been shown in fig. 31 below.

6.2.2 Effect of catalyst loading

The catalyst loading is meant by the weight of enzyme added per unit weight of vegetable oil. With the increase in catalyst loading the viscosity significantly decreases but all this happened up to a catalyst loading of 1% after that there was no increase in yield had been noticed. Fig. 32 depicts the variation in S/N ratio with enzyme loading such that an increase in catalyst loading from 0.5% to 1.25% there has been a decrease in S/N ratio. Therefor an increased catalyst loading of 1.25 % will be an uneconomic option.

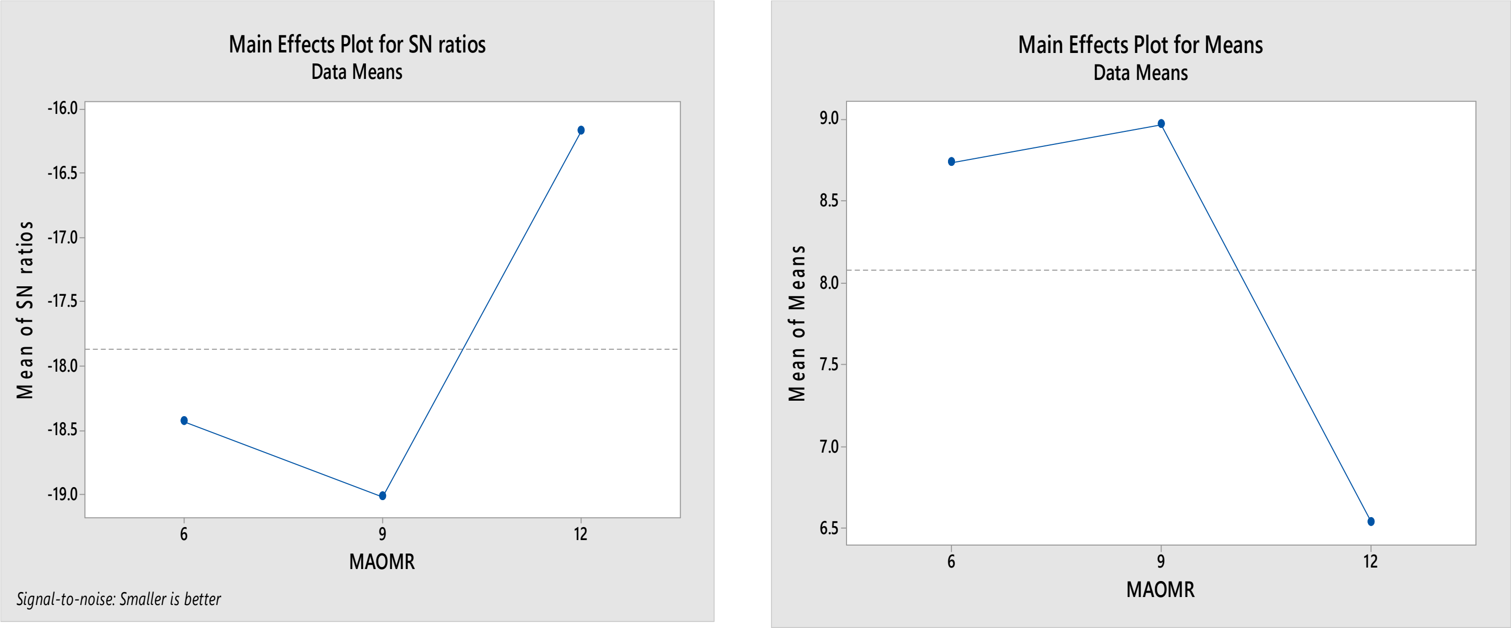


Fig. 31 S/N ratio and mean biodiesel viscosity for different methyl acetate to oil molar ratios

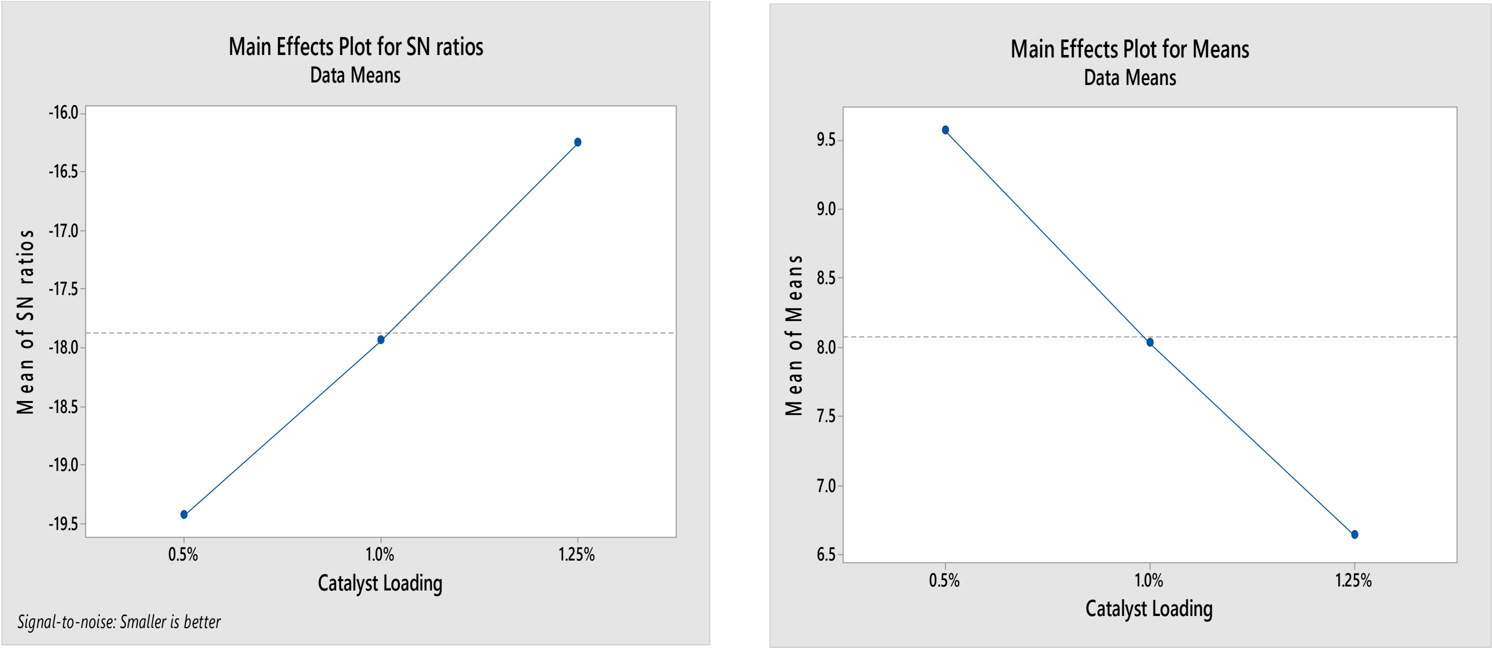


Fig. 32 S/N ratio and mean biodiesel viscosity for different enzyme loading

6.2.3 Effect of temperature

The effect of process temperature on biodiesel viscosity has been investigated for three different temperatures viz. 30 ° C, 40° C, 50 ° C. The viscosity got decreased significantly after increasing temperature from 30° C to 40° C. It is due to the fact that increased temperature increases the kinetic energy of molecules thus increases the interaction among the reactants. But further increase in temperature decreases the extent of decrease in viscosity after reaching the threshold temperature because at this temperature the enzyme works at its maximum capacity beyond which its activity starts decaying and falls substantially. A slight increase in viscosity has been observed over 50° C process temperature.

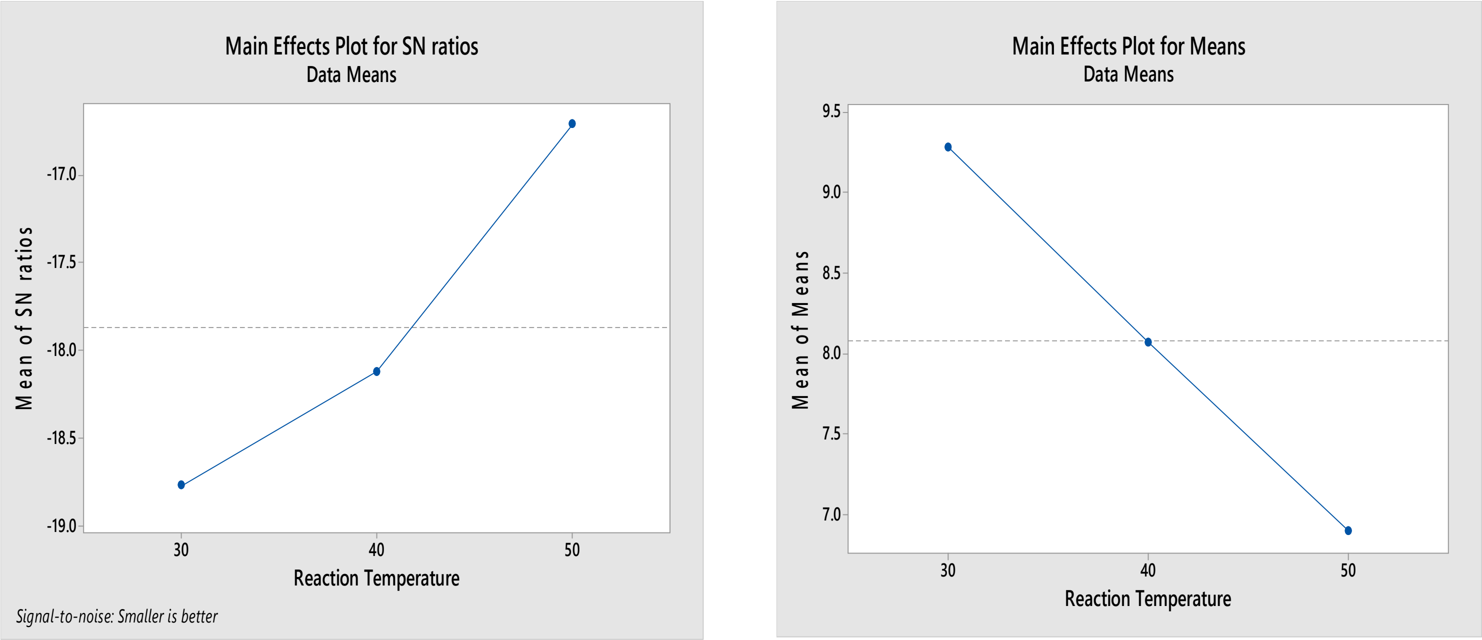


Fig. 33 S/N ratio and mean biodiesel viscosity for different reaction temperatures

6.2.4 Effect of Reaction time

The reaction time is taken in three intervals of 60 min., 120 min and 180 min. The result obtained are shown in the fig. 34 below. As depicted by S/N ratio curve there is a magnificent effect of reaction time on biodiesel viscosity when it is increased from 60 min to 120 min. but the effect got diminished for further increase in process time to 180 min. If we look at the average viscosity curve too the decrease is very low for increasing reaction time from 120 min to 180 min.

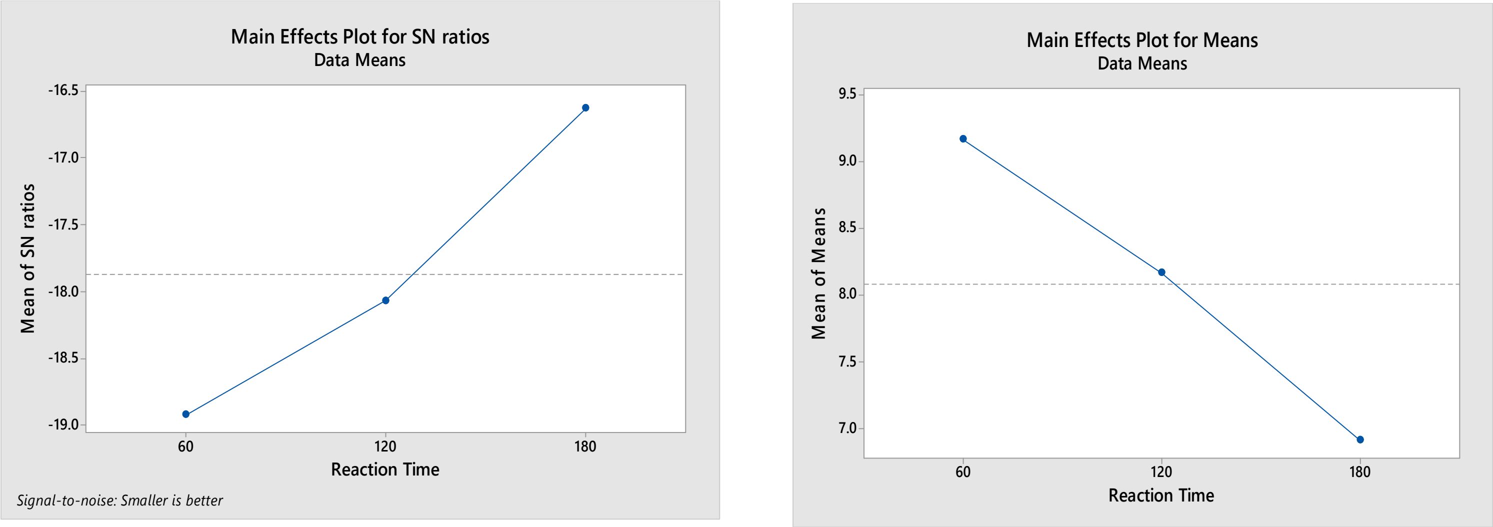


Fig. 34 S/N ratio and mean biodiesel viscosity at different reaction times

6.2.5 Taguchi design method result

Table 9 Taguchi method result table

Exp. No.	**Column no. & factor assigned**				**S/N ratio η (%)**
	Reaction Temperature (° C) (A)	**MAOMR (B)**	**Catalyst loading (C)**	**Reaction time (min.) (D)**	
1	30	6	0.5%	60	-21.938
2	30	9	1.0%	120	-20.172
3	30	12	1.25%	180	-14.202
4	40	6	1.0%	180	-17.501
5	40	9	1.25%	60	-18.690
6	40	12	0.5%	120	-18.170
7	50	6	1.25%	120	-15.848
8	50	9	0.5%	180	-18.170
9	50	12	1.0%	60	-16.124

Table 10 S/N ratios for various reaction parameters

Level	S/N Ratios for different reaction parameters			
	Temperature	MAOMR	Reaction time	Catalyst loading
1	9.277	8.733	9.567	9.167
2	8.067	8.967	8.033	8.167
3	6.900	6.543	6.643	6.910
Delta	2.377	2.423	2.923	2.257
Rank	3	2	1	4

Chapter 7
Conclusion

7.1 Conclusion

- A marginal decrease in reaction time by using ultrasonic assisted enzymatic inter-esterification as compared to conventional enzymatic inter-esterification.
- The biodiesel fuel mixture (FAME+TA) properties confirm the ASTM D6751 standards.
- The effect of the triacetin presence in FAME negligibly affect the fuel properties as compared to biodiesel from trans-esterification.
- The effect of various reaction parameters is calculated. The reaction time is most effective parameter among all followed by MAOMR, process temperature and finally the catalyst loading.
- The optimum reaction conditions for ultrasonic assisted enzymatic inter-esterification are
    - Process temperature - 30° C
    - Methyl acetate to oil molar ratio - 12:1
    - Reaction time - 180 minute
    - Catalyst loading - 1.25%
    - Duty cycle - 60%

7.2 Recommendation for the future work

The present work can be extended in following directions –

- Analysis of yield using microwaves
- Instead of ultrasonic cavitation, use of hydrodynamic cavitation
- Value analysis of the process
- Fuel performance testing on IC engine
- Developing the production process for industrial biodiesel production
- Developing a continuous flow type enzymatic inter-esterification method

References

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1. H. L. Mac Leana, L. B. Laveb, Evaluating automobile fuel/propulsion system technologies, Progress of Energy Combustion Science 2003; 29:1-69
2. A. Demirbas, Biodiesel fuels from vegetable oils via catalytic and non-catalytic supercritical alcohol transesterifications and other methods: a survey. Energy Convers Manage 2003;44:2093–109
3. S Shah, S Sharma and MN Gupta, Development of bio based products, Bio-energy Resource 2003;91:1-29
4. Z. Helwani, M. R. Othman, N. Aziz, J. Kim and W. J. N. Fernando, Solid Heterogeneous Catalysts for trans-esterification of Triglycerides with Methanol, Applied Catalysis A: General 2009;363: 110
5. S. Nakagaki, A. Bail, V. C. Dossantos, V. H. R. Desouza, H. Vrubel, F. S. Nune and L. P. Ramos, Use of Anhydrous Sodium Molybdate as an Efficient Heterogeneous Catalyst for Soybean Oil Methanolysis, Applied Catalysis A: General 2008; 351: 267-274
6. B. K. Bala, Studies on biodiesels from transformation of vegetable oils for diesel engines, Energy Education Science Technology 2005;15:1–43
7. A. Demirbas, Biodiesel production from vegetable oils via catalytic and non-catalytic supercritical methanol trans-esterification methods, Progress in Energy and Combustion Science 2005; 31; 466–487
8. M. Canakci, J. Van Gerpen, Biodiesel production via acid catalysts, Transactions of the ASAE. 1999; VOL. 42(5);1203-1210
8. Obie Farobie, Y. Matsumura, A comparative study of biodiesel production using methanol, ethanol and tert-butyl ether (MTBE) under supercritical conditions, Bioresourse Technology 2015;191:306-311

9. A. Demirbas, Biodiesel from vegetable oils via trans-esterification in supercritical methanol, Energy conversion and management 2002; 43:2349-2356

10. A. Demirbas, Biodiesel production via non-catalytic SCF method and biodiesel fuel characteristics, Energy conversion and management 2006; 47:2271-2282

11. Abraham Casas, María Jesús Ramos and Ángel Pérez, Methanol-enhanced chemical inter-esterification of sunflower oil with methyl acetate, Fuel 2013; 106:869-872

12. Z. Sustere, R. Murnieks and V. Kampars, Chemical inter-esterification of rapeseed oil with methyl, ethyl, propyl and isopropyl acetates and fuel properties of obtained mixtures, Fuel Processing Technology 2016;149: 320–325

13. Abraham Casas, María Jesús Ramos and Ángel Pérez, New trends in biodiesel production: Chemical inter-esterification of sunflower oil with methyl acetate, Biomass and bio energy 2011; 35:1702-1709

14. Ganesh L. Maddikeri, Aniruddha B. Pandit, Parag R. Gogate, Ultrasound assisted inter-esterification of waste cooking oil and methyl acetate for biodiesel and triacetin production, Fuel Processing Technology 2013; 116: 241-249

15. Preeti B. Subhedar, Parag R. Gogate, Ultrasound assisted intensification of biodiesel production using enzymatic inter-esterification, Ultrasonics Sonochemistry 2016;29:67-75

16. Roberta Claro da Silva, Fabiana Andreia Schaffer De Martini Soares, Thaı´s Gonzaga Fernandes, Anna Laura Donadi Castells, Kelly Caroline Guimara˜es da Silva, Maria Ine^s Almeida Gonc¸alves, Chiu Chih Ming, Lireny Aparecida Guaraldo Gonc¸alves and Luiz Antonio Gioielli, Inter-esterification of Lard and Soybean Oil Blends Catalyzed by Immobilized Lipase in a Continuous Packed Bed Reactor, Journal of the American Oil Chemists' Society 2011; DOI 10.1007/s11746-011-1869-x

17. H. N. Pandey, Development of a New Catalyst for Bio-diesel Production (ID: 2010/NFIB/163), (2008); 2.

18. Y. M. Park, J. Y. Lee, S. H. Chung, I. S. Park, S. Y. Lee, D. K. Kim, J. S. Lee and K. Y. Lee, Esterification of used Vegetable Oils using the Heterogeneous WO_3/ZrO Catalyst for Production of Biodiesel, Bioresource Tech. 2010; 101(1): S59-S61

19. Mittelbach M, Gangl S. Long storage stability of biodiesel made from rapeseed and used frying oil. J Am Oil ChemSoc 2001;78:573–577

20. Kusdiana D, Saka S., Effects of water on biodiesel fuel production by supercritical methanol treatment, Bioresourse Technology 2004;91:289‑95

21. Vicente G, Martinez M, Aracil J. Integrated biodiesel production: a comparison of different homogeneous catalysts systems. Bioresourse Technology 2004;92:297–305

22. Ashok Pandey, Handbook of plant based biofuels, 2009 (CRC Press, ISBN 13: 978-1-56022-175-3, 186-189

23. Garg Paterson, Thesis on ion-exchange resins as heterogeneous catalysts in biodiesel production, copyright Greg Paterson 2012,19-22

24. Ashok Pandey, Handbook of plant based biofuels, 2009 (CRC Press, ISBN 13: 978-1-56022-175-3, 24-27

25. Shiro Saka, Yohei Isayama, A new process for catalyst-free production of biodiesel using supercritical methyl acetate, Fuel 2009;88:1307-1313

26. DT Johnson, KA. Taconi, The glycerin glut: Options for the value-added conversion of crude glycerol resulting from biodiesel production, Environmental Progress and Sustainable Energy 2007;26: 338–48

27. Cholada Komintarachat, Ruengwit Sawangkeaw, Somkiat Ngamprasertsith; Continuous production of palm biofuel under supercritical ethyl acetate; Energy Conversion and Management 2015; 95:332-338

28. Shiro Saka, Yohei Isayama; A new process for catalyst-free production of biodiesel using supercritical methyl acetate; Fuel 2009;88:1307-1313

29. http://www.afdc.energy.gov/fuels/biodiesel_specifications.html assessed on June 26, 2016; 3:43 PM.

30. R. S. Mishra, Amit Pal, Anand Prakash Mall; Application of Taguchi Experimental Design for the optimization of effective parameters on the Neem oil Methyl Ester (Biodiesel) production; International Journal of Advance Research and Innovation 2015;3:490-497